

Literature on Genetic Effects of Non-Ionizing Radiation

ELF-EMF/Static Field Genetic Effects Studies (current to April 23, 2022)

Of 307 total studies: E = 257 (84%) NE = 50 (16%)

RFR Genetic Effects Studies. (current to April 24, 2022)

Of 423 total studies: E – 291 (68%) NE = 132 (32%)

(E = reported biological effect NE = reported no biological effect)

Key: (VT-in vitro study; VO- in vivo study; HU- human study; LE- long term/repeated exposure; AE- acute exposure; LI- low intensity; GT- genotoxic effect, e.g., DNA damage, micronucleus formation, chromosome alterations; GE- gene expression; OX- oxidative effects, i.e., involvement of free radicals and oxidative enzymes; IX- interaction with other factors to cause genetic effects; DE- effects on developing animals; RP- reproduction, e.g., sperm damage; WS- waveform specific effect, e.g., modulation and frequency; CS- cell type specific effect; EP-epigenetic effect).

(E) Agliassa C, Narayana R, Berteau CM, Rodgers CT, Maffei ME. Reduction of the geomagnetic field delays *Arabidopsis thaliana* flowering time through downregulation of flowering-related genes. *Bioelectromagnetics*. 39(5):361-374, 2018. (VO, LE, GE)

Variations in magnetic field (MF) intensity are known to induce plant morphological and gene expression changes. In *Arabidopsis thaliana* Col-0, near-null magnetic field (NNMF, i.e., <100 nT MF) causes a delay in the transition to flowering, but the expression of genes involved in this response has been poorly studied. Here, we showed a time-course quantitative analysis of the expression of both leaf (including clock genes, photoperiod pathway, GA20ox, SVP, and vernalization pathway) and floral meristem (including GA20ox, SOC1, AGL24, LFY, AP1, FT, and FLC) genes involved in the transition to flowering in *A. thaliana* under NNMF. NNMF induced a delayed flowering time and a significant reduction of leaf area index and flowering stem length, with respect to controls under geomagnetic field. Generation experiments (F₁ - and F₂ -NNMF) showed retention of flowering delay. The quantitative expression (qPCR) of some *A. thaliana* genes expressed in leaves and floral meristem was studied during transition to flowering. In leaves and flowering meristem, NNMF caused an early downregulation of clock, photoperiod, gibberellin, and vernalization pathways and a later down regulation of TSF, AP1, and FLC. In the floral meristem, the downregulation of AP1, AGL24, FT, and FLC in early phases of floral

development was accompanied by a down regulation of the gibberellin pathway. The progressive upregulation of AGL24 and AP1 was also correlated to the delayed flowering by NNMF. The flowering delay is associated with the strong downregulation of FT, FLC, and GA20ox in the floral meristem and FT, TSF, FLC, and GA20ox in leaves.

(E) Ahuja YR, Vijayashree B, Saran R, Jayashri EL, Manoranjani JK, Bhargava SC. In vitro effects of low-level, low-frequency electromagnetic fields on DNA damage in human leucocytes by comet assay. Indian J Biochem Biophys. 36(5):318-322, 1999. (VT, AE, GT)

The sources for the effects of electromagnetic fields (EMFs) have been traced to time varying as well as steady electric and magnetic fields, both at low and high to ultra high frequencies. Of these, the effects of low-frequency (50/60 HZ) magnetic fields, directly related to time-varying currents, are of particular interest as exposure to some fields may be commonly experienced. In the present study, investigations have been carried out at low-level (mT) and low-frequency (50 Hz) electromagnetic fields in healthy human volunteers. Their peripheral blood samples were exposed to 5 doses of electromagnetic fields (2,3,5,7 and 10mT at 50 Hz) and analysed by comet assay. The results were compared to those obtained from unexposed samples from the same subjects. 50 cells per treatment per individual were scored for comet-tail length which is an estimate of DNA damage. Data from observations among males were pooled for each flux density for analysis. At each flux density, with one exception, there was a significant increase in the DNA damage from the control value. When compared with a similar study on females carried out by us earlier, the DNA damage level was significantly higher in the females as compared to the males for each flux density.

(E) Akbarnejad Z, Eskandary H, Dini L, Vergallo C, SN, Farsinejad A, MFS, Ahmadi M. Cytotoxicity of temozolomide on human glioblastoma cells is enhanced by the concomitant exposure to an extremely low-frequency electromagnetic field (100Hz, 100G). Biomed Pharmacother 92:254-264, 2017. (VT,AE,GE, IX)

Glioblastoma multiforme (GBM) is the most malignant brain cancer that causes high mortality in humans. It responds poorly to the most common cancer treatments, such as surgery, chemo- and radiation therapy. Temozolomide (TMZ) is an alkylating agent that has been widely used to treat GBM; resistance to this drug is often found. One unexplored possibility for overcoming this resistance is a treatment based on concomitant exposure to electromagnetic fields (EMF) and TMZ. Indeed, many evidences show that EMF affects cancer cells and drug performance. In this study, we evaluated the potential synergistic effect of 100 μ M TMZ and EMF (100Hz, 100G) on two human glioma cells line, i.e., U87 and T98G above single treatments, TMZ or EMF. Co-treatment synergistically enhanced apoptosis in U87 and T98G cells, by increasing the expression of P53, Bax, and Caspase-3 and decreasing that of Bcl-2 and Cyclin-D1. We also observed an increase in reactive oxygen species (ROS) production and the overexpression of the heme oxygenase-1 (HO-1) gene in comparison to controls. In conclusion, since EMF enhanced

the apoptotic effect of TMZ, possibly through a redox regulation mechanism, the TMZ/EMF combination may be effective for glioma cancer treating. Further studies are needed to reveal the action mechanism of this possible novel therapeutic approach.

(NE) Albert GC, McNamee JP, Marro L, Bellier PV, Prato FS, Thomas AW. Assessment of genetic damage in peripheral blood of human volunteers exposed (whole-body) to a 200 μ T, 60 Hz magnetic field. Int J Radiat Biol. 85(2):144-152, 2009. (HU, AE, GT)

AIM: To investigate the extent of damage in nucleated cells in peripheral blood of healthy human volunteers exposed to a whole-body 60 Hz, 200 microT magnetic field. **MATERIALS AND METHODS:** In this study, 10 male and 10 female healthy human volunteers received a 4 h whole-body exposure to a 200 microT, 60 Hz magnetic field. In addition, five males and five females were treated in a similar fashion, but were exposed to sham conditions. For each subject, a blood sample was obtained prior to the exposure period and aliquots were used as negative- (pre-exposure) and positive- [1.5 Gray (Gy) (⁶⁰Cobalt ((⁶⁰Co) gamma-irradiation] controls. At the end of the 4 h exposure period, a second blood sample was obtained. The extent of DNA damage was assessed in peripheral human blood leukocytes from all samples using the alkaline comet assay. To detect possible clastogenic effects, the incidence of micronuclei was assessed in phytohemagglutinin (PHA)-stimulated lymphocytes using the cytokinesis-block micronucleus assay. **RESULTS:** There was no evidence of either increased DNA damage, as indicated by the alkaline comet assay, or increased incidence of micronuclei (MN) in the magnetic field exposed group. However, an in vitro exposure of 1.5 Gy gamma-irradiation caused a significant increase in both DNA damage and MN induction. **CONCLUSIONS:** This study found no evidence that an acute, whole-body exposure to a 200 microT, 60 Hz magnetic field for 4 hours could cause DNA damage in human blood.

(E) Alcaraz M, Olmos E, Alcaraz-Saura M, Achel DG, Castillo J. Effect of long-term 50 Hz magnetic field exposure on the micronucleated polychromatic erythrocytes of mice. Electromagn Biol Med. 33(1):51-57, 2014. (VO, LE, GT, OX)

In recent years extremely low-frequency magnetic fields (ELF-EMF) have become widely used in human activities, leading to an increased chance of exposure to ELF-EMF. There are few reports on in vivo mammalian genotoxic effects using micronucleus (MN) assays, which generally have been used as a short-term screening system. We analyzed the possible genotoxic effect induced by long-term exposure (7, 14, 21, 28 d) of a 50 Hz ELM-MF to mice by measuring the increase in frequency of micronucleated polychromatic erythrocyte in their bone marrow (MNPCEs) and we compared it with that induced by 50 cGy of X-rays. Subsequently, we tried to reduce this chromosomal damage by administering four antioxidants substances with radioprotective capacities: dimethyl sulfoxide (DMSO), 6-n-propyl-2-thiouracil (PTU), grape-procyanidins (P) and citrus flavonoids extract (CE). The increase in micronucleated cells was higher in both physical treatments (Control < ELF-EMF (p < 0.01) < X-rays (p > 0.001)); however, the antioxidant substances only showed a genoprotective capacity against the damage induced by ionizing radiation (Ci > PTU = DMSO (p < 0.001) > P = CE (p < 0.001). The 50 Hz ELM-MF increased MNPCEs in mouse bone marrow, expressing a genotoxic capacity.

Administration of antioxidant substances with radioprotective capacities known to act through the elimination of free radicals did not diminish the genotoxic effect induced by ELM-MF.

(E) Al-Huqail AA, Abdelhaliem E. Evaluation of genetic variations in maize seedlings exposed to electric field based on protein and DNA markers. Biomed Res Int. 2015:874906, 2015. (VO, AE, GT) (electric field)

The current study analyzed proteins and nuclear DNA of electric fields (ELF) exposed and nonexposed maize seedlings for different exposure periods using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), isozymes, random amplified polymorphic DNA (RAPD), and comet assay, respectively. SDS-PAGE analysis revealed total of 46 polypeptides bands with different molecular weights ranging from 186.20 to 36.00 KDa. It generated distinctive polymorphism value of 84.62%. Leucine-aminopeptidase, peroxidase, and catalase isozymes showed the highest values of polymorphism (100%) based on zymograms number, relative front (R f), and optical intensity while esterase isozyme generated polymorphism value of 83.33%. Amino acids were analyzed using high-performance liquid chromatography, which revealed the presence of 17 amino acids of variable contents ranging from 22.65% to 28.09%. RAPD revealed that 78 amplified DNA products had highly polymorphism value (95.08%) based on band numbers, with variable sizes ranging from 120 to 992 base pairs and band intensity. Comet assay recorded the highest extent of nuclear DNA damage as percentage of tailed DNA (2.38%) and tail moment unit (5.36) at ELF exposure of maize nuclei for 5 days. The current study concluded that the longer ELF exposing periods had genotoxic stress on macromolecules of maize cells and biomarkers used should be augmented for reliable estimates of genotoxicity after exposure of economic plants to ELF stressors.

(E) Amara S, Abdelmelek H, Garrel C, Guiraud P, Douki T, Ravanat JL, Favier A, Sakly M, Ben Rhouma K. Effects of subchronic exposure to static magnetic field on testicular function in rats. Arch Med Res. 37(8):947-952, 2006. (VO, CE, GT)

Background: The aim of this study was to investigate the effect of static magnetic field (SMF) exposure on testicular function, antioxidant status and DNA oxidation in rats. **Methods:** Male adult rats were exposed to SMF (128 mT; 1 h/day for 30 days). After sacrifice, the epididymal sperm number was counted. Testosterone concentration in plasma and testis was measured by radioimmunoassay. MDA level and GPx, CAT and SOD activities were used as markers of oxidative stress in testis. The 8-oxo-dG level is measured by the HPLC-EC system.

Results: Subchronic exposure to SMF has no effect on epididymal sperm count, spermatozoa motility and genital organ weight. In contrast, SMF induces a decrease of testicular and plasmatic testosterone levels, respectively (1.48 +/- 0.56 vs. 4.66 +/- 0.51 ng/g, p<0.05; 0.97 +/- 0.16 vs. 1.64 +/- 0.18 ng/mL, p<0.05). Exposed rats displayed an increase of malondialdehyde (2.01 +/- 0.03 vs. 1.47 +/- 0.06 micromol/g protein, p<0.05), metallothioneins (1.04 +/- 0.22 vs. 0.37 +/- 0.06 microg/g, p<0.05) and 8-oxo-dG concentrations (3.38 +/- 0.30 vs. 2.36 +/- 0.28 8-oxo-dG/10(6) bases, p<0.05) in the testis. In the gonad, SMF decreases the CAT (14.33 +/- 1.16 vs. 21.67 +/- 2.05 U/mg protein, p<0.05), GPx (177.40 +/- 5.97 vs. 237.20 +/- 15.65 U/mg

protein, $p < 0.05$) and mitochondrial Mn-SOD (2.95 ± 0.10 vs. 3.53 ± 0.29 U/mg protein, $p < 0.05$) activities. However, cytosolic CuZn-SOD activity is unaffected.

Conclusions: Subchronic exposure to SMF failed to alter spermatogenesis in rat testis. In contrast, the same treatment decreased testosterone levels and induced DNA oxidation.

(E) Amara S, Douki T, Ravanat JL, Garrel C, Guiraud P, Favier A, Sakly M, Ben Rhouma K, Abdelmelek H. Influence of a static magnetic field (250 mT) on the antioxidant response and DNA integrity in THP1 cells. Phys Med Biol. 52(4):889-898, 2007a. (VT, AE, GT)

The aim of this study was to investigate the effect of static magnetic field (SMF) exposure in antioxidant enzyme activity, the labile zinc fraction and DNA damage in THP1 cells (monocyte line). Cell culture flasks were exposed to SMF (250 mT) during 1 h (group 1), 2 h (group 2) and 3 h (group 3). Our results showed that cell viability was slightly lower in SMF-exposed groups compared to a sham exposed group. However, SMF exposure failed to alter malondialdehyde (MDA) concentration (+6%, $p > 0.05$) and glutathione peroxidase (GPx) (-5%, $p > 0.05$), catalase (CAT) (-6%, $p > 0.05$) and superoxide dismutase (SOD) activities (+38%, $p > 0.05$) in group 3 compared to the sham exposed group. DNA analysis by single cell gel electrophoresis (comet assay) revealed that SMF exposure did not exert any DNA damage in groups 1 and 2. However, it induced a low level of DNA single strand breaks in cells of group 3. To further explore the oxidative DNA damage, cellular DNA for group 3 was isolated, hydrolyzed and analysed by HPLC-EC. The level of 8-oxodGuo in this group remained unchanged compared to the sham exposed group (+6.5%, $p > 0.05$). Cells stained with zinc-specific fluorescent probes zinpyr-1 showed a decrease of labile zinc fraction in all groups exposed to SMF. Our data showed that SMF exposure (250 mT, during 3 h) did not cause oxidative stress and DNA damage in THP1 cells. However, SMF could alter the intracellular labile zinc fraction.

(E) Amara S, Abdelmelek H, Garrel C, Guiraud P, Douki T, Ravanat JL, Favier A, Sakly M, Ben Rhouma K. Zinc supplementation ameliorates static magnetic field-induced oxidative stress in rat tissues. Environ Toxicol Pharmacol. 23(2):193-197, 2007b. (VO, CE, GT, OX, IX)

The present study was undertaken to find out the effect of zinc supplementation on the antioxidant enzymatic system, lipid peroxidation and DNA oxidation in liver and kidney of static magnetic field (SMF) exposed rats. The exposure of rats to SMF (128mT, 1h/day during 30 consecutive days) decreased the activities of glutathione peroxidase (GPx), catalase (CAT) and the superoxide dismutase (SOD) in liver and kidney. By contrast, sub-chronic exposure to SMF increased the malondialdehyde (MDA) concentration in liver and kidney. Our results revealed an increase of the 8-oxo-7,8-dihydro-2'-desoxyguanosine (8-oxodGuo) in kidney of SMF-exposed rats. However, this biomarker of DNA oxidation remained unchanged in liver. Zinc supplementation (ZnCl₂, 40mg/l, per os) in SMF-exposed rats restored the activities of GPx, CAT and SOD in liver to those of control group. However, only CAT activity was restored in kidney. Moreover, zinc administration was able to bring down the elevated levels of MDA in the liver but not in the kidney. Interestingly, zinc supplementation attenuated DNA oxidation

induced by SMF in kidney to the control level. Our investigations suggested that zinc supplementation minimizes oxidative damage induced by SMF in rat tissues.

(NE) Amara S, Douki T, Garel C, Favier A, Sakly M, Rhouma KB, Abdelmelek H. Effects of static magnetic field exposure on antioxidative enzymes activity and DNA in rat brain. Gen Physiol Biophys. 28(3):260-265, 2009. (VO, CE, GT)

The present study was undertaken in order to investigate the effects of static magnetic field (SMF) exposure on the antioxidative enzymes activity, malondialdehyde (MDA) concentration and DNA oxidation in male rat brain. The exposure of rats to SMF (128 mT, 1 h/day during 30 consecutive days) decreased the glutathione peroxidase (GPx; -39%, $p < 0.05$), CuZn superoxide dismutase (CuZn-SOD; -35%, $p < 0.05$) and catalase (-59%, $p < 0.05$) activities in frontal cortex. The same treatment decreased the CuZn-SOD (-51%, $p < 0.05$) and Mn-SOD (-13%, $p < 0.05$) activities in hippocampus. However, the glutathione levels remained unchanged in the both brain structures. In the hippocampus, SMF exposure increased MDA concentration (+32%, $p < 0.05$). Interestingly, exposed-rats to SMF displayed a significant increase of metallothioneins level in frontal cortex (+100%, $p < 0.05$), while the 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo) concentration remained unaffected, indicating the absence of DNA oxidation. Our results indicated that sub-chronic exposure to SMF induced oxidative stress in rat hippocampus and frontal cortex. Metallothionein induction protected probably DNA against oxidative damage.

(NE) Amara S, Douki T, Garrel C, Favier A, Ben Rhouma K, Sakly M, Abdelmelek H. Effects of static magnetic field and cadmium on oxidative stress and DNA damage in rat cortex brain and hippocampus. Toxicol Ind Health. 27(2):99-106, 2011. (VO, LE, GT, OX, IX)

The present study was undertaken to determine the effect of co-exposure to static magnetic field (SMF) and cadmium (Cd) on the antioxidant enzymes activity and DNA integrity in rat brain. Sub-chronic exposure to CdCl₂ (CdCl₂, 40 mg/L, per os) for 30 days resulted in a significant reduction in antioxidant enzyme activity such as the glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) in frontal cortex and hippocampus. Total GSH were decreased in the frontal cortex of the Cd-exposed group. Cd exposure induced an increase in malondialdehyde (MDA) concentration in the frontal cortex and hippocampus. Moreover, the same exposure increased 8-oxo-7,8-dihydro-2'-desoxyguanosine (8-oxodGuo) level in rat brain. Interestingly, the combined effect of SMF (128 mT, 1 hour/day for 30 consecutive days) and CdCl₂ (40 mg/L, per os) decreased the SOD activity and glutathione level in frontal cortex as compared with the Cd group. Moreover, the association between SMF and Cd increased MDA concentration in frontal cortex as compared with Cd-exposed rats. DNA analysis revealed that SMF exposure failed to alter 8-oxodGuo concentration in Cd-exposed rats. Our data showed that Cd exposure altered the antioxidant enzymes activity and induced oxidative DNA lesions in rat brain. The combined effect of SMF and Cd increased oxidative damage in rat brain as compared with Cd-exposed rats.

(E) Arruda-Neto J.D.T., Friedberg, E.C., Bittencourt-Oliveira M.C. et al. (2009). Static electric fields interfere in the viability of cells exposed to ionising radiation. Int. J. Rad. Biol. 85:314-321. (VT, AE, GT, IX)

Purpose: The interference of electric fields (EF) with biological processes is an issue of considerable interest. No studies have as yet been reported on the combined effect of EF plus ionising radiation. Here we report studies on this combined effect using the prokaryote *Microcystis panniformis*, the eukaryote *Candida albicans* and human cells. *Materials and methods:* Cultures of *Microcystis panniformis* (Cyanobacteria) in glass tubes were irradiated with doses in the interval 0.5–5 kGy, using a ^{60}Co gamma source facility. Samples irradiated with 3 kGy were exposed for 2 h to a $20 \text{ V} \cdot \text{cm}^{-1}$ static electric field and viable cells were enumerated. Cultures of *Candida albicans* were incubated at 36°C for 20 h, gamma-irradiated with doses from 1–4 kGy, and submitted to an electric field of $180 \text{ V} \cdot \text{cm}^{-1}$. Samples were examined under a fluorescence microscope and the number of unviable (red) and viable (apple green fluorescence) cells was determined. For crossing-check purposes, MRC5 strain of lung cells were irradiated with 2 Gy, exposed to an electric field of 1250 V/cm, incubated overnight with the antibody anti-phospho-histone H2AX and examined under a fluorescence microscope to quantify nuclei with γ -H2AX foci. *Results:* In cells exposed to EF, death increased substantially compared to irradiation alone. In *C. albicans* we observed suppression of the DNA repair shoulder. The effect of EF in growth of *M. panniformis* was substantial; the number of surviving cells on day-2 after irradiation was 12 times greater than when an EF was applied. By the action of a static electric field on the irradiated MRC5 cells the number of nuclei with γ -H2AX foci increased 40%, approximately. *Conclusions:* Application of an EF following irradiation greatly increases cell death. The observation that the DNA repair shoulder in the survival curve of *C. albicans* is suppressed when cells are exposed to irradiation + EF suggests that EF likely inactivate cellular recovering processes. The result for the number of nuclei with γ -H2AX foci in MRC5 cells indicates that an EF interferes mostly in the DNA repair mechanisms. A molecular ad-hoc model is proposed

(E) Ashta A, Motalleb G, Ahmadi-Zeidabadi M. Evaluation of frequency magnetic field, static field, and Temozolomide on viability, free radical production and gene expression (p53) in the human glioblastoma cell line (A172) Electromagn Biol Med 39: 298-309, 2020. (VT, AE, GE, IX)

Thirteen million cancer deaths and 21.7 million new cancer cases are expected in the world by 2030. Glioblastoma is the most common primary malignant tumor of the central nervous system which is the most lethal type of primary brain tumor in adults with the survival time of 12-15 months after the initial diagnosis. Glioblastoma is the most common and most malignant type of brain tumor, and despite surgery, chemotherapy and radiation treatment, the average survival of patients is about 14 months. The current research showed that the frequency magnetic field (FMF) and static magnetic field (SMF) can influence cancer cell proliferation and coupled with anticancer drugs may provide a new strategy for cancer therapy. At the present study, we investigated the effects of FMF (10 Hz, 50 G), SMF (50 G) and Temozolomide (200 μm) on viability, free radical production, and p53 followed by p53 protein expression in the human glioblastoma cell line (A172) by MTT, NBT, RT-PCR and Western blot. Results showed that the

effect of Temozolomide (TMZ) with SMF and FMF together increased the cytotoxicity, free radical production, and *p53* followed by p53 protein expression in the human glioblastoma cell line (A172).

(E) Baek S, Choi H, Park H, Cho B, Kim S, Kim J. Effects of a hypomagnetic field on DNA methylation during the differentiation of embryonic stem cells. Sci Rep. 9(1):1333, 2019. (VT, CE, GT, EP)

It has been reported that hypomagnetic fields (HMFs) have a negative influence on mammalian physiological functions. We previously reported that HMFs were detrimental to cell fate changes during reprogramming into pluripotency. These studies led us to investigate whether HMFs affect cell fate determination during direct differentiation. Here, we found that an HMF environment attenuates differentiation capacity and is detrimental to cell fate changes during the *in vitro* differentiation of embryonic stem cells (ESCs). Moreover, HMF conditions cause abnormal DNA methylation through the dysregulation of DNA methyltransferase3b (Dnmt3b) expression, eventually resulting in incomplete DNA methylation during differentiation. Taken together, these results suggest that an appropriate electromagnetic field (EMF) environment may be essential for favorable epigenetic remodeling during cell fate determination via differentiation.

(E) Bagheri Hosseinabadi M, Khanjani N, Mirzaii M, Norouzi P, Atashi A. DNA damage from long-term occupational exposure to extremely low frequency electromagnetic fields among power plant workers. Mutat Res. 846:403079, 2019.(HU, LE, GT)

Extremely low frequency electromagnetic fields (ELF-EMFs) are not known as definite occupational carcinogens, but some studies have reported the genotoxic effects of these fields on cell lines. The present study aimed to evaluate the effects of long-term occupational exposure to these fields on DNA damage. In this cross-sectional study, blood samples were taken from 102 thermal power plant workers as the exposure group and 136 subjects as the unexposed group. DNA damage was evaluated using alkaline comet assay and flow cytometry. Exposure to ELF-EMFs was measured based on spot measurements and the IEEE Std C95.3.1 standard. The indices of comet assay, tail DNA percent, tail factor (%), and damage index were significantly higher in the exposed group compared to the unexposed group. Increased exposure to magnetic fields enhanced comet assay indices, except tail length; while exposure to electric fields had no significant effect on such indices. The percentage of cells at early apoptosis and late apoptosis phases caused by exposure to magnetic fields, respectively, decreased and increased significantly. Long-term occupational exposure to ELF-EMFs can probably cause genotoxic effects.

(E) Bagheri Hosseinabadi M, Khanjani N, Atashi A, Norouzi P, Mirbadie SR, Mirzaii M. The effect of vitamin E and C on comet assay indices and apoptosis in power plant workers: A double blind randomized controlled clinical trial. Mutat Res. 850-851:503150, 2020. (HU, LE, GT, OX)

Extremely low frequency electromagnetic fields have been classified as a possible human carcinogen by the International Agency for Research on Cancer and this has raised some concern about its health effects on employees extensively exposed to these fields at thermal power plants. In this study, the effect of using vitamin E and C supplements have been examined on employees working at a thermal power plant. In this randomized controlled, double-blind clinical trial, 81 employees from different parts of the thermal power plant were enrolled between July and November 2017, and divided into four groups: Group 1 received vitamin E (400 units/day), Group 2: vitamin C (1000 mg/day), Group 3: vitamin E + C and Group 4: no intervention. DNA damage was measured in peripheral blood lymphocytes using comet assay and apoptosis, using flow cytometry. Based on the results, tail intensity and tail length in the vitamin E group, and all comet assay indices in the vitamin E + C and vitamin C groups (except DNA damage index) significantly decreased after the intervention, while the comet assay indices did not change significantly in the control group. None of the flow cytometry indices including early apoptosis, late apoptosis and necrosis changed after intervention in either group. The use of antioxidant vitamins such as E and C, can increase the activity of the non-enzymatic antioxidant defense system, and protect DNA from damage caused by exposure to extremely low frequency magnetic fields. But, taking these vitamins has no effect on apoptosis. It seems that consumption of vitamin E affected all investigated comet assay indices and can be probably considered as the best intervention.

(E) Bai W, Li M, Xu W, Zhang M. Comparison of effects of high- and low-frequency electromagnetic fields on proliferation and differentiation of neural stem cells. Neurosci Lett 741:135463, 2021.(VT, LE, GE)

To compare the effects of high- (HF-EMF) and low-frequency electromagnetic fields (LF-EMF) on the proliferation and differentiation of neural stem cells (NSCs). NSCs were obtained from SD rat hippocampus and cultured in suspension and adherent differentiation media. NSCs were exposed to LF-EMF (5 m T, 50 Hz, 30 min daily), HF-EMF (maximum magnetic induction 2.5 T, 40% MO, 50 Hz, 10 min daily) and no electromagnetic field. At 3 d, cell viability and quantity of NSCs in suspension were detected by CCK-8 assay and cell counting plate. Immunofluorescence staining and qRT-PCR were performed to detect the percentage of Tuj-1 and GFAP-positive NSCs and the expression of Tuj-1 and GFAP mRNA. The P3 NSCs were positive with Nestin and induced NSCs expressed Tuj-1, GFAP and oligodendrocyte markers (MBP). CCK-8 assay and cell counting showed that the OD value and quantity of LF-EMF group were significantly higher than those in other two groups (both $P < 0.05$). Compared with the control group, the OD value and quantity were significantly higher in the HF-EMF group ($P < 0.05$). Immunofluorescence staining and qRT-PCR revealed that the percentage of Tuj-1 positive cells and the expression of Tuj-1 mRNA of NSCs exposed to LF-EMF were the highest (both $P < 0.05$). The proportion of GFAP-positive NSCs and the expression of GFAP mRNA did not significantly differ among three groups (all $P > 0.05$). Both 50 Hz LF-EMF and HF-EMF can promote the proliferation of NSCs in vitro and LF-EMF can accelerate NSCs to differentiate into neurons.

(E) Balamuralikrishnan B, Balachandar V, Kumar SS, Stalin N, Varsha P, Devi SM, Arun M, Manikantan P, Venkatesan C, Sasikala K, Dharwadkar SN. Evaluation of chromosomal alteration in electrical workers occupationally exposed to low frequency of electro magnetic field (EMFs) in coimbatore population, India. Asian Pac J Cancer Prev. 13(6):2961-2966, 2012. (HU, LE, GT)

Extremely low frequency electromagnetic fields (EMFs) have been classified as possibly carcinogenic to humans by the International Agency for Research on Cancer. An increased number of chromosomal alterations in peripheral lymphocytes are correlated with elevated incidence of cancer. The aim of the present study was to assess occupationally induced chromosomal damage in EMF workers exposed to low levels of radiation. We used conventional metaphase chromosome aberration (CA) analysis and the micronucleus (MN) assay as biological indicators of nonionizing radiation exposure. In the present study totally 70 subjects were selected including 50 exposed and 20 controls. Informed written consent was obtained from all participants and the study was performed in accordance with the Declaration of Helsinki and the approval of the local ethical committee. A higher degree of CA and MN was observed in exposed subjects compared to controls, the frequency of CA being significantly enhanced with long years of exposure ($P < 0.05$). Moreover increase in CA and MN with age was noted in both exposed subjects and controls, but was significantly greater in the former. The results of this study demonstrated that a significant induction of cytogenetic damage in peripheral lymphocytes of workers occupationally exposed to EMFs in electric transformer and distribution stations. In conclusion, our findings suggest that EMFs possess genotoxic capability, as measured by CA and MN assays; CA analysis appeared more sensitive than other cytogenetic end-points. It can be concluded that chronic occupational exposure to EMFs may lead to an increased risk of genetic damage among electrical workers.

(E) Barati M, Javidi MA, Darvishi B, Shariatpanahi SP, Moosavi ZSM, Ghadirian R, Khani T, Sanati H, Simaee H, Barough MS, Farahmand L, Ansari AM. Necroptosis triggered by ROS accumulation and Ca^{2+} overload, partly explains the inflammatory responses and anti-cancer effects associated with 1Hz, 100 mT ELF-MF in vivo. Free Radic Biol Med 2021 Jun;169:84-98. (VT, LE, GE)

Whereas the anti-neoplastic activity of extremely low frequency magnetic fields (ELF-EMF) is well-documented in literature, little is known about its underlying anti-cancer mechanisms and induced types of cell death. Here, for the first time, we reported induction of necroptosis, a specific type of programmed necrotic cell death, in MC4-L2 breast cancer cell lines following a 2 h/day exposure to a 100 Hz, 1 mT ELF-EMF for five days. For in vivo assessment, inbred BALB/c mice bearing established MC-4L2 tumors were exposed to 100 mT, 1 Hz ELF-EMF 2 h daily for a period of 28-day, following which tumors were dissected and fixed for evaluation of tumor biomarkers expression and types of cell death induced using TUNEL assay, Immunohistochemistry and H&E staining. Peripheral blood samples were also collected for assessing pro-inflammatory cytokine profile following exposure. An exaggerated proinflammatory response evident from enhancement of IFN- γ (4.8 ± 0.24 folds) and TNF- α (3.1 ± 0.19 folds) and number of tumors infiltrating lymphocytes (TILs), specially CD8⁺ T_h cells (~20 folds), proposed occurrence of necroptosis in vivo. Meanwhile, exposure could effectively

suppress tumor growth and expression of Ki-67, CD31, VEGFR2 and MMP-9. In vitro studies on ELF-EMF exposed MC-4L2 cells demonstrated a meaningful increase in phosphorylation of RIPK1/RIPK3/MLKL proteins and cleavage of caspase-9/caspase-3, confirming occurrence of both necroptosis and apoptosis. Complementary in vitro studies by treating ELF-EMF exposed MC-4L2 cells with verapamil (a calcium channel inhibitor), N-acetyl cysteine (a ROS scavenger) or calcium chloride confirmed the role of elevated intracellular calcium and ROS levels in ELF-EMF induced necroptosis.

(E) Baraúna RA, Santos AV, Graças DA, Santos DM, Ghilardi R Júnior, Pimenta AM, Carepo MS, Schneider MP, Silva A. Exposure to an extremely low-frequency electromagnetic field only slightly modifies the proteome of *Chromobacterium violaceum* ATCC 12472. *Genet Mol Biol.* 38(2):227-230, 2015. (VT, AE, GE)

Several studies of the physiological responses of different organisms exposed to extremely low-frequency electromagnetic fields (ELF-EMF) have been described. In this work, we report the minimal effects of in situ exposure to ELF-EMF on the global protein expression of *Chromobacterium violaceum* using a gel-based proteomic approach. The protein expression profile was only slightly altered, with five differentially expressed proteins detected in the exposed cultures; two of these proteins (DNA-binding stress protein, Dps, and alcohol dehydrogenase) were identified by MS/MS. The enhanced expression of Dps possibly helped to prevent physical damage to DNA. Although small, the changes in protein expression observed here were probably beneficial in helping the bacteria to adapt to the stress generated by the electromagnetic field.

(E) Belyaev IY, Hillert L, Protopopova M, Tamm C, Malmgren LO, Persson BR, Selivanova G, Harms-Ringdahl M. 915 MHz microwaves and 50 Hz magnetic field affect chromatin conformation and 53BP1 foci in human lymphocytes from hypersensitive and healthy persons. *Bioelectromagnetics* 26:173-184, 2005. (VT, AE, GT)

We used exposure to microwaves from a global system for mobile communication (GSM) mobile phone (915 MHz, specific absorption rate (SAR) 37 mW/kg) and power frequency magnetic field (50 Hz, 15 μ T peak value) to investigate the response of lymphocytes from healthy subjects and from persons reporting hypersensitivity to electromagnetic field (EMF). The hypersensitive and healthy donors were matched by gender and age and the data were analyzed blind to treatment condition. The changes in chromatin conformation were measured with the method of anomalous viscosity time dependencies (AVTD). 53BP1 protein, which has been shown to colocalize in foci with DNA double strand breaks (DSBs), was analyzed by immunostaining in situ. Exposure at room temperature to either 915 MHz or 50 Hz resulted in significant condensation of chromatin, shown as AVTD changes, which was similar to the effect of heat shock at 41 degrees C. No significant differences in responses between normal and hypersensitive subjects were detected. Neither 915 MHz nor 50 Hz exposure induced 53BP1 foci. On the contrary, a distinct decrease in background level of 53BP1 signaling was observed upon these exposures as well as after heat shock treatments. This decrease correlated with the AVTD data and may indicate decrease in accessibility of 53BP1 to antibodies because of stress-induced chromatin condensation. Apoptosis was determined by morphological changes and by apoptotic fragmentation of DNA as analyzed by pulsed-field gel electrophoresis (PFGE). No

apoptosis was induced by exposure to 50 Hz and 915 MHz microwaves. In conclusion, 50 Hz magnetic field and 915 MHz microwaves under specified conditions of exposure induced comparable responses in lymphocytes from healthy and hypersensitive donors that were similar but not identical to stress response induced by heat shock.

(E) Bernardini C, Zannoni A, Turba ME, Bacci ML, Forni M, Mesirca P, Remondini D, Castellani G, Bersani F. Effects of 50 Hz sinusoidal magnetic fields on Hsp27, Hsp70, Hsp90 expression in porcine aortic endothelial cells (PAEC). Bioelectromagnetics 28(3):231-237, 2007. (VT, AE, GE)

The aim of the present study was to investigate the influence of 50 Hz sinusoidal magnetic field on Hsp27, Hsp70, and Hsp90 expression in a model of primary culture of porcine aortic endothelial cells (PAEC). We took into consideration the Hsp profile in terms of mRNA expression, protein expression and protein localization inside the cells. The choice of the cell system was motivated by the involvement of the endothelial cells in the onset of many diseases; moreover, only few reports describe the effects of extremely low frequency magnetic fields (ELF-MFs) on such cells. ELF-MF exposure induced an increase in the mRNA levels of the three proteins, which was statistically significant for Hsp70. On the contrary, we did not observe any influence on Hsp27, Hsp70, and Hsp90 protein levels. Analysis in situ by immunofluorescence revealed that ELF-MF exposure affected the cellular distribution of Hsp27; in particular a partial relocalization in the nucleus was observed.

(E) Berteza, C.M., Narayana, R., Agliassa, C., Rodgers, C.T., Maffei, M.E. Geomagnetic field (Gmf) and plant evolution: investigating the effects of Gmf reversal on *Arabidopsis thaliana* development and gene expression. J. Vis. Exp. (105), e53286, 2015. (VT, LE, GE, OX)

One of the most stimulating observations in plant evolution is a correlation between the occurrence of geomagnetic field (GMF) reversals (or excursions) and the moment of the radiation of Angiosperms. This led to the hypothesis that alterations in GMF polarity may play a role in plant evolution. Here, we describe a method to test this hypothesis by exposing *Arabidopsis thaliana* to artificially reversed GMF conditions. We used a three-axis magnetometer and the collected data were used to calculate the magnitude of the GMF. Three DC power supplies were connected to three Helmholtz coil pairs and were controlled by a computer to alter the GMF conditions. Plants grown in Petri plates were exposed to both normal and reversed GMF conditions. Sham exposure experiments were also performed. Exposed plants were photographed during the experiment and images were analyzed to calculate root length and leaf areas. *Arabidopsis* total RNA was extracted and Quantitative Real Time-PCR (qPCR) analyses were performed on gene expression of *CRUCIFERIN 3 (CRU3)*, *copper transport protein1 (COTP1)*, *Redox Responsive Transcription Factor1 (RRTF1)*, *Fe Superoxide Dismutase 1, (FSD1)*, *Catalase3 (CAT3)*, *Thylakoidal Ascorbate Peroxidase (TAPX)*, a cytosolic *Ascorbate Peroxidase1 (APX1)*, and *NADPH/respiratory burst oxidase protein D (RbohD)*. Four different reference genes were analysed to normalize the results of the qPCR. The best of the four genes was selected and the most stable gene for normalization was used. Our data show

for the first time that reversing the GMF polarity using triaxial coils has significant effects on plant growth and gene expression. This supports the hypothesis that GMF reversal contributes to inducing changes in plant development that might justify a higher selective pressure, eventually leading to plant evolution.

(E) Borhani N, Rajaei F, Salehi Z, Javadi A. Analysis of DNA fragmentation in mouse embryos exposed to an extremely low-frequency electromagnetic field. Electromagn Biol Med. 30(4):246-252, 2011. (VO, LE, GT, DE)

Effects of extremely low-frequency electromagnetic fields (ELF-EMFs) on DNA damage in biological systems are still a matter of dispute. The aim of the present study was to investigate the possible effect of electromagnetic field exposure on DNA fragmentation in cells (blastomers) of mouse blastocysts. Eighty female NMRI mice were randomly divided into 2 groups of 40 animals each. The control group was left unexposed whereas the animals in the EMF-group were exposed to a 50-Hz EMF at 0.5 mT 4 h per day, 6 days a week for a duration of 2 weeks. After the 8(th) day of exposure, the female mice in both groups were superovulated (with injections of pregnant mare serum gonadotropin and human chorionic gonadotropin) and then mated overnight. At approximately 4 days after mating (102 h after the human chorionic gonadotropin treatment), blastocysts were obtained by flushing the uterus horns. The mean numbers of pregnant mice, blastocysts after flushing, blastomers within the blastocysts, and the DNA fragmentation index following staining in both groups were compared using statistical methods (SPSS, the Chi-square test, the Student's t-test and the Mann-Whitney U-test, $P < 0.05$). The results showed that the mean number of blastocysts after flushing was significantly decreased in the EMF-group compared to that of the control group ($P < 0.03$). The DNA fragmentation index was significantly increased in the EMF-group compared to control (10.53% vs. 7.14%; $P < 0.001$). However, there was no significant difference in the mean numbers of blastomers and numbers of pregnant mice between the EMF-exposed and control group. Our findings indicate that the EMF exposure in preimplantation stage could have detrimental effects on female mouse fertility and embryo development by decreasing the number of blastocysts and increasing the blastocysts DNA fragmentation.

(NE) Brix G, Günther E, Rössler U, Endesfelder D, Kamp A, Beer A, Eiber M. Double-strand breaks in lymphocyte DNA of humans exposed to [¹⁸F]fluorodeoxyglucose and the static magnetic field in PET/MRI. EJNMMI Res. 10(1):43, 2020. (HU, AE,GT)

BACKGROUND: Given the increasing clinical use of PET/MRI, potential risks to patients from simultaneous exposure to ionising radiation and (electro)magnetic fields should be thoroughly investigated as a precaution. With this aim, the genotoxic potential of 2-deoxy-2-[¹⁸F]fluoro-D-glucose ([¹⁸F]FDG) and a strong static magnetic field (SMF) were evaluated both in isolation and in combination using the γ H2AX assay detecting double-strand breaks in lymphocyte DNA. **METHODS:** Thirty-two healthy young volunteers allocated to three study arms were exposed to [¹⁸F]FDG alone, to a 3-T SMF alone or to both combined over 60 min at a PET/CT or a PET/MRI system. Blood samples taken after in vivo exposure were incubated up to 60 min to extend the irradiation of blood by residual [¹⁸F]FDG within the samples and the time to monitor the γ H2AX response. Absorbed doses to lymphocytes delivered in vivo and in vitro were estimated individually for each volunteer exposed to [¹⁸F]FDG. γ H2AX foci were

scored automatically by immunofluorescence microscopy. **RESULTS:** Absorbed doses to lymphocytes exposed over 60 to 120 min to [¹⁸F]FDG varied between 1.5 and 3.3 mGy. In this time interval, the radiotracer caused a significant median relative increase of 28% in the rate of lymphocytes with at least one γ H2AX focus relative to the background rate ($p = 0.01$), but not the SMF alone ($p = 0.47$). Simultaneous application of both agents did not result in a significant synergistic or antagonistic outcome ($p = 0.91$). **CONCLUSION:** There is no evidence of a synergism between [¹⁸F]FDG and the SMF that may be of relevance for risk assessment of PET/MRI.

(E) Buldak RJ, Polaniak R, Buldak L, Zwirska-Korczala K, Skonieczna M, Monsiol A, Kukla M, Duława-Buldak A, Birkner E. Short-term exposure to 50 Hz ELF-EMF alters the cisplatin-induced oxidative response in AT478 murine squamous cell carcinoma cells. Bioelectromagnetics. 33(8):641-651, 2012. (VT, AE, GT, IX, OX)

The aim of this study was to assess the influence of cisplatin and an extremely low frequency electromagnetic field (ELF-EMF) on antioxidant enzyme activity and the lipid peroxidation ratio, as well as the level of DNA damage and reactive oxygen species (ROS) production in AT478 carcinoma cells. Cells were cultured for 24 and 72 h in culture medium with cisplatin. Additionally, the cells were irradiated with 50 Hz/1 mT ELF-EMF for 16 min using a solenoid as a source of the ELF-EMF. The amount of ROS, superoxide dismutase (SOD) isoenzyme activity, glutathione peroxidase (GSH-Px) activity, DNA damage, and malondialdehyde (MDA) levels were assessed. Cells that were exposed to cisplatin exhibited a significant increase in ROS and antioxidant enzyme activity. The addition of ELF-EMF exposure to cisplatin treatment resulted in decreased ROS levels and antioxidant enzyme activity. A significant reduction in MDA concentrations was observed in all of the study groups, with the greatest decrease associated with treatment by both cisplatin and ELF-EMF. Cisplatin induced the most severe DNA damage; however, when cells were also irradiated with ELF-EMF, less DNA damage occurred. Exposure to ELF-EMF alone resulted in an increase in DNA damage compared to control cells. ELF-EMF lessened the effects of oxidative stress and DNA damage that were induced by cisplatin; however, ELF-EMF alone was a mild oxidative stressor and DNA damage inducer. We speculate that ELF-EMF exerts differential effects depending on the exogenous conditions. This information may be of value for appraising the pathophysiological consequences of exposure to ELF-EMF.

(E) Burgos-Molina AM, Mercado-Sáenz S, Sendra-Portero F, Ruiz-Gómez MJ. Effect of low frequency magnetic field on efficiency of chromosome break repair. Electromagn Biol Med. 39(1):30-37, 2020. (VT, LE, GT)

DNA repair is essential to maintain genome integrity. There is scientific evidence that exposure to magnetic fields (MF) can produce alterations in DNA repair without clear conclusions. This work aims to study the cellular response to and repair of a very deleterious type of DNA damage, the DNA double strand break (DSB), in *S. cerevisiae*, under MF exposure. In *S. cerevisiae* cells, pairs of DSB were induced enzymatically by HO endonuclease by plating the cells on Galactose-containing media. The repair processes took place under exposure to a 50Hz, 2.45mT sinusoidal MF during 21 days. MF was generated by a pair of Helmholtz coils. MF induced 1.29- and 1.5-

fold increase in the number of colonies grown at day 21 of exposure in relation to untreated controls for *Pho91* and *Rmd5* strain, respectively. In relation to the kinetics of DSB repair during MF exposure, a higher increase (55.56-fold) in DNA reparation was observed at day 15 for *Rmd5* strain in relation to the slight increment (1.18-fold) found for *Pho91* strain. The results suggest that long-term MF exposure could increase the DNA repair activity and there may be a relationship between the position of the DSB and the distance to the centromere.

(E) Calabrò E, Condello S, Magazù S, Ientile, R. Static and 50 Hz electromagnetic fields effects on human neuronal-like cells vibration bands in the mid-infrared region. J Electromagnetic Analysis and Applications 3(2) 69-78, 2011. (VT, AE, GT)

Human neuronal-like cells were exposed to static and 50 Hz electromagnetic fields at the intensities of 2 mT and 1 mT, respectively. The effects of exposure were investigated in the mid-infrared region by means of Fourier self deconvolution spectroscopic analysis. After exposure of 3 hours to static and 50 Hz electromagnetic fields, the vibration bands of CH₂ methylene group increased significantly after both exposures, suggesting a relative increase of lipid related to conformational changes in the cell membrane due to electromagnetic fields. In addition, PO₂-stretching phosphate bands decreased after both exposures, suggesting that alteration in DNA/RNA can be occurred. In particular, exposure of 3 hours to 50 Hz electromagnetic fields produced significant increases in β -sheet contents in amide I, and around the 1740 cm⁻¹ band assigned to non-hydrogen-bonded ester carbonyl stretching mode, that can be related to unfolding processes of proteins structure and cells death. Further exposure up to 18 hours to static magnetic field produced an increase in β -sheet contents as to α -helix components of amide I region, as well.

(E) Calabrò E, Goswami HK, Salvatore Magazù S. Chromosome aberration in typical biological systems under exposure to low- and high-intensity magnetic fields. Electromagn Biol Med. (March 5, 2020) (VT, AE, GT)

The aim of this study was to investigate the response of chromosomes in typical human and plant cells under applied low-frequency magnetic fields at low and high intensities. Neuronal-like cells and roots of *Allium sativum* and *Vicia faba* were used to investigate chromosome's response to a static and 50 Hz magnetic fields at intensities ranging from 1 mT to 0.8 T, generated by two Helmholtz coils driven by direct current or alternate current voltage. Vertex spectrometer and Olympus microscope with camera were used. A significant decrease in intensity of the phosphate bands in the DNA infrared region was observed by FTIR spectroscopy analysis after exposure of neuronal-like cells to static and 50 Hz magnetic field at low intensity of 1 mT, which can be explained assuming that uncoiling and unpackaging of chromatin constituents occurred after exposure. This effect was directly observed by microscope in roots of *Allium sativum* and *Vicia faba* under exposure to a static magnetic field at high intensity of 0.8 T. These findings can be explained assuming that exposure to both low- and high-intensity magnetic fields of chromosomes in typical human and plant cells induces uncoiling and unpackaging of chromatin constituents, followed by chromosome alignment towards the

direction of applied magnetic field, providing further demonstration that magnetic fields can induce the orientation of organic macromolecules even at low-intensity values.

(NE) Cantoni O, Sestili P, Fiorani M, Dacha M. Effect of 50 Hz sinusoidal electric and/or magnetic fields on the rate of repair of DNA single strand breaks in cultured mammalian cells exposed to three different carcinogens: methylmethane sulphonate, chromate and 254 nm U.V. radiation. Biochem Mol Biol Int. 38(3):527-533, 1996. (VT, AE, GT, IX)

Treatment of cultured mammalian cells with three different carcinogens, namely methylmethane sulphonate (MMS), chromate and 254 U.V. radiation, produces DNA single strand breaks (SSB) in cultured mammalian cells. The rate of removal of these lesions is not affected by exposure to 50 Hz electric (0.2 - 20 kV/m), magnetic (0.0002-0.2 mT), or combined electric and magnetic fields. These results indicate that, under the experimental conditions utilized in this study, 50 Hz electric, magnetic and electromagnetic fields (over a wide range of intensities) do not affect the machinery involved in the repair of DNA SSBs generated by different carcinogens in three different cultured mammalian cell lines, making it unlikely that field exposure enhances the ability of these carcinogens to induce transformation via inhibition of DNA repair.

(E) Celikler S, Aydemir N, Vatan O, Kurtuldu S, Bilaloglu R. A biomonitoring study of genotoxic risk to workers of transformers and distribution line stations. Int J Environ Health Res. 19(6):421-430, 2009. (HU, LE, GT)

A cytogenetic monitoring study was carried out on a group of workers from transformer and distribution line stations in the Bursa province of Turkey, to investigate the genotoxic risk of occupational exposure to extremely low frequency electric (ELF) and magnetic fields (EMF). Cytogenetic analysis, namely chromosomal aberrations (CAs) and micronucleus (MN) tests were performed on a strictly selected group of 55 workers and compared to 17 controls. CA and MN frequencies in electrical workers appeared significantly higher than in controls ($p < 0.001$, 0.05, respectively). The frequency of CA in exposed groups were significantly enhanced with the years of exposure ($p < 0.01$). The effect of smoking on the level of CA and MN was not significant in the control and exposure groups. The results of this study demonstrated that a significant induction of cytogenetic damage in peripheral lymphocytes of workers engaged to occupational exposure to ELMF in electric transformer and distribution stations.

(NE) Cellini L, Grande R, Di Campli E, Di Bartolomeo S, Di Giulio M, Robuffo I, Trubiani O, Marigliò MA. Bacterial response to the exposure of 50 Hz electromagnetic fields. Bioelectromagnetics. 29(4):302-311, 2008. (VO, AE, GT)

To investigate the ability of prokaryotic microorganisms to activate strategies in adapting themselves to the environmental stress induced by exposure to extremely low frequency electromagnetic fields (ELF-EMF), cultures of *Escherichia coli* ATCC 700926 exposed at 50 Hz EMF (0.1, 0.5, 1.0 mT), and the respective sham-exposed controls were studied for: the total and

culturable counts, the viability status, the antimicrobial susceptibility pattern, the morphological analysis, the genotypical and transcriptional profile. Exposed samples and controls displayed similar total and culturable counts, whereas an increased cell viability was observed in exposed samples re-incubated for 24 h outside of the solenoid compared to the corresponding controls. An exposure to 50 Hz EMF of 20-120 min produced a significant change of *E. coli* morphotype with a presence of coccoid cells also aggregated in clusters after re-incubation of 24 h outside of the solenoid. Atypical lengthened bacterial forms were also observed suggesting a probable alteration during cell division. No changes among DNA fingerprintings and some differences in RNA-AFLP analysis were observed for each 50 Hz EMF intensities evaluated. Our results indicate that an exposure to 50 Hz EMF acts as a stressing factor on bacteria which can represent a suitable model to investigate acute and chronic effects related to ELF-EMF exposure.

(NE) Chahal R, Craig DQ, Pinney RJ. Investigation of potential genotoxic effects of low frequency electromagnetic fields on Escherichia coli. J Pharm Pharmacol. 45(1):30-33, 1993. (VT, AE, GT)

Exposure of growing cells of *Escherichia coli* strain AB1157 to a frequency of 1 Hz with field strengths of 1 or 3 kV m⁻¹ did not affect spontaneous or ultraviolet light (UV)-induced mutation frequencies to rifampicin resistance. Neither did growth in the presence of charge alter the sensitivities of strains AB1157, TK702 umuC or TK501 umuC uvrB to UV. Similarly, although the resistance of strains TK702 umuC and TK501 umuC uvrB to UV was increased by the presence of plasmid pKM101, which carries DNA repair genes, pre-growth of plasmid-containing strains in electric fields did not increase UV resistance. Finally, growth in a low frequency field in the presence of sub-inhibitory concentrations of mitomycin C did not affect mitomycin C-induced mutation frequencies. It is concluded that low frequency electromagnetic fields do not increase spontaneous mutation, induce DNA repair or increase the mutagenic effects of UV or mitomycin C.

(E) Chang WH-S, Chen L-T, Sun J-S, Lin F-H. Effect of pulse-burst electromagnetic field stimulation on osteoblast cell activities. Bioelectromagnetics 2004 Sep;25(6):457-65. (VT, CE, GE)

Electric stimulation has been used successfully to treat a wide range of bone disorders. However, the mechanism by which the electric fields can influence the bone cells behavior remains poorly understood. The purpose of this research was to assess the possible mechanism of the stimulatory effect of pulsed electromagnetic field (PEMF) on bone cells. A PEMF with a frequency of 15 Hz (1 G [0.1 mT]; electric field strength 2 mV/cm) were applied to neonatal mouse calvarial bone cell cultures for 14 days. The temporal effects of PEMF on the osteoblasts were evaluated by the status of proliferation, differentiation, mineralization, and gene expression on the 3rd, 5th, 7th, and 14th days of culture. Our results demonstrated that PEMF stimulation significantly increased the osteoblasts' proliferation by 34.0, 11.5, and 13.3% over the control group after 3, 5, and 7 days' culture. Although the alkaline phosphatase (ALP) staining and the mineralization nodules formation did not change, the ALP activity of the bone cells decreased significantly after PEMF stimulation. Under the PEMF stimulation, there was no effect on the extracellular matrix synthesis, while the osteoprotegerin (OPG) mRNA expression was up regulated and the receptor

activator of NF-kappaB ligand (RANKL) mRNA expression were down regulated, compared to the control. In conclusion, the treatment by PEMF of osteoblasts may accelerate cellular proliferation, but did not affect the cellular differentiation. The effect of PEMF stimulation on the bone tissue formation was most likely associated with the increase in the number of cells, but not with the enhancement of the osteoblasts' differentiation.

(E) Chen GD, Lu DQ, Jiang H, Xu ZP.[Effects of 50 Hz magnetic fields on gene expression in MCF-7 cells]. *Zhejiang Da Xue Xue Bao Yi Xue Ban.* 37(1):15-22, 2008. [Article in Chinese] **(VT, AE, GT, GE)**

OBJECTIVE: To investigate whether 50 Hz magnetic fields (MF) can change the gene expression profile in MCF-7 cells and to screen MF responsive genes. **METHODS:** In vitro cultured MCF-7 cells were continuously exposed or sham-exposed to 0.4 mT of 50 Hz MF for 24 hours. Affymetrix Human Genome Genechips (U133A) were applied to analyze gene expression profiles in MF exposed and sham-exposed MCF-7 cells and the data were processed with Genechip data analysis software MAS 5.0 and DMT 3.0. Real-time RT-PCR assay was employed to examine the differentially expressed genes. **RESULT:** Thirty differentially expressed genes were screened with 100 % consistency change calls in the MF exposed MCF-7 cells. Six independent real-time RT-PCR analyses showed that SCNN1A, METTL3 and GPR137B were slightly but statistically significantly changed in MCF-7 cells after exposure to 50 Hz MF ($P < 0.05$), while other analyzed genes exhibited slight up-and down-fluctuations in expressions and no increase or decrease in each gene expression reached statistical significance ($P > 0.05$). **CONCLUSION:** The present study identified three 50 Hz MF responsive genes in MCF-7 cells and the biological consequences of expression changes in these MF responsive genes need to be further investigated. 0.4 mT 50 Hz MF exposure for longer duration might induce DNA double-strand breaks in human lens epithelial cells in vitro.

(NE) Chen G, Lu D, Chiang H, Leszczynski D, Xu Z. Using model organism *Saccharomyces cerevisiae* to evaluate the effects of ELF-MF and RF-EMF exposure on global gene expression. *Bioelectromagnetics.* 33(7):550-560, 2012. **(VO, AE, GE)**

The potential health hazard of exposure to electromagnetic fields (EMF) continues to cause public concern. However, the possibility of biological and health effects of exposure to EMF remains controversial and their biophysical mechanisms are unknown. In the present study, we used *Saccharomyces cerevisiae* to identify genes responding to extremely low frequency magnetic fields (ELF-MF) and to radiofrequency EMF (RF-EMF) exposures. The yeast cells were exposed for 6 h to either 0.4 mT 50 Hz ELF-MF or 1800 MHz RF-EMF at a specific absorption rate of 4.7 W/kg. Gene expression was analyzed by microarray screening and confirmed using real-time reverse transcription-polymerase chain reaction (RT-PCR). We were unable to confirm microarray-detected changes in three of the ELF-MF responsive candidate genes using RT-PCR ($P > 0.05$). On the other hand, out of the 40 potential RF-EMF responsive genes, only the expressions of structural maintenance of chromosomes 3 (SMC3) and aquaporin 2 (AQY2 (m)) were confirmed, while three other genes, that is, halotolerance protein 9 (HAL9), yet another kinase 1 (YAK1) and one function-unknown gene (open reading frame: YJL171C),

showed opposite changes in expression compared to the microarray data ($P < 0.05$). In conclusion, the results of this study suggest that the yeast cells did not alter gene expression in response to 50 Hz ELF-MF and that the response to RF-EMF is limited to only a very small number of genes. The possible biological consequences of the gene expression changes induced by RF-EMF await further investigation.

(E) Chen J, Guan L, Fan P, Liu X, Liu R, Liu Y, Bai H. In vitro study of the effects of DC electric fields on cell activities and gene expression in human choriocarcinoma cells. Electromagn Biol Med 2020 Nov 12;1-16. doi: 10.1080/15368378.2020.1846555. Online ahead of print. (VT, AE, GE)

Physiological electric fields (EFs), as one of the environmental cues influencing both normal and tumor cells, have profound effects on tumor cell malignancy potential. The cellular responses to EFs by choriocarcinoma cells and their underlying mechanisms are unknown. In this study, the migration/motility, cell cycle progression and proliferation of choriocarcinoma cells in electric field culture showed that choriocarcinoma cells migrated cathodally in an applied EF, and EF stimulation influenced cell cycle progression through G2/M arrest and therefore induced a reduction in cellular proliferation. The transcriptome of choriocarcinoma cells subjected to EF stimulation (150 mV/mm) was analyzed using RNA sequencing (RNA-Seq), and the results were verified by reverse transcription quantitative polymerase chain reaction. A Kyoto Encyclopedia of Genes and Genomes pathway analysis revealed that ErbB and HIF-1 signaling pathways that are involved in cell migration/motility, cell cycle progression and proliferation were significantly altered in cells treated with an EF of 150 mV/mm compared with control cells, and in addition, the downstream pathways of these signaling pathways such as AKT and P42/P44 MAPK (ERK1/2) showed primary activation by Western blotting. This study's results suggest that an applied EF is an effective cue in regulating cellular phenotypes of choriocarcinoma cells and that transcriptional analysis contributes to the understanding of the mechanism of EF-guided cell functions.

(E) Chen WF, Qi H, Sun RG, Liu Y, Zhang K, Liu JQ. Static magnetic fields enhanced the potency of cisplatin on k562 cells. Cancer Biother Radiopharm. 25(4):401-408, 2010. (VT, AE, GT, IX)

Purpose: This study investigates whether 8.8 mT static magnetic fields (SMFs) can enhance the killing potency of cisplatin (DDP) on human leukemic cells (K562). **Methods:** The cell proliferation, cell cycle distribution, DNA damage, and the change in cell surface ultrastructure after K562 cells were exposed to 8.8 mT SMFs with or without DDP were analyzed. **Results:** The results show that SMFs enhanced the killing effect of DDP on K562 cells, reducing the efficient killing concentration of DDP on K562 cells from 20 to 10 microg/mL. Atomic force microscope observation showed that the cell surface ultrastructure was altered. The results of fluorescence-activated cell sorting analysis indicated that K562 cells treated with SMF plus DDP were arrested at the S phase. The SMF exposure induced DNA to become thicker than controls, and breakage of DNA occurred in the DDP group; however, DNA breakage was increased in the SMF + DDP group. **Conclusions:** The results show that SMFs enhanced the anticancer effect of

DDP on K562 cells. The mechanism correlated with the DNA damage model. This study also shows the potentiality of SMFs as an adjunctive treatment method for chemotherapy.

(E) Chen Y, M Menger MM, Braun BJ, Schweizer S, Linnemann C, Falldorf K, Ronniger M, Wang H, Histing T, Nussler AK, Ehnert S. Modulation of Macrophage Activity by Pulsed Electromagnetic Fields in the Context of Fracture Healing. Bioengineering (Basel) 8(11):167, 2021. (VT, LE, GE, OX, WS)

Delayed fracture healing and fracture non-unions impose an enormous burden on individuals and society. Successful healing requires tight communication between immune cells and bone cells. Macrophages can be found in all healing phases. Due to their high plasticity and long life span, they represent good target cells for modulation. In the past, extremely low frequency pulsed electromagnetic fields (ELF-PEMFs) have been shown to exert cell-specific effects depending on the field conditions. Thus, the aim was to identify the specific ELF-PEMFs able to modulate macrophage activity to indirectly promote mesenchymal stem/stromal cell (SCP-1 cells) function. After a blinded screening of 22 different ELF-PEMF, two fields (termed A and B) were further characterized as they diversely affected macrophage function. These two fields have similar fundamental frequencies (51.8 Hz and 52.3 Hz) but are emitted in different groups of pulses or rather send-pause intervals. Macrophages exposed to field A showed a pro-inflammatory function, represented by increased levels of phospho-Stat1 and CD86, the accumulation of ROS, and increased secretion of pro-inflammatory cytokines. In contrast, macrophages exposed to field B showed anti-inflammatory and pro-healing functions, represented by increased levels of Arginase I, increased secretion of anti-inflammatory cytokines, and growth factors are known to induce healing processes. The conditioned medium from macrophages exposed to both ELF-PEMFs favored the migration of SCP-1 cells, but the effect was stronger for field B. Furthermore, the conditioned medium from macrophages exposed to field B, but not to field A, stimulated the expression of extracellular matrix genes in SCP-1 cells, i.e., *COL1A1*, *FNI*, and *BGN*. In summary, our data show that specific ELF-PEMFs may affect immune cell function. Thus, knowing the specific ELF-PEMFs conditions and the underlying mechanisms bears great potential as an adjuvant treatment to modulate immune responses during pathologies, e.g., fracture healing.

(E) Chen Z, Wen J, Ju H, Fang Z. Magnetic nano-Fe₃O₄ particles targeted gathering and bio-effects on nude mice loading human hepatoma Bel-7402 cell lines model under external magnetic field exposure in vivo. Electromagn Biol Med 34(4):309-316, 2015. (VT, AE, GE, IX)

Magnetic nano-Fe₃O₄ particles (MNPs), static magnetic field (SMF) and extremely low-frequency altering electric magnetic field (ELFF) were utilized to treat nude mice loading hepatoma Bel-7402 cell lines to investigate the therapeutic values of MNPs combined with ELFF in vivo. Magnetic resonance image (MRI) figures showed that about 98.9% MNPs injected into mice body through tail vein were gathered in tumor focal by SMF directing exposure. Single ELFF and MNPs treatments did not influence mice physiological function obviously. However, gathered MNPs combined with ELFF treatment prolonged mice survival time and inhibited loading tumor cells proliferation significantly compared to other mice groups ($p < 0.05$);

furthermore, the tumor cells early apoptosis ratio of mice group was significantly higher than other groups ($p < 0.05$), and ELFF combined with gathered MNPs treatment improved tumor cells early apoptosis associated with Bcl group protein expression: Bax protein expression was higher than Bcl-2 and the combined treatment improved cells Heat shock protein-27 (Hsp-27) expression which could protect cells avoiding early apoptosis. The possible mechanism that this kind of combination inducing more cells into early apoptosis could be due to ELFF exposure influencing cells ion metabolism, MNPs strengthening the effects, and the ELFF vibrating MNPs to generate extra heat and activate cellular heat shock signal channel.

(E) Cheng L, Yang B, Du H, Zhou T, Li Y, Wu J, Cao Z, Xu A. Moderate intensity of static magnetic fields can alter the avoidance behavior and fat storage of *Caenorhabditis elegans* via serotonin. *Environ Sci Pollut Res Int* 2022 Jan 29. doi: 10.1007/s11356-022-18898-5. Online ahead of print. (VO, AE, GE)

Man-made static magnetic fields (SMFs) widely exist in human life as a physical environmental factor. However, the biological responses to moderate SMFs exposure and their underlying mechanisms are largely unknown. The present study was focused on exploring the nervous responses to moderate-intensity SMFs at 0.5 T and 1 T in *Caenorhabditis elegans* (*C. elegans*). We found that SMFs at either 0.5 T or 1 T had no statistically significant effects on the locomotor behaviors, while the 1 T magnetic field increased pharyngeal pumping. The avoidance behavior of the pathogenic *Pseudomonas aeruginosa* was greatly decreased in either 0.5 T or 1 T SMFs exposed nematodes, and the learning index was reduced from 0.52 ± 0.11 to 0.23 ± 0.17 and 0.16 ± 0.11 , respectively. The total serotonin level was increased by 17.08% and 16.45% with the treatment of 0.5 T and 1 T SMF, compared to the control group; however, there were minimal effects of SMFs on other three neurotransmitters including choline, γ -aminobutyric acid (GABA), dopamine. RT-qPCR was used to further investigate the expression of serotonin-related genes, including rate-limiting enzymes, transcription factors and transport receptors. The expression levels of *tph-1* and *unc-86* genes were increased by SMF exposure, while those of *ocr-2*, *osm-9*, *ser-1* and *mod-1* genes were decreased. With the staining of lipid in either wild-type N2 or *tph-1* mutants, we found that 0.5 T and 1 T SMFs decreased fat storage in *C. elegans* via serotonin pathway. Our study demonstrated that moderate-intensity SMFs induced neurobehavioral disorder and the reduction of fat storage by disturbing the secretion of serotonin in *C. elegans*, which provided new insights into elucidating nervous responses of *C. elegans* to moderate-intensity SMFs.

(E) Cheng Y, Qu Z, Fu X, Jiang Q, Fei J. Hydroxytyrosol contributes to cell proliferation and inhibits apoptosis in pulsed electromagnetic fields treated human umbilical vein endothelial cells in vitro. *Mol Med Rep* 16(6):8826-8832, 2017. (VT, AE, GE)

A variety of pulsed electromagnetic fields (PEMFs) have been experimentally and clinically used in an effort to promote wound healing, although the mechanisms involved remain unknown. The aim of the present study was to investigate the action of a novel protocol of co-treatment with PEMFs and hydroxytyrosol (HTY) on the proliferation and differentiation potential of human umbilical vein endothelial cells (HUVECs). The HUVECs were assigned randomly into three

groups: Control, PEMF-treated and PEMF + HT-treated. The intensity of the electromagnetic field used in this protocol was 2.25 mT, the frequency of the bursts was 50 Hz and the application time was 15 min. A Cell Counting kit-8 (CCK-8) assay was used to assess cell proliferation, and cell apoptosis was analyzed by TUNEL apoptosis assay kit and calcein-acetoxymethyl/propidium iodide dual-staining assay. In addition, protein and mRNA expression levels of protein kinase B (Akt), mechanistic target of rapamycin (mTOR), transforming growth factor (TGF)- β 1 and p53 were determined by western blotting and reverse transcription-quantitative polymerase chain reaction assays, respectively. The CCK-8 assay demonstrated that HTY contributed to HUVEC proliferation mediated by PEMFs in a time-dependent manner. The Transwell assay and scratch wound results demonstrated that co-treatment of HTY and PEMFs could increase HUVEC migration. Furthermore, the levels of apoptotic cells were reversed by pre-incubation with HTY in the PEMF treatment group, while PEMF treatment alone had no such effect. The proteins and mRNA expression levels of Akt, mTOR, TGF- β 1 were elevated in co-treatment of HTY and PEMFs, whereas there was no effect on levels of p53. Therefore, the results indicated that combined exposure of HUVECs to PEMFs and HTY exerted protective effects in HUVECs by promoting cell proliferation and inhibiting apoptosis. In conclusion, to the best of our knowledge, the present study was the first to demonstrate the beneficial roles of HTY and PEMF combined treatment in HUVECs, which may represent an effective treatment for wound healing.

(E) Cho S, Lee Y, Lee S, Choi YJ, Chung HW. Enhanced cytotoxic and genotoxic effects of gadolinium following ELF-EMF irradiation in human lymphocytes. Drug Chem Toxicol. 37(4):440-447, 2014. (VT, AE, GT, IX)

Gadolinium (Gd) and its chelated derivatives are widely utilized for various industrial and medical purposes, particularly as a contrast agent for magnetic resonance imaging (MRI). There are many studies of Gd nephrotoxicity and neurotoxicity, whereas research on cyto- and genotoxicity in normal human lymphocytes is scarce. It is important to investigate the effect of extremely low-frequency electromagnetic fields (ELF-EMF) on Gd toxicity, as patients are co-exposed to Gd and ELF-EMF generated by MRI scanners. We investigated the cytotoxicity and genotoxicity of Gd and the possible enhancing effect of ELF-EMF on Gd toxicity in cultured human lymphocytes by performing a micronuclei (MN) assay, trypan blue dye exclusion, single cell gel electrophoresis, and apoptosis analyses using flow cytometry. Isolated lymphocytes were exposed to 0.2-1.2 mM of Gd only or in combination with a 60-Hz ELF-EMF of 0.8-mT field strength. Exposing human lymphocytes to Gd resulted in a concentration- and time-dependent decrease in cell viability and an increase in MN frequency, single strand DNA breakage, apoptotic cell death, and ROS production. ELF-EMF (0.8 mT) exposure also increased cell death, MN frequency, olive tail moment, and apoptosis induced by Gd treatment alone. These results suggest that Gd induces DNA damage and apoptotic cell death in human lymphocytes and that ELF-EMF enhances the cytotoxicity and genotoxicity of Gd.

(E) Cho YH, Jeon HK, Chung HW. Effects of extremely low-frequency electromagnetic fields on delayed chromosomal instability induced by bleomycin in normal human fibroblast cells. J Toxicol Environ Health A. 70(15-16):1252-1258, 2007. (VT, AE, GT, IX)

This study was carried out to examine the interaction of extremely low-frequency electromagnetic fields (ELF-EMF) on delayed chromosomal instability by bleomycin (BLM) in human fibroblast cells. A micronucleus-centromere assay using DNA probes for chromosomes 1 and 4 was performed and a 60-Hz ELF-EMF of 0.8 mT field strength was applied either alone or with BLM throughout the culture period. The frequencies of micronuclei (MN) and aneuploidy were analyzed at 28, 88, and 240 h after treatment with BLM. The coexposure of cells to BLM and ELF-EMF led to a significant increase in the frequencies of MN and aneuploidy compared to the cells treated with BLM alone. No difference was observed between field-exposed and sham-exposed control cells. The frequency of MN induced by BLM was increased at 28 h, and further analysis showed a persistent increase up to 240 h, but the new levels were not significantly different from the level at 28 h. BLM increased the frequencies of aneuploidy at 28, 88, and 240 h, and significantly higher frequency of aneuploidy was observed in the cells analyzed at 240 h compared to the cells examined at 28 h. No interaction of ELF-EMF on delayed chromosomal instability by BLM was observed. Our results suggest that ELF-EMF enhances the cytotoxicity of BLM. BLM might induce delayed chromosomal instability, but no effect of ELF-EMF was observed on the BLM-induced delayed chromosomal instability in fibroblast cells.

(E) Chow K, Tung WL Magnetic field exposure enhances DNA repair through the induction of DnaK/J synthesis. FEBS Lett. 478(1-2):133-136, 2000a. (VT, AE, GT)

In contrast to the common impression that exposure to a magnetic field of low frequency causes mutations to organisms, we have demonstrated that a magnetic field can actually enhance the efficiency of DNA repair. Using Escherichia coli strain XL-1 Blue as the host and plasmid pUC8 that had been mutagenized by hydroxylamine as the vector for assessment, we found that bacterial transformants that had been exposed to a magnetic field of 50 Hz gave lower percentages of white colonies as compared to transformants that had not been exposed to the magnetic field. This result was indicative that the efficiency of DNA repair had been improved. The improvement was found to be mediated by the induced overproduction of heat shock proteins DnaK/J (Hsp70/40).

(E) Chow KC, Tung WL. Magnetic field exposure stimulates transposition through the induction of DnaK/J synthesis. Biochem Biophys Res Commun. 270(3):745-748. 2000b. (VT, AE, GT)

Like some naturally occurring environmental stress factors such as heat shock and UV irradiation, magnetic field exposure is also stimulatory to transposition activity. This feature could be illustrated by a bacterial conjugation study using an Escherichia coli strain that carries the transposable element Tn5 as the donor. When the donor cultures were exposed to a low-frequency (50 Hz) magnetic field of 1.2 mT, Tn5 located on the bacterial chromosome was stimulated to transpose and settled on the extrachromosomal episome, and eventually transferred to the recipient cell through conjugation. Such transposition activity stimulation was mediated by the induced synthesis and accumulation of the heat shock proteins DnaK/J.

(E) Cichoń N, Bijak M, Czarny P, Miller E, Synowiec E, Sliwinski T, Saluk-Bijak J. Increase in Blood Levels of Growth Factors Involved in the Neuroplasticity Process by Using an Extremely Low Frequency Electromagnetic Field in Post-stroke Patients. Front Aging Neurosci. 10:294, 2018. (HU, CE, GE)

Background: Neuroplasticity ensures the improvement of functional status in patients after stroke. The aim of this study was to evaluate the effect of extremely low-frequency electromagnetic field therapy (ELF-EMF) on brain plasticity in the rehabilitation of patients after stroke. Methods: Forty-eight patients were divided into two groups underwent the same rehabilitation program, but in the study group, the patients additionally were exposed to a standard series of 10 ELF-EMF treatments. To determine the level of neuroplasticity, we measured the plasma level of the brain-derived neurotrophic factor (BDNF), the vascular-endothelial growth factor, as well as BDNF mRNA expression. Additionally, we determined the molecule levels for hepatocyte growth factor, stem cell factor, stromal cell-derived factor 1 α , nerve growth factor β , and leukemia inhibitory factor, using 5plex cytokine panel in plasma. After 4 weeks, during which patients had undergone neurorehabilitation and neurological examinations, we assessed functional recovery using the Barthel Index, Mini-Mental State Examination (MMSE), Geriatric Depression Scale, National Institutes of Health Stroke Scale (NIHSS), and the modified Rankin Scale (mRS). Results: We observed that ELF-EMF significantly increased growth factors and cytokine levels involved in neuroplasticity, as well as promoted an enhancement of functional recovery in post-stroke patients. Additionally, we presented evidence that **these effects could be related to the increase of gene expression on the mRNA level**. Moreover, a change of BDNF plasma level was positively correlated with the Barthel Index, MMSE, and negatively correlated with GDS. Conclusion: Extremely low-frequency electromagnetic field therapy improves the effectiveness of rehabilitation of post-stroke patients by improving neuroplasticity processes.

(E) Cichon N, Saluk-Bijak J, Miller E, Sliwinski T, Synowiec E, Wigner P, Bijak M. Evaluation of the effects of extremely low frequency electromagnetic field on the levels of some inflammatory cytokines in post-stroke patients. J Rehabil Med 2019 Dec 16;51(11):854-860. (HU, LE, GE)

Background: Activation of immunologically competent cells results in the overproduction of pro-inflammatory factors, and causes progression of nerve tissue damage. However, the potential neuroprotective effects of these factors in brain damage have not been well investigated. Objective: To evaluate the effect of extremely low frequency electromagnetic field (ELF-EMF) treatment on the molecular mechanism of inflammatory cytokine activity in post-stroke patients. Methods: All patients underwent the same rehabilitation program, but the ELF-EMF group were also given ELF-EMF treatment. Both groups have been used in our previous studies. In order to determine the plasma level of cytokines, the levels of interleukin 1 β (IL-1 β), interleukin 2 (IL-2), interferon- γ (INF- γ) and transforming growth factor β (TGF- β) were evaluated, and the level of IL-1 β mRNA expression was determined. Results: After ELF-EMF treatment, both IL-1 β plasma level and IL-1 β mRNA expression level, as well as IL-2 plasma level increased, while IFN- γ and TGF- β levels did not change. Conclusion: The increased expression of IL-1 β found in this study may be a response to ELF-EMF stimulation. It is hypothesized that a neuroprotective role of this

cytokine may occur due to IL-1 β -dependent regulation of neurotrophic factors. Further research is needed to explore this hypothesis.

(E) Cichon N, Synowiec E, Miller E, Sliwinski T, Ceremuga M, Saluk-Bijak J, Bijak M Effect of Rehabilitation with Extremely Low Frequency Electromagnetic Field on Molecular Mechanism of Apoptosis in Post-Stroke Patients. Brain Sci 2020 Apr 30;10(5):266. (HU, CE, GE)

Apoptosis in acute stroke is associated with a negative prognosis and is correlated with the severity of the neurological deficit. However, there is no evidence that indicates that, in the subacute phase of the stroke, the apoptosis process might activate neuroplasticity. Therefore, in this study, we investigated the effect of an extremely low frequency electromagnetic field (ELF-EMF) on the molecular mechanism of apoptosis, as used in the rehabilitation of post-stroke patients. Patients with moderate stroke severity ($n = 48$), 3-4 weeks after incident, were enrolled in the analysis and divided into ELF-EMF and non-ELF-EMF group. The rehabilitation program in both groups involves the following: kinesiotherapy-30 min; psychological therapy-15 min; and neurophysiological routines-60 min. Additionally, the ELF-EMF group was exposed to an ELF-EMF (40 Hz, 5 mT). In order to assess the apoptosis gene expression level, we measured the mRNA expression of *BAX*, *BCL-2*, *CASP8*, *TNF α* , and *TP53*. We found that ELF-EMF significantly increased the expression of *BAX*, *CASP8*, *TNF α* , and *TP53*, whereas the *BCL-2* mRNA expression after ELF-EMF exposition remained at a comparable level in both groups. Thus, we suggest that increasing the expression of pro-apoptotic genes in post-stroke patients promotes the activation of signaling pathways involved in brain plasticity processes. However, further research is needed to clarify this process.

(E) Ciombor DM, Lester G, K Aaron RK, Neame P, Caterson B. Low frequency EMF regulates chondrocyte differentiation and expression of matrix proteins. J Orthop Res 20(1):40-50, 2002. (VT, LE, GE)

This study describes the enhancement of chondrogenic differentiation in endochondral ossification by extremely low frequency pulsed electric/magnetic fields (EMFs). The demineralized bone matrix (DBM)-induced endochondral ossification model was used to examine the effects of EMF stimulation. [³⁵S]-Sulfate and [³H]-thymidine incorporation and glycosaminoglycan (GAG) content were determined by standard methods. Proteoglycan (PG) and GAG molecular size and composition were determined by gel chromatography and sequential enzyme digestion. Immunohistochemical and Western blot analysis of PGs were done with antibodies 2B6, 3B3, 2D3 and 5D4. Northern analysis of total RNA extracts was performed for aggrecan, and type II collagen. All data was compared for significance by Student's t- or analysis of variance (ANOVA)-tests. The EMF field accelerated chondrogenesis as evidenced by an increase in: (1) ³⁵SO₄ incorporation and GAG content, (2) the number of chondrocytes at day 8 of development, (3) the volumetric density of cartilage and (4) the extent of immunostaining for 3B3 and 5D4. No differences in DNA content or [³H]-thymidine incorporation were observed between control and stimulated ossicles, suggesting the absence of enhanced cell proliferation or recruitment as a mechanism for the acceleration. PG and GAG molecular sizes

and GAG chemical composition were similar in stimulated and control ossicles, indicating that stimulation resulted in an accelerated synthesis of normal cartilage molecules. The increased expression of PG and type II collagen mRNA as well as a greater immunoreactivity of 3B3 and 5D4 suggest an increase in the rate of differentiation of chondrocytes and enhanced phenotypic maturation.

(E) Collard JF, Lazar C, Nowé A, Hinsenkamp M. Statistical validation of the acceleration of the differentiation at the expense of the proliferation in human epidermal cells exposed to extremely low frequency electric fields. Prog Biophys Mol Biol. 111(1):37-45, 2013. (VT, AE, GE)

An acceleration of differentiation at the expense of proliferation is observed in our previous publications and in the literature after exposure of various biological models to low frequency and low-amplitude electric and electromagnetic fields. This observation is related with a significant modification of genes expression. We observed and compared over time this modification. This study use microarray data obtained on epidermis cultures harvested from human abdominoplasty exposed to ELF electric fields. This protocol is repeated with samples collected on three different healthy patients. The sampling over time allows comparison of the effect of the stimulus at a given time with the evolution of control group. After 4 days, we observed a significant difference of the genes expression between control (D4C) and stimulated (D4S) ($p < 0.05$). On the control between day 4 and 7, we observed another group of genes with significant difference ($p < 0.05$) in their expression. We identify the common genes between these two groups and we select from them those expressing no difference between stimulate at 4 days (D4S) and control after 7 days (D7C). The same analysis was performed with D4S-D4C-D12C and D7S-D7C-D12C. The lists of genes which follow this pattern show acceleration in their expressions under stimulation appearing on control at a later time. In this list, genes such as DKK1, SPRR3, NDRG4, and CHEK1 are involved in cell proliferation or differentiation. Numerous other genes are also playing a function in mitosis, cell cycle or in the DNA replication transcription and translation.

(E) Consales C, Cirotti C, Filomeni G, Panatta M, Butera A, Merla C, Lopresto V, Pinto R, Marino C, Benassi B. Fifty-hertz magnetic field affects the epigenetic modulation of the miR-34b/c in neuronal cells. Mol Neurobiol. 55(7):5698-5714, 2018. (VT, AE, OX, GE, EP)

The exposure to extremely low-frequency magnetic fields (ELF-MFs) has been associated to increased risk of neurodegenerative diseases, although the underlying molecular mechanisms are still undefined. Since epigenetic modulation has been recently encountered among the key events leading to neuronal degeneration, we here aimed at assessing if the control of gene expression mediated by miRNAs, namely miRs-34, has any roles in driving neuronal cell response to 50-Hz (1 mT) magnetic field in vitro. We demonstrate that ELF-MFs drive an early reduction of the expression level of miR-34b and miR-34c in SH-SY5Y human neuroblastoma cells, as well as in mouse primary cortical neurons, by affecting the transcription of the common pri-miR-34. This modulation is not p53 dependent, but attributable to the hyper-

methylation of the CpG island mapping within the miR-34b/c promoter. Incubation with N-acetyl-L-cysteine or glutathione ethyl-ester fails to restore miR-34b/c expression, suggesting that miRs-34 are not responsive to ELF-MF-induced oxidative stress. By contrast, we show that miRs-34 control reactive oxygen species production and affect mitochondrial oxidative stress triggered by ELF-MFs, likely by modulating mitochondria-related miR-34 targets identified by in silico analysis. We finally demonstrate that ELF-MFs alter the expression of the α -synuclein, which is specifically stimulated upon ELF-MFs exposure via both direct miR-34 targeting and oxidative stress. Altogether, our data highlight the potential of the ELF-MFs to tune redox homeostasis and epigenetic control of gene expression in vitro and shed light on the possible mechanism(s) producing detrimental effects and predisposing neurons to degeneration.

(E) Consales C, Merla C, Benassi B, Garcia-Sanchez T, Muscat A, André FM, Marino C, Lluís M Mir LM. Biological effects of ultrashort electric pulses in a Neuroblastoma cell line: the energy density role. *Int J Radiat Biol* 2021 Oct 29;1-40. doi: 10.1080/09553002.2022.1998704. Online ahead of print. (VT, AE, GE)

Background: Despite the numerous literature results about biological effects of electromagnetic field exposure, the interaction mechanisms of these fields with organisms are still a matter of debate. Extremely low frequency magnetic fields can modulate redox homeostasis and we showed that 24 hours exposure to 50 Hz-1 mT has a pro-oxidant effect and effects on the epigenome of SH-SY5Y cells, decreasing miR-34b/c expression through the hypermethylation of their promoter. **Methods:** Here, we investigated the role of the electromagnetic deposited energy density during exposures lasting 24 hours to 1mT amplitude magnetic fields at a frequency of 50 Hz in inducing the above mentioned effects. To this end, we delivered ultrashort electric pulses, in the range of microsecond and nanosecond duration, with the same energy density of the previously performed magnetic exposure to SH-SY5Y cells. Furthermore, we explored the effect of higher deposited energy densities. Analysis of i) gene and microRNA expression, ii) cell morphology, iii) reactive oxygen species (ROS) generation, and iv) apoptosis were carried out. **Results:** We observed significant changes in *egr-1* and *c-fos* expression at very low deposited energy density levels, but no change of the ROS production, miR-34b/c expression, nor the appearance of indicators of apoptosis. We thus sought investigating changes in *egr-1* and *c-fos* expression caused by ultrashort electric pulses at increasing deposited energy density levels. The pulses with the higher deposited energy density caused cell electroporation and even other morphological changes such as cell fusion. The changes in *egr-1* and *c-fos* expression were more intense, but, again, no change of the ROS production, miR-34b/c expression, nor apoptosis induction was observed. **Conclusion:** These results, showing that extremely low levels of electric stimulation (never investigated until now) can cause transcriptional changes, also reveal the safety of the electroporating pulses used in biomedical applications and open up the possibility to further therapeutic applications of this technology.

*(E) Coskun C, Ocal I, Gunay I A Low-Frequency Pulsed Magnetic Field Reduces Neuropathic Pain by Regulating NaV_{1.8} and NaV_{1.9} Sodium Channels at the Transcriptional Level in Diabetic Rats. *Bioelectromagnetics* 2021 Jul;42(5):357-370. (VO, LE, GE)

Low-frequency pulsed magnetic field (LF-PMF) application is a non-invasive, easy, and inexpensive treatment method in pain management. However, the molecular mechanism underlying the effect of LF-PMF on pain is not fully understood. Considering the obvious dysregulations of gene expression observed in certain types of voltage-gated sodium channels (VGSCs) in pain conditions, the present study tested the hypothesis that LF-PMF shows its pain-relieving effect by regulating genes that code VGSCs proteins. Five experimental rat groups (Control, Streptozotocin-induced experimental painful diabetic neuropathy (PDN), PDN Sham, PDN 10 Hz PMF, and PDN 30 Hz PMF) were established. After the pain formation in PDN groups, the magnetic field groups were exposed to 10/30 Hz, 1.5 mT PMF for 4 weeks, an hour daily. Progression of pain was evaluated using behavioral pain tests during the entire experimental processes. After the end of PMF treatment, SCN9A (NaV_{1.7}), SCN10A (NaV_{1.8}), SCN11A (NaV_{1.9}), and SCN3A (NaV_{1.3}) gene expression level changes were determined by analyzing real-time polymerase chain reaction results. We found that 10 Hz PMF application was more effective than 30 Hz on pain management. In addition, NaV_{1.7} and NaV_{1.3} transcriptions were upregulated while NaV_{1.8} and NaV_{1.9} were downregulated in painful conditions. Notably, the downregulated expression of the genes encoding NaV_{1.8} and NaV_{1.9} were re-regulated and increased to control level by 10 Hz PMF application. Consequently, it may be deduced that 10 Hz PMF application reduces pain by modulating certain VGSCs at the transcriptional level.

(E) Costantini E, Sinjari B, D'Angelo C, Murmura G, Reale M, Caputi S. Human Gingival Fibroblasts Exposed to Extremely Low-Frequency Electromagnetic Fields: In Vitro Model of Wound-Healing Improvement. Int J Mol Sci 20(9):2108, 2019. (VT, AE, GE, WS)

Several clinical studies have suggested the impact of sinusoidal and pulsed electromagnetic fields in quickening wound repair processes and tissue regeneration. The clinical use of extremely low-frequency electromagnetic fields could represent a novel frontier in tissue repair and oral health, with an interesting clinical perspective. The present study aimed to evaluate the effect of an extremely low-frequency sinusoidal electromagnetic field (SEMF) and an extremely low-frequency pulsed electromagnetic field (PEMF) with flux densities of 1 mT on a model of oral healing process using gingival fibroblasts. An in vitro mechanical injury was produced to evaluate wound healing, migration, viability, metabolism, and the expression of selected cytokines and protease genes in fibroblasts exposed to or not exposed to the SEMF and the PEMF. Interleukin 6 (IL-6), transforming growth factor beta 1 (TGF- β), metalloproteinase 2 (MMP-2), monocyte chemoattractant protein 1 (MCP-1), inducible nitric oxide synthase (iNOS), and heme oxygenase 1 (HO-1) are involved in wound healing and tissue regeneration, favoring fibroblast proliferation, chemotaxis, and activation. Our results show that the exposure to each type of electromagnetic field increases the early expression of IL-6, TGF- β , and iNOS, driving a shift from an inflammatory to a proliferative phase of wound repair. Additionally, a later induction of MMP-2, MCP-1, and HO-1 was observed after electromagnetic field exposure, which quickened the wound-healing process. Moreover, electromagnetic field exposure influenced the proliferation, migration, and metabolism of human gingival fibroblasts compared to sham-exposed cells. This study suggests that exposure to SEMF and PEMF could be an interesting new non-invasive treatment option for wound healing. However, additional studies

are needed to elucidate the best exposure conditions to provide the desired in vivo treatment efficacy

(NE) Coulton LA, Harris PA, Barker AT, Pockley AG. Effect of 50 Hz electromagnetic fields on the induction of heat-shock protein gene expression in human leukocytes. Radiat Res 161(4):430-434, 2004. (VT, AE, GE)

Although evidence is controversial, exposure to environmental power-frequency magnetic fields is of public concern. Cells respond to some abnormal physiological conditions by producing cytoprotective heat-shock (or stress) proteins. In this study, we determined whether exposure to power-frequency magnetic fields in the range 0-100 microT rms either alone or concomitant with mild heating induced heat-shock protein gene expression in human leukocytes, and we compared this response to that induced by heat alone. Samples of human peripheral blood were simultaneously exposed to a range of magnetic-field amplitudes using a regimen that was designed to allow field effects to be distinguished from possible artifacts due to the position of the samples in the exposure system. Power-frequency magnetic-field exposure for 4 h at 37 degrees C had no detectable effect on expression of the genes encoding HSP27, HSP70A or HSP70B, as determined using reverse transcriptase-PCR, whereas 2 h at 42 degrees C elicited 10-, 5- and 12-fold increases, respectively, in the expression of these genes. Gene expression in cells exposed to power-frequency magnetic fields at 40 degrees C was not increased compared to cells incubated at 40 degrees C without field exposure. These findings and the extant literature suggest that power-frequency electromagnetic fields are not a universal stressor, in contrast to physical agents such as heat.

(E) Cuccurazzu B, Leone L, Podda MV, Piacentini R, Riccardi E, Ripoli C, Azzena GB, Grassi C. Exposure to extremely low-frequency (50 Hz) electromagnetic fields enhances adult hippocampal neurogenesis in C57BL/6 mice. Exp Neurol. 226(1):173-182, 2010. (VO, LE, GE, DE)

Throughout life, new neurons are continuously generated in the hippocampus, which is therefore a major site of structural plasticity in the adult brain. We recently demonstrated that extremely low-frequency electromagnetic fields (ELFEFs) promote the neuronal differentiation of neural stem cells in vitro by up-regulating Ca(v)1-channel activity. The aim of the present study was to determine whether 50-Hz/1 mT ELFEF stimulation also affects adult hippocampal neurogenesis in vivo, and if so, to identify the molecular mechanisms underlying this action and its functional impact on synaptic plasticity. ELFEF exposure (1 to 7 h/day for 7 days) significantly enhanced neurogenesis in the dentate gyrus (DG) of adult mice, as documented by increased numbers of cells double-labeled for 5-bromo-deoxyuridine (BrdU) and double cortin. Quantitative RT-PCR analysis of hippocampal extracts revealed significant ELFEF exposure-induced increases in the transcription of pro-neuronal genes (Mash1, NeuroD2, Hes1) and genes encoding Ca(v)1.2 channel α (1C) subunits. Increased expression of NeuroD1, NeuroD2 and Ca(v)1 channels was also documented by Western blot analysis. Immunofluorescence experiments showed that, 30 days after ELFEF stimulation, roughly half of the newly generated immature neurons had survived and become mature dentate granule cells (as shown by their immunoreactivity for both BrdU and NeuN) and were integrated into the granule cell layer of the DG. Electrophysiological

experiments demonstrated that the new mature neurons influenced hippocampal synaptic plasticity, as reflected by increased long-term potentiation. Our findings show that ELFEF exposure can be an effective tool for increasing in vivo neurogenesis, and they could lead to the development of novel therapeutic approaches in regenerative medicine.

(E) D'Angelo C, Costantini E, Kamal MA, Reale M. Experimental model for ELF-EMF exposure: Concern for human health. Saudi J Biol Sci. 2015 Jan;22(1):75-84. (VT, AE, GE)

Low frequency (LF) electromagnetic fields (EMFs) are abundantly present in modern society and in the last 20 years the interest about the possible effect of extremely low frequency (ELF) EMFs on human health has increased progressively. Epidemiological studies, designed to verify whether EMF exposure may be a potential risk factor for health, have led to controversial results. The possible association between EMFs and an increased incidence of childhood leukemia, brain tumors or neurodegenerative diseases was not fully elucidated. On the other hand, EMFs are widely used, in neurology, psychiatry, rheumatology, orthopedics and dermatology, both in diagnosis and in therapy. In vitro studies may help to evaluate the mechanism by which LF-EMFs affect biological systems. In vitro model of wound healing used keratinocytes (HaCaT), neuroblastoma cell line (SH-SY5Y) as a model for analysis of differentiation, metabolism and functions related to neurodegenerative processes, and monocytic cell line (THP-1) was used as a model for inflammation and cytokines production, while leukemic cell line (K562) was used as a model for hematopoietic differentiation. MCP-1, a chemokine that regulates the migration and infiltration of memory T cells, natural killer (NK), monocytes and epithelial cells, has been demonstrated to be induced and involved in various diseases. Since, varying the parameters of EMFs different effects may be observed, we have studied MCP-1 expression in HaCaT, SH-SY5Y, THP-1 and K562 exposed to a sinusoidal EMF at 50 Hz frequency with a flux density of 1 mT (rms). Our preliminary results showed that EMF-exposure differently modifies the expression of MCP-1 in different cell types. Thus, the MCP-1 expression needs to be better determined, with additional studies, with different parameters and times of exposure to ELF-EMF.

(E) Dehghani-Soltani S, Eftekhar-Vaghefi SH, Babaee A, Basiri M, Mohammadipoor-Ghasemabad L, Vosough P, Ahmadi-Zeidabadi M. Pulsed and Discontinuous Electromagnetic Field Exposure Decreases Temozolomide Resistance in Glioblastoma by Modulating the Expression of O⁶-Methylguanine-DNA Methyltransferase, Cyclin-D1, and p53. Cancer Biother Radiopharm 36(7):579-587, 2021.(VT, AE, GE, IX,CS)

Background: Glioblastoma is a malignant and very aggressive brain tumor with a poor prognosis. Despite having chemotherapy concomitant with surgery and/or radiation therapy, the median survival of glioblastoma-affected people is less than 1 year. Temozolomide (TMZ) is a chemotherapeutic used as a first line treatment of glioblastoma. Several studies have reported that resistance to TMZ due to overexpression of O⁶-methylguanine-DNA methyltransferase (MGMT) is the main reason for treatment failure. Several studies described that pulsed-electromagnetic field (EMF) exposure could induce cell death and influence gene expression. **Materials and Methods:** In this study the authors assessed the effects of EMF (50

Hz, 70 G) on cytotoxicity, cell migration, gene expression, and protein levels in TMZ-treated T98 and A172 cell lines. **Results:** In this study, the authors show that treatment with a combination of TMZ and EMF enhanced cell death and decreased the migration potential of T98 and A172 cells. The authors also observed overexpression of the p53 gene and downregulation of cyclin-D1 protein in comparison to controls. In addition, T98 cells expressed the MGMT protein following treatment, while the A172 cells did not express MGMT. **Conclusion:** Their data indicate that EMF exposure improved the cytotoxicity of TMZ on T98 and A172 cells and could partially affect resistance to TMZ in T98 cells.

(E) de Kleijn S, G. Ferwerda, M. Wiese, J. Trentelman, J. Cuppen, T Kozicz, L. de Jager, PWM Hermans, BML Verburg-van Kemenade. A short-term extremely low frequency electromagnetic field exposure increases circulating leukocyte numbers and affects HPA-axis signaling in mice Bioelectromagnetics 2016 Oct;37(7):433-443.(AS, CE, GE)

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There is still uncertainty whether extremely low frequency electromagnetic fields (ELF-EMF) can induce health effects like immunomodulation. Despite evidence obtained in vitro, an unambiguous association has not yet been established in vivo. Here, mice were exposed to ELF-EMF for 1, 4, and 24 h/day in a short-term (1 week) and long-term (15 weeks) set-up to investigate whole body effects on the level of stress regulation and immune response. ELF-EMF signal contained multiple frequencies (20-5000 Hz) and a magnetic flux density of 10 μ T. After exposure, blood was analyzed for leukocyte numbers (short-term and long-term) and adrenocorticotrophic hormone concentration (short-term only). Furthermore, in the short-term experiment, stress-related parameters, corticotropin-releasing hormone, proopiomelanocortin (POMC) and CYP11A1 gene-expression, respectively, were determined in the hypothalamic paraventricular nucleus, pituitary, and adrenal glands. In the short-term but not long-term experiment, leukocyte counts were significantly higher in the 24 h-exposed group compared with controls, mainly represented by increased neutrophils and CD4 \pm lymphocytes. POMC expression and plasma adrenocorticotrophic hormone were significantly lower compared with unexposed control mice. In conclusion, short-term ELF-EMF exposure may affect hypothalamic-pituitary-adrenal axis activation in mice. Changes in stress hormone release may explain changes in circulating leukocyte numbers and composition.

(E) De Mattei M, Gagliano N, Moscheni C, Dellavia C, Calastrini C, Pellati A, Gioia M, Caruso A, Stabellini G. Changes in polyamines, c-myc and c-fos gene expression in osteoblast-like cells exposed to pulsed electromagnetic fields. Bioelectromagnetics 26(3):207-214, 2005. (VT, AE, GE)

Pulsed electromagnetic field (PEMF) stimulation promotes the healing of fractures in humans, though its effect is little known. The processes of tissue repair include protein synthesis and cell differentiation. The polyamines (PA) are compounds playing a relevant role in both protein synthesis processes and cell differentiation through c-myc and c-fos gene activation. Since several studies have demonstrated that PEMF acts on embryonic bone cells, human osteoblast-

like cells and osteosarcoma TE-85 cell line, in this study we analyzed the effect on cell PAs, proliferation, and c-myc and c-fos gene expression of MG-63 human osteoblast-like cell cultures exposed to a clinically useful PEMF. The cells were grown in medium with 0.5 or 10% fetal calf serum (FCS). c-myc and c-fos gene expressions were determined by RT-PCR. Putrescine (PUT), spermidine (SPD), or spermine (SPM) levels were evaluated by HPLC. [(3)H]-thymidine was added to cultures for DNA analysis. The PEMF increased [(3)H]-thymidine incorporation ($P < \text{or} = .01$), while PUT decreased after treatment ($P < \text{or} = .01$); SPM and SPD were not significantly affected. c-myc was activated after 1 h and downregulated thereafter, while c-fos mRNA levels increased after 0.5 h and then decreased. PUT, SPD, SPM trends, and [(3)H]-thymidine incorporation were significantly related to PEMF treatment. These results indicate that exposure to PEMF exerts biological effects on the intracellular PUT of MG-63 cells and DNA synthesis, influencing the genes encoding c-myc and c-fos gene expression. These observations provide evidence that in vitro PEMF affects the mechanisms involved in cell proliferation and differentiation.

(E) Del Re B, Bersani F, Mesirca P, Giorgi G. Synthesis of DnaK and GroEL in Escherichia coli cells exposed to different magnetic field signals. Bioelectrochemistry. 69(1):99-103, 2006. (VT, AE, GE, WS)

The effects of extremely low frequency magnetic field (ELF-MF)(1 mT, 50 Hz) on the heat shock protein (HSP) synthesis in Escherichia coli were investigated. Two magnetic field signals were studied: sinusoidal (SMF) and pulsed square wave (PMF). It was found that bacteria exposed to SMF showed a significantly higher level of DnaK and GroEL proteins as compared to sham-exposed bacteria as revealed by Western blot, whereas a lower level was observed after PMF exposure. Similar results were obtained when bacterial cells were exposed to heat shock (HS) after ELF-MF exposure: again SMF and PMF resulted in an increase and in a reduction of HSP amount in comparison with sham control, respectively. In conclusion, the MF influences the synthesis of HSPs in E. coli in a way that critically depends on the signal characteristics.

(E) Delimaris J, Tsilimigaki S, Messini-Nicolaki N, Ziros E, Piperakis SM Effects of pulsed electric fields on DNA of human lymphocytes. Cell Biol Toxicol. 22(6):409-415, 2006. (VT, AE, GT) (electric field)

The effects of pulsed electric fields of low frequency (50 Hz) on DNA of human lymphocytes were investigated. The influence of additional external factors, such as hydrogen peroxide (H_2O_2) and gamma-irradiation, as well as the repair efficiency in these lymphocytes, was also evaluated. The comet assay, a very sensitive and rapid method for detecting DNA damage at the single cells level was the method used. A significant amount of damage was observed after exposure to the electric fields, compared to the controls. After 2 h incubation at 37 degrees C, a proportion of damage was repaired. H_2O_2 and gamma-irradiation increased the damage to lymphocytes exposed to pulsed electric fields according to the dose used, while the amount of the repair was proportional to the damage.

(E) Di Campli E, Di Bartolomeo S, Grande R, Di Giulio M, Cellini L. Effects of extremely low-frequency electromagnetic fields on Helicobacter pylori biofilm. Curr Microbiol. 60(6):412-418, 2010. (VT, AE, GE)

The aim of this work was to investigate the effects of exposure to extremely low-frequency electromagnetic fields (ELF-EMF) both on biofilm formation and on mature biofilm of *Helicobacter pylori*. Bacterial cultures and 2-day-old biofilm of *H. pylori* ATCC 43629 were exposed to ELF-EMF (50 Hz frequency-1 mT intensity) for 2 days to assess their effect on the cell adhesion and on the mature biofilm detachment, respectively. All the exposed cultures and the respective sham exposed controls were studied for: the cell viability status, the cell morphological analysis, the biofilm mass measurement, the genotypic profile, and the luxS and amiA gene expression. The ELF-EMF acted on the bacterial population during the biofilm formation displaying significant differences in cell viability, as well as, in morphotypes measured by the prevalence of spiral forms (58.41%) in respect to the controls (33.14%), whereas, on mature biofilm, no significant differences were found when compared to the controls. The measurement of biofilm cell mass was significantly reduced in exposed cultures in both examined experimental conditions. No changes in DNA patterns were recorded, whereas a modulation in amiA gene expression was detected. An exposure to ELF-EMF of *H. pylori* biofilm induces phenotypic changes on adhering bacteria and decreases the cell adhesion unbalancing the bacterial population therefore reducing the *H. pylori* capability to protect itself.

(E) Dominici L, Villarini M, Fatigoni C, Monarca S, Moretti M. Genotoxic hazard evaluation in welders occupationally exposed to extremely low-frequency magnetic fields (ELF-MF). Int J Hyg Environ Health. 215(1):68-75, 2011. (HU, LE, GT)

Electric arc welding is known to involve considerable exposure to extremely low-frequency magnetic fields (ELF-MF). A cytogenetic monitoring study was carried out in a group of welders to investigate the genotoxic risk of occupational exposure to ELF-MF. This study assessed individual occupational exposure to ELF-MF using a personal magnetic-field dosimeter, and the cytogenetic effects were examined by comparing micronuclei (MN) and sister chromatid exchange (SCE) frequencies in the lymphocytes of the exposed workers with those of non-exposed control subjects (blood donors) matched for age and smoking habit. Cytogenetic analyses were carried out on 21 workers enrolled from two different welding companies in Central Italy and compared to 21 controls. Some differences between the groups were observed on analysis of SCE and MN, whereas replication indices in the exposed were found not to differ from the controls. In particular, the exposed group showed a significantly higher frequency of MN (group mean \pm SEM: 6.10 \pm 0.39) compared to the control group (4.45 \pm 0.30). Moreover, the increase in MN is associated with a proportional increase in ELF-MF exposure levels with a dose-response relationship. A significant decrease in SCE frequency was observed in exposed subjects (3.73 \pm 0.21) compared to controls (4.89 \pm 0.12). The hypothesis of a correlation between genotoxic assays and ELF-MF exposure value was partially supported, especially as regards MN assay. Since these results are derived from a small-scale pilot study, a larger scale study should be undertaken.

(E) Dong D, Yang J, Zhang G, Huyan T, Shang P. 16 T high static magnetic field inhibits receptor activator of nuclear factor kappa-B ligand-induced osteoclast differentiation by regulating iron metabolism in Raw264.7 cells. J Tissue Eng Regen Med 13(12):2181-2190, 2019. (VT, AE, GE)

High static magnetic fields (HiSMFs) are usually defined as those SMFs with intensities ≥ 1 T. Although many studies have indicated that SMFs have positive effects on bone tissue, there were limited studies that investigate the effects of cells, including osteoclasts, to illustrate the effect of HiSMF on osteoclast differentiation, and whether iron involve in the altered osteoclast formation and resorption ability under HiSMF. 16 T HiSMF generated from a superconducting magnet was used. Osteoclastogenesis, bone resorption, acting ring formation, messenger ribonucleic acid expression, and protein expression were determined by tartrate-resistant acid phosphatase staining, pits formation assay, rhodamine-conjugated phalloidine staining, quantitative real-time polymerase chain reaction, and western blot, respectively. The changes induced by HiSMF in the level of iron and the concentration of mitochondrial protein, adenosine triphosphate, reactive oxygen species, malonaldehyde, and glutathione were examined by atomic absorption spectrometry and corresponding commercial kits, respectively. The results showed that HiSMF significantly inhibited osteoclastic formation and resorption ability and reduced cellular iron content during osteoclast differentiation. Mitochondrial concentration and oxidative stress levels in osteoclasts were decreased under HiSMF. Mechanistically, HiSMF markedly blocked the expression of osteoclast-associated transcription factors and osteoclast marker genes and inhibited iron absorption and iron storage-related protein expression. These findings demonstrated that the effect of HiSMF on iron metabolism of osteoclasts was involved in the inhibition of HiSMF on osteoclast differentiation.

(E) Dong L, Xiang J, Guo J, Chen G, Di G. Can static electric fields increase the activity of nitric oxide synthase and induce oxidative stress and damage of spleen? Environ Sci Pollut Res Int. 2021 Aug 16. doi: 10.1007/s11356-021-15853-8. (VO, LE, GE, OX)

With the rapid development of ultra-high-voltage (UHV) direct-current (DC) transmissions, the impact of static electric fields (SEF) in the vicinity of overhead UHV DC transmission lines on health has aroused much public concern. This study explored the effects of 56.3kV/m SEF on the spleen of mice. Results showed that SEF exposure of 21days significantly increased malonic dialdehyde content, superoxide dismutase activity, calcineurin activity, nitric oxide synthase (NOS) activity, and the mRNA expression levels of tumor necrosis factor- α (TNF- α) and nuclear factor- κ B (NF- κ B) in the spleen and caused the separation of nucleus and nuclear membrane, the disappearance of mitochondrial membrane, and the deficiency of mitochondrial cristae in splenic lymphocytes. By analysis and discussion, it was deduced that SEF could induce oxidative stress of the spleen by increasing the activity of NOS. Oxidative stress could further cause ultrastructural changes of splenic lymphocytes. Moreover, oxidative stress could cause the increase of the mRNA expression levels of TNF- α and NF- κ B, which contributed to the occurrence of spleen inflammation.

(E) Dong Y, Suryani L, Zhou X, Muthukumaran P, Rakshit M, Yang F, Wen F, Hassanbhai AM, Parida K, Simon DT, Iandolo D, Lee PS, Ng KW, Teoh SH Synergistic Effect of PVDF-Coated PCL-TCP Scaffolds and Pulsed Electromagnetic Field on Osteogenesis. Int J Mol Sci 021 Jun 16;22(12):6438. (VT, LE, GE)

Bone exhibits piezoelectric properties. Thus, electrical stimulations such as pulsed electromagnetic fields (PEMFs) and stimuli-responsive piezoelectric properties of scaffolds have been investigated separately to evaluate their efficacy in supporting osteogenesis. However, current understanding of cells responding under the combined influence of PEMF and piezoelectric properties in scaffolds is still lacking. Therefore, in this study, we fabricated piezoelectric scaffolds by functionalization of polycaprolactone-tricalcium phosphate (PCL-TCP) films with a polyvinylidene fluoride (PVDF) coating that is self-polarized by a modified breath-figure technique. The osteoinductive properties of these PVDF-coated PCL-TCP films on MC3T3-E1 cells were studied under the stimulation of PEMF. Piezoelectric and ferroelectric characterization demonstrated that scaffolds with piezoelectric coefficient $d_{33} = -1.2$ pC/N were obtained at a powder dissolution temperature of 100 °C and coating relative humidity (RH) of 56%. DNA quantification showed that cell proliferation was significantly enhanced by PEMF as low as 0.6 mT and 50 Hz. Hydroxyapatite staining showed that cell mineralization was significantly enhanced by incorporation of PVDF coating. Gene expression study showed that the combination of PEMF and PVDF coating promoted late osteogenic gene expression marker most significantly. Collectively, our results suggest that the synergistic effects of PEMF and piezoelectric scaffolds on osteogenesis provide a promising alternative strategy for electrically augmented osteoinduction. The piezoelectric response of PVDF by PEMF, which could provide mechanical strain, is particularly interesting as it could deliver local mechanical stimulation to osteogenic cells using PEMF.

(E) Drzewiecka EM, Kozłowska W, Paukzsto L, Zmijewska A, Wydorski PJ, JP, Franczak A. Effect of the Electromagnetic Field (EMF) Radiation on Transcriptomic Profile of Pig Myometrium during the Peri-Implantation Period-An In Vitro Study. Int J Mol Sci 2021 Jul 7;22(14):7322.(VT, AE, GE)

The electromagnetic field (EMF) affects the physiological processes in mammals, but the molecular background of the observed alterations remains not well established. In this study was tested the effect of short duration (2 h) of the EMF treatment (50 Hz, 8 mT) on global transcriptomic alterations in the myometrium of pigs during the peri-implantation period using next-generation sequencing. As a result, the EMF treatment affected the expression of 215 transcript active regions (TARs), and among them, the assigned gene protein-coding biotype possessed 90 ones (differentially expressed genes, DEGs), categorized mostly to gene ontology terms connected with defense and immune responses, and secretion and export. Evaluated DEGs enrich the KEGG *TNF signaling pathway*, and *regulation of IFNA signaling* and *interferon-alpha/beta signaling* REACTOME pathways. There were evaluated 12 differentially expressed long non-coding RNAs (DE-lnc-RNAs) and 182 predicted single nucleotide variants (SNVs) substitutions within RNA editing sites. In conclusion, the EMF treatment in the myometrium collected during the peri-implantation period affects the expression of genes involved in defense

and immune responses. The study also gives new insight into the mechanisms of the EMF action in the regulation of the transcriptomic profile through lnc-RNAs and SNVs.

(E) Du XG, Xu SS, Chen Q, Lu DQ, Xu ZP, Zeng QL. [Effects of 50 Hz magnetic fields on DNA double-strand breaks in human lens epithelial cells]. Zhejiang Da Xue Xue Bao Yi Xue Ban. 37(1):9-14, 2008. [Article in Chinese] (VT, AE, GT)

OBJECTIVE: To investigate the effects of 50 Hz magnetic fields (MF) on DNA double-strand breaks in human lens epithelial cells (hLECs). **METHODS:** The cultured human lens epithelial cells were exposed to 0.4 mT 50 Hz MF for 2 h, 6 h, 12 h, 24 h and 48 h. Cells exposed to 4-nitroquinoline-1-oxide, a DNA damage agent, at a final concentration of 0.1 micromol/L for 1 h were used as positive controls. After exposure, cells were fixed with 4 % paraformaldehyde and for H2AX (gamma H2AX) immunofluorescence measurement. gamma H2AX foci were detected at least 200 cells for each sample. Cells were classified as positive when more than three foci per cell were observed. Mean values of foci per cell and percentage of foci positive cells were adopted as indexes of DNA double-strand breaks. **RESULT:** The mean value of foci per cell and the percentage of gamma H2AX foci positive cells in 50 Hz MF exposure group for 24 h were (2.93 +/-0.43) and (27.88 +/-2.59)%, respectively, which were significantly higher than those of sham-exposure group [(1.77 +/-0.37) and (19.38 +/-2.70)%, P <0.05], and the mean value of foci per cell and the percentage of gamma H2AX foci positive cells in 50 Hz MF exposure group for 48 h were (3.14 +/-0.35) and (31.00 +/-3.44)%, which were significantly higher than those of sham-exposure group (P <0.01). However there was no significant difference between 50 Hz MF exposure groups for 2 h, 6 h, 12 h and sham-exposure group for above two indexes (P >0.05). **CONCLUSION:** 0.4 mT 50 Hz MF exposure for longer duration might induce DNA double-strand breaks in human lens epithelial cells in vitro.

(E) Duan W, Liu C, Zhang L, He M, Xu S, Chen C, Pi H, Gao P, Zhang Y, Zhong M, Yu Z, Zhou Z. Comparison of the genotoxic effects induced by 50 Hz extremely low-frequency electromagnetic fields and 1800 MHz radiofrequency electromagnetic fields in GC-2 cells. Radiat Res. 183(3):305-314, 2015. (VT, AE, GT, OX, WS, RP)

Extremely low-frequency electromagnetic fields (ELF-EMF) and radiofrequency electromagnetic fields (RF-EMF) have been considered to be possibly carcinogenic to humans. However, their genotoxic effects remain controversial. To make experiments controllable and results comparable, we standardized exposure conditions and explored the potential genotoxicity of 50 Hz ELF-EMF and 1800 MHz RF-EMF. A mouse spermatocyte-derived GC-2 cell line was intermittently (5 min on and 10 min off) exposed to 50 Hz ELF-EMF at an intensity of 1, 2 or 3 mT or to RF-EMF in GSM-Talk mode at the specific absorption rates (SAR) of 1, 2 or 4 W/kg. After exposure for 24 h, we found that neither ELF-EMF nor RF-EMF affected cell viability using Cell Counting Kit-8. Through the use of an alkaline comet assay and immunofluorescence against γ -H2AX foci, we found that ELF-EMF exposure resulted in a significant increase of DNA strand breaks at 3 mT, whereas RF-EMF exposure had insufficient energy to induce such effects. Using a formamidopyrimidine DNA glycosylase (FPG)-modified alkaline comet assay, we observed that RF-EMF exposure significantly induced oxidative DNA base damage at a SAR value of 4 W/kg, whereas ELF-EMF exposure did not. Our results suggest that both ELF-EMF

and RF-EMF under the same experimental conditions may produce genotoxicity at relative high intensities, but they create different patterns of DNA damage. Therefore, the potential mechanisms underlying the genotoxicity of different frequency electromagnetic fields may be different.

(E) El-Bialy NS, Rageh MM. Extremely low-frequency magnetic field enhances the therapeutic efficacy of low-dose cisplatin in the treatment of Ehrlich carcinoma. Biomed Res Int. 2013;189352, 2013. (Vo, LE, GT, IX)

The present study examines the therapeutic efficacy of the administration of low-dose cisplatin (cis) followed by exposure to extremely low-frequency magnetic field (ELF-MF), with an average intensity of 10 mT, on Ehrlich carcinoma in vivo. The cytotoxic and genotoxic actions of this combination were studied using comet assay, mitotic index (MI), and the induction of micronucleus (MN). Moreover, the inhibition of tumor growth was also measured. Treatment with cisplatin and ELF-MF (group A) increased the number of damaged cells by 54% compared with 41% for mice treated with cisplatin alone (group B), 20% for mice treated by exposure to ELF-MF (group C), and 9% for the control group (group D). Also the mitotic index decreased significantly for all treated groups ($P < 0.001$). The decrement percent for the treated groups (A, B, and C) were 70%, 65%, and 22%, respectively, compared with the control group (D). Additionally, the rate of tumor growth at day 12 was suppressed significantly ($P < 0.001$) for groups A, B, and C with respect to group (D). These results suggest that ELF-MF enhanced the cytotoxic activity of cisplatin and potentiate the benefit of using a combination of low-dose cisplatin and ELF-MF in the treatment of Ehrlich carcinoma.

(E) Erdal N, Gürgül S, Celik A. Cytogenetic effects of extremely low frequency magnetic field on Wistar rat bone marrow. Mutat Res. 630(1-2):69-77, 2007. (VO, AE, LE, GT)

In this study, the genotoxic and cytotoxic potential of extremely low frequency magnetic fields (ELF-MF) was investigated in Wistar rat tibial bone marrow cells, using the chromosomal aberration (CA) and micronucleus (MN) test systems. In addition to these test systems, we also investigated the mitotic index (MI), and the ratio of polychromatic erythrocytes (PCEs) to normochromatic erythrocytes (NCEs). Wistar rats were exposed to acute (1 day for 4h) and long-term (4h/day for 45 days) to a horizontal 50Hz, 1mT uniform magnetic field generated by a Helmholtz coil system. Mitomycin C (MMC, 2mg/kg BW) was used as positive control. Results obtained by chromosome analysis do not show any statistically significant differences between the negative control and both acute and long-term ELF-MF exposed samples. When comparing the group mean CA of long-term exposure with the negative control and acute exposure, the group mean of the long-term exposed group was higher, but this was not statistically significant. However, the mean micronucleus frequency of the longer-term exposed group was considerably higher than the negative control and acutely exposed groups. This difference was statistically significant ($p < 0.01$). The results of the MI in bone marrow showed that the averages of both A-MF and L-MF groups significantly decreased when compared to those in the negative control ($p < 0.001$ and $p < 0.01$, respectively). No significant differences were found between the group mean MI of A-MF exposure with L-MF. We found that the average of PCEs/NCEs ratios of A-MF exposed group was significantly lower than the negative control and L-MF exposed groups ($p < 0.001$ and $p < 0.01$, respectively). In addition, the group mean of the PCEs/NCEs ratios of L-

MF was significantly lower than negative control ($p < 0.01$). We also found that the MMC treated group showed higher the number of CA and the frequency of MN formation when compared to those in all other each groups (p -values of all each groups < 0.01) and also MMC treated group showed lower MI and the PCEs/NCEs ratios when compared to those in all other each groups (p -values of all groups < 0.01). These observations indicate the in vivo susceptibility of mammals to the genotoxicity potential of ELF-MF.

(E) Erdal ME, Yilmaz SG, Gürgül S, Uzun C, Dericı D, Erdal N. miRNA expression profile is altered differentially in the rat brain compared to blood after experimental exposure to 50 Hz and 1 mT electromagnetic field. Prog Biophys Mol Biol 132:35-42, 2018. (VO, CE, GE, EP)

Common complex diseases are a result of host and environment interactions. One such putative environmental factor is the electromagnetic field exposure, especially the occupational extremely low frequency (ELF) magnetic field, 50 Hz, 1 mT, whose neurobiological relevance remains elusive. We evaluated the effects of long-term (60 days) ELF-MF exposure on miRNAs previously related to brain and human diseases (miR-26b-5p, miR-9-5p, miR-29a-3p, miR-106b-5p, miR-107, miR-125a-3p). A total of 64 young (3 weeks-old) and mature (10 weeks-old) male/female Wistar-Albino rats were divided into sham and ELF-MF exposed groups. After sacrifice of the animals, blood samples from rat's tail vein and brain tissues were collected. The expression levels of miRNAs were investigated with Real-Time PCR technique and TaqMan probe Technology. All miRNA expression levels of the young female rats show a significant decrease in blood according to brain samples ($p < 0.05$), but fewer miRNAs displayed a similar significant decrease in the blood. In conclusion, these new observations might inform future clinical biological psychiatry studies of long-term electromagnetic field exposure, and the ways in which host-environment interactions contribute to brain diseases.

(E) Fadel MA, El-Gebaly RH, Mohamed SA, Abdelbacki AMM. Biophysical control of the growth of Agrobacterium tumefaciens using extremely low frequency electromagnetic waves at resonance frequency. Biochem Biophys Res Commun. 494(1-2):365-371, 2017. (VO, AE, GT)

Isolated *Agrobacterium tumefaciens* was exposed to different extremely low frequencies of square amplitude modulated waves (QAMW) from two generators to determine the resonance frequency that causes growth inhibition. The carrier was 10 MHz sine wave with amplitude ± 10 Vpp which was modulated by a second wave generator with a modulation depth of ± 2 Vpp and constant field strength of 200 V/m at 28 °C. The exposure of *A. tumefaciens* to 1.0 Hz QAMW for 90 min inhibited the bacterial growth by 49.2%. In addition, the tested antibiotics became more effective against *A. tumefaciens* after the exposure. Furthermore, results of DNA, dielectric relaxation and TEM showed highly significant molecular and morphological changes due to the exposure to 1.0 Hz QAMW for 90 min. An in-vivo study has been carried out on healthy tomato plants to test the pathogenicity of *A. tumefaciens* before and after the exposure to QAMW at the inhibiting frequency. Symptoms of crown gall and all pathological symptoms were more aggressive in tomato plants treated with non-exposed bacteria, comparing with those treated with exposed bacteria. We concluded that, the exposure of *A. tumefaciens* to 1.0 Hz QAMW for 90

min modified its cellular activity and DNA structure, which inhibited the growth and affected the microbe pathogenicity.

(NE) Fairbairn DW, O'Neill KL The effect of electromagnetic field exposure on the formation of DNA single strand breaks in human cells. *Cell Mol Biol (Noisy-legrand)*. **40(4):561-567, 1994. (VT, AE, GT)**

Electromagnetic fields (EMF) have been reported to be associated with human cancers in a number of epidemiological studies. Agents that are associated with cancer affect DNA in an adverse manner. This is a report of a DNA damage study in human cells exposed to EMFs. Single strand breaks in DNA are proposed to be necessary events in both mutagenesis and carcinogenesis. The single cell gel assay is a sensitive and accurate technique that was used in this study for single strand break detection. The EMF exposure system used here appeared to have no direct effect on DNA damage induction in a series of experiments. Moreover, EMF did not have a significant effect in potentiating DNA damage in cells treated with oxidative stresses.

(E) Fan W, Qian F, Ma Q, Zhang P, Chen T, Chen C, Zhang Y, Deng P, Zhou Z, Yu Z. 50 Hz electromagnetic field exposure promotes proliferation and cytokine production of bone marrow mesenchymal stem cells. *Int J Clin Exp Med*. **8(5):7394-7404. 2015. (VT, CE, GE)**

OBJECTIVE: To investigate the effects of extremely low frequency electromagnetic field (ELF-EMF) on the proliferation and cytokine production of mesenchymal stem cells (MSC) and the effects of mesenchymal stem cell conditioned medium (MSC-CM) on the proliferation and migration of macrophagocytes (RAW264.7). METHODS: Bone marrow derived-mesenchymal stem cells (rBMSC) were isolated from rats, cultured and randomly divided into two groups: SHAM group (absence of electromagnetic field exposure) and EMF group. Cells in EMF group were exposed to ELF-EMF (50 Hz, 1 mT, 4 h/d) under sXc-ELF. Mouse mesenchymal stem cells (mMSC) were exposed to EMF for 3 days. RESULTS: The cell viability, DNA synthesis and proportion of cells in S phase in EMF group increased markedly when compared with SHAM group ($P<0.05$). When compared with SHAM group, the mRNA expressions of M-CSF and SCF increased markedly at 2 days after EMF exposure ($P<0.05$), the mRNA expressions of SCF, M-CSF, TPO, LIF, IL-11 and IL-7 increased dramatically, but the mRNA expressions of IL-6, SDF-1, IFN- γ and TNF- α remained unchanged ($P>0.05$) in mMSCs at 3 days after EMF exposure. In EMF group, the viability of RAW264.7 after MSC-CM treatment increased markedly as compared to SHAM group ($P<0.05$), and the ability to migrate of RAW264.7 after MSC-CM treatment in EMF group also increased significantly when compared with SHAM group ($P<0.05$). CONCLUSION: EMF is able to promote the proliferation of rBMSCs, up-regulate the expressions of hematopoietic growth factors in rBMSC and mMSC and increase the mMSC induced proliferation and migration of RAW264.7.

(E) Fan W, Huang Z, Fan B. Effects of prolonged exposure to moderate static magnetic field and its synergistic effects with alkaline pH on *Enterococcus faecalis*. *Microb Pathog* **115:117-122, 2018. (VT, AE, GE, IX)**

Static magnetic field (SMF) has been shown to biologically affect various microorganisms, but its effects on *Enterococcus faecalis*, which is associated with multiple dental infections, have not been reported yet. Besides, *Enterococcus faecalis* was found to be resistant to the alkaline environment provided by a major dental antimicrobial, calcium hydroxide. Therefore, the antibacterial activity of prolonged exposure to moderate SMF (170 mT) and its possible synergistic activity with alkaline pH (pH = 9) were evaluated in the study. The ability to form a biofilm under these conditions was examined by crystal violet assay. Real-time quantitative PCR was performed to evaluate the relative expression of stress (*dnaK* and *groEL*) and virulence (*efaA*, *ace*, *gelE* and *fsrC*) related genes. As the results indicated, cell proliferation was inhibited after 120 h of SMF exposure. What's more, the combined treatment of SMF and alkaline pH showed significantly improved antimicrobial action when compared to single SMF and alkaline pH treatment for more than 24 h and 72 h respectively. However, the ability to form a biofilm was also enhanced under SMF and alkaline pH treatments. SMF can induce stress response by up-regulating the expression of *dnaK* and elevate virulence gene expression (*efaA* and *ace*). These responses were more significant and more genes were up-regulated including *groEL*, *gelE* and *fsrC* when exposed to SMF and alkaline pH simultaneously. Hence, combination of SMF and alkaline pH could be a promising disinfection strategy in dental area and other areas associated with *Enterococcus faecalis* infections.

(E) Fathi E, Farahzadi R Enhancement of osteogenic differentiation of rat adipose tissue-derived mesenchymal stem cells by zinc sulphate under electromagnetic field via the PKA, ERK1/2 and Wnt/ β -catenin signaling pathways. PLoS One 12(3):e0173877, 2017. (VT, LE, GE)

Zinc ion as an essential trace element and electromagnetic fields (EMFs) has been reported to be involved in the regulation of bone metabolism. The aim of this study was to elucidate the effects of zinc sulphate ($ZnSO_4$) on the osteogenic differentiation of adipose tissue-derived mesenchymal stem cells (ADSCs) in the presence of EMF as a strategy in osteoporosis therapy. Alkaline phosphatase (ALP) activity measurement, calcium assay and expression of several osteoblastic marker genes were examined to assess the effect of $ZnSO_4$ on the osteogenic differentiation of ADSCs under EMF. The expression of cAMP and PKA was evaluated by ELISA. The expression of β -catenin, Wnt1, Wnt3a, low-density lipoprotein receptor-related protein 5 (LRP5) and reduced dickkopf1 (DKK1) genes were used to detect the Wnt/ β -catenin pathway. It was found that $ZnSO_4$, in the presence of EMF, resulted in an increase in the expression of osteogenic genes, ALP activity and calcium levels. EMF, in the presence of $ZnSO_4$, increased the cAMP level and protein kinase A (PKA) activity. Treatment of ADSCs with (MAPK)/ERK kinase 1/2 inhibitor, or PKA inhibitor, significantly inhibited the promotion of osteogenic markers, indicating that the induction of osteogenesis was dependent on the ERK and PKA signaling pathways. Real-time PCR analysis showed that $ZnSO_4$, in the presence of EMF, increased the mRNA expressions of β -catenin, Wnt1, Wnt3a, LRP5 and DKK1. In this study, it was shown that 0.432 $\mu g/ml$ $ZnSO_4$, in the presence of 50 Hz, 20 mT EMF, induced the osteogenic differentiation of ADSCs via PKA, ERK1/2 and Wnt/ β -catenin signaling pathways.

(E) Fatigoni C, Dominici L, Moretti M, Villarini M, Monarca S. Genotoxic effects of extremely low frequency (ELF) magnetic fields (MF) evaluated by the Tradescantia-micronucleus assay Environ Toxicol. 20(6):585-91, 2005. (VT, AE, GT)

Extremely low frequency (ELF) electric fields (EF) and magnetic fields (MF) are generated during the production, transmission, and use of electrical energy. Although epidemiology studies suggest that there is a cancer risk associated with exposure to ELF-MF, short-term genotoxicity assays with bacteria and mammalian cells have produced inconsistent results. In the present study, we investigated the possible genotoxicity of ELF-MF by using the Tradescantia-micronucleus (Trad-MN) assay, a sensitive, reproducible, well-standardized assay for genotoxicity testing. A 50 Hz ELF-MF was generated by a laboratory exposure system consisting of a pair of parallel coils in a Helmholtz configuration. Exposure of Tradescantia (clone # 4430) inflorescences to the ELF-MF, at a flux density (B) corresponding to 1 mT, for 1, 6, and 24 h resulted in a time-dependent increase in MN frequency. The results indicate that a 50 Hz MF of 1 mT field strength is genotoxic in the Trad-MN bioassay and suggest that this assay may be suitable as a biomonitor for detecting the genotoxicity of ELF-MF in the field.

(E) Fedrowitz M, Löscher W. Gene expression in the mammary gland tissue of female Fischer 344 and Lewis rats after magnetic field exposure (50 Hz, 100 μ T) for 2 weeks. Int J Radiat Biol. 88(5):425-429, 2012. (VO, LE, GE) See also: Fedrowitz M, Hass R, Löscher W. Effects of 50 Hz magnetic field exposure on the stress marker α -amylase in the rat mammary gland. Int J Radiat Biol. 88(7):556-564, 2012.

PURPOSE: The issue of whether exposure to environmental power-frequency magnetic fields (MF) has impact on breast cancer development still remains equivocal. Previously, we observed rat strain differences in the MF response of breast tissue, so that the genetic background plays a role in MF effects. The present experiment aimed to elucidate candidate genes involved in MF effects by comparison of MF-susceptible Fischer 344 (F344) rats and MF-insensitive Lewis rats. **MATERIALS AND METHODS:** Female F344 and Lewis rats were exposed to MF (50 Hz, 100 μ T) for two weeks, and a whole genome microarray analysis in the mammary gland tissue was performed. **RESULTS:** A remarkably decreased α -amylase gene expression, decreases in carbonic anhydrase 6 and lactoperoxidase, both relevant for pH regulation, and an increased gene expression of cystatin E/M, a tumor suppressor, were observed in MF-exposed F344, but not in Lewis rats. **CONCLUSION:** The MF-exposed F344 breast tissue showed alterations in gene expression, which were absent in Lewis and may therefore be involved in the MF-susceptibility of F344. Notably α -amylase might serve as a promising target to study MF effects, because first experiments indicate that MF exposure alters the functionality of this enzyme in breast tissue.

(E) Feng B, Ye C, Qiu L, Chen L, Fu Y, Sun W. Mitochondrial ROS release and subsequent Akt activation potentially mediated the anti-apoptotic effect of a 50-Hz magnetic field on FL cells. Cell Physiol Biochem 38(6):2489-2499, 2016. (VT, AE, GE)

Background/aims: Our previous study showed that exposure to a 50-Hz magnetic field (MF) could induce transient mitochondrial permeability transition (MPT) in cells. In the present study, the aim was to explore the possible biological implications of MF-induced transient MPT.

Materials and methods: Human amniotic (FL) cells were exposed to MF for different durations or intensities followed by incubation with staurosporine for 4 h. After MF exposure, cell early apoptosis, cell viability mitochondrial ROS and the level of phosphorylated Akt were assessed. After MF exposure followed by incubation with staurosporine, cell early apoptosis was assessed. **Results:** MF exposure had a protective effect against early apoptosis induced by staurosporine, which could be abolished by MPT inhibitors, although MF exposure alone had no significant effect on early apoptosis or viability of cells. In addition, exposing cells to MF increased the level of mitochondrial ROS which were released into cytoplasm through mitochondrial permeability transition pores (mPTP), and induced ROS-dependent phosphorylation of Akt. Furthermore, the anti-apoptotic effect of MF exposure was completely eliminated when Akt was inhibited. **Conclusions:** The present study indicated a possibility that mitochondrial ROS release through mPTP and subsequent Akt activation were necessary for the anti-apoptotic effect of MF.

Ferreira de Sousa, Liciane S Menezes, Wilton Mitsunari Takeshita. Genotoxic and cytotoxic effects of mobile phone use on the oral epithelium: a systematic review with meta-analysis. Gen Dent. Nov-Dec 2020;68(6):70-74. PMID: 33136050. (Review)

The use of mobile phones is based on radiofrequency (RF) waves, and the devices act as transmitters and receivers of non-ionizing energy. The micronucleus test was developed to identify increases in the occurrence of mutations in cells exposed to various agents. This systematic review with meta-analysis adhered to the following protocol: defining the objective, outlining the search method (PICO model), conducting the search, identifying literature, selecting articles, and extracting data. The study aimed to answer the following research question: Does non-ionizing radiation emitted by mobile phones have genotoxic and/or cytotoxic effects on the oral epithelium? The search for evidence published 2009-2019 was conducted in the MEDLINE, PubMed, Scopus, LILACS, Google Scholar, PROSPERO, and Cochrane Library databases. The following inclusion criteria were defined: investigations of effects on the oral mucosa related to RF; investigations of cytotoxic and/or genotoxic effects; investigations involving humans; and investigations using cells exfoliated from the oral epithelium. Investigations related to the parotid gland were excluded. The search strategy found 464 articles; after application of the eligibility criteria, 358 abstracts were analyzed and 351 abstracts excluded. After 7 full texts were reviewed, 1 study was excluded. The 6 included studies were classified as level 5 quality of evidence (observational studies). The meta-analysis included 2 studies that compared the frequency of micronuclei on the side exposed to RF electromagnetic fields (RF-EMFs) to that on the unexposed side. The studies evaluated presented a low degree of evidence, but the meta-analysis indicated that no genotoxic effects are associated with mobile phone use. However, observations of other nuclear abnormalities in some studies suggest the occurrence of cytotoxic effects caused by exposure to the RF-EMFs emitted by mobile phones. More studies are necessary to prove or refute this association.

(NE)Fiorani M, Cantoni O, Sestili P, Conti R, Nicolini P, Vetrano F, Dacha M. Electric and/or magnetic field effects on DNA structure and function in cultured human cells. Mutat Res. 282(1):25-29, 1992. (VT, AE, GT)

Exposure of cultured K562 cells to 50 Hz electric (0.2-20 kV/m), magnetic (0.002-2 G),

or combined electric and magnetic fields for up to 24 h did not result in the production of detectable DNA lesions, as assayed by the filter elution technique. The rate of cell growth was also unaffected as well as the intracellular ATP and NAD⁺ levels. These results indicate that, under the experimental conditions utilized in this study, 50 Hz electric, magnetic and electromagnetic fields are not geno- and cyto-toxic in cultured mammalian cells.

(E) Focke F, Schuermann D, Kuster N, Schär P. DNA fragmentation in human fibroblasts under extremely low frequency electromagnetic field exposure. *Mutat Res.* 683(1-2):74-83, 2010. (VT, AE, GT, WS)

Extremely low frequency electromagnetic fields (ELF-EMFs) were reported to affect DNA integrity in human cells with evidence based on the Comet assay. These findings were heavily debated for two main reasons; the lack of reproducibility, and the absence of a plausible scientific rationale for how EMFs could damage DNA. Starting out from a replication of the relevant experiments, we performed this study to clarify the existence and explore origin and nature of ELF-EMF induced DNA effects. Our data confirm that intermittent (but not continuous) exposure of human primary fibroblasts to a 50 Hz EMF at a flux density of 1 mT induces a slight but significant increase of DNA fragmentation in the Comet assay, and we provide first evidence for this to be caused by the magnetic rather than the electric field. Moreover, we show that EMF-induced responses in the Comet assay are dependent on cell proliferation, suggesting that processes of DNA replication rather than the DNA itself may be affected. Consistently, the Comet effects correlated with a reduction of actively replicating cells and a concomitant increase of apoptotic cells in exposed cultures, whereas a combined Fpg-Comet test failed to produce evidence for a notable contribution of oxidative DNA base damage. Hence, ELF-EMF induced effects in the Comet assay are reproducible under specific conditions and can be explained by minor disturbances in S-phase processes and occasional triggering of apoptosis rather than by the generation of DNA damage.

(NE) Frahm J, Lantow M, Lupke M, Weiss DG, Simkó M. Alteration in cellular functions in mouse macrophages after exposure to 50 Hz magnetic fields. *J Cell Biochem.* 99(1):168-177, 2006. (VT, AE, GT, OX)

The aim of the present study is to investigate whether extremely low frequency electromagnetic fields (ELF-EMF) affect certain cellular functions and immunologic parameters of mouse macrophages. In this study, the influence of 50 Hz magnetic fields (MF) at 1.0 mT was investigated on the phagocytic activity and on the interleukin-1beta (IL-1beta) production in differentiated macrophages. MF-exposure led to an increased phagocytic activity after 45 min, shown as a 1.6-fold increased uptake of latex beads in MF-exposed cells compared to controls. We also demonstrate an increased IL-1beta release in macrophages after 24 h exposure (1.0 mT MF). Time-dependent IL-1beta formation was significantly increased already after 4 h and reached a maximum of 12.3-fold increase after 24 h compared to controls. Another aspect of this study was to examine the genotoxic capacity of 1.0 mT MF by analyzing the micronucleus (MN) formation in long-term (12, 24, and 48 h) exposed macrophages. Our data show no significant differences in MN formation or irregular mitotic activities in exposed cells. Furthermore, the

effects of different flux densities (ranging from 0.05 up to 1.0 mT for 45 min) of 50 Hz MF was tested on free radical formation as an endpoint of cell activation in mouse macrophage precursor cells. All tested flux densities significantly stimulated the formation of free radicals. Here, we demonstrate the capacity of ELF-EMF to stimulate physiological cell functions in mouse macrophages shown by the significantly elevated phagocytic activity, free radical release, and IL-1beta production suggesting the cell activation capacity of ELF-EMF in the absence of any genotoxic effects.

(NE) Frazier ME, Reese JA, Morris JE, Jostes RF, Miller DL Exposure of mammalian cells to 60-Hz magnetic or electric fields: analysis of DNA repair of induced, singlestrand breaks. Bioelectromagnetics. 11(3):229-234, 1990. (VT, AE, GT)

DNA damage was induced in isolated human peripheral lymphocytes by exposure at 5 Gy to ⁶⁰Co radiation. Cells were permitted to repair the DNA damage while exposed to 60-Hz fields or while sham-exposed. Exposed cells were subjected to magnetic (B) or electric (E) fields, alone or in combination, throughout their allotted repair time. Repair was stopped at specific times, and the cells were immediately lysed and then analyzed for the presence of DNA single-strand breaks (SSB) by the alkaline-elution technique. Fifty to 75 percent of the induced SSB were repaired 20 min after exposure, and most of the remaining damage was repaired after 180 min. Cells were exposed to a 60-Hz ac B field of 1 mT; an E field of 1 or 20 V/m; or combined E and B fields of 0.2 V/m and 0.05 mT, 6 V/m and 0.6 mT, or 20 V/m and 1 mT. None of the exposures was observed to affect significantly the repair of DNA SSB.

(E) Frisch P, Li GC, McLeod K, Laramee CB. Induction of heat shock gene expression in RAT1 primary fibroblast cells by ELF electric fields. Bioelectromagnetics. 34(5):405-413, 2013. (VT, AE, GE) (Electric field)

Recent studies have demonstrated that the Ku70 gene fragment can be placed in the anti-sense orientation under the control of a heat-inducible heat shock protein 70 (HSP70) promoter and activated through heat shock exposure. This results in attenuation of the Ku70 protein expression, inhibiting cellular repair processes, and sensitizing the transfected cells to exposures such as the ionizing radiation exposures used clinically. However, achieving the tissue temperatures necessary to thermally induce the HSP70 response presents significant limitations to the clinical application of this strategy. Previous findings suggest an alternative approach to inducing a heat shock response, specifically through the use of extremely low frequency (ELF) electrical field stimulation. To further pursue this approach, we investigated HSP70 responses in transfected rat primary fibroblast (RAT1) cells exposed to 10 Hz electric fields at intensities of 20-500 V/m. We confirmed that low frequency electric fields can induce HSP70 heat shock expression, with peak responses obtained at 8 h following a 2 h field exposure. However, the approximate threefold increase in expression is substantially lower than that obtained using thermal stimulation, raising questions of the clinical utility of the response.

(E) Garip AI, Akan Z. Effect of ELF-EMF on number of apoptotic cells; correlation with reactive oxygen species and HSP. Acta Biol Hung 61(2):158-167, 2010. (VT, AE, GE, OX)

It is by now accepted that extremely low frequency electromagnetic fields ELF-EMF (0-300 Hz) affect biological systems although the mechanism has not been elucidated yet. In this study the effect of ELFEMF on the number of apoptotic cells of K562 human leukemia cell line induced or not with oxidative stress and the correlation with heat-shock protein 70 (hsp70) levels was investigated. One sample was treated with H₂O₂ while the other was left untreated. ELF-EMF (1 mT, 50 Hz) was applied for 3 hours. ELF-EMF alone caused a decrease in the number of apoptotic cells and a slight increase in viability. However, it increased the number of apoptotic cells. In cells treated with H₂O₂. hsp70 and reactive oxygen species (ROS) levels were increased by ELF-EMF. These results show that the effect of ELF-EMF on biological systems depends on the status of the cell: while in cells not exposed to oxidative stress it is able to decrease the number of apoptotic cells by inducing an increase in hsp levels, it increases the number of apoptotic cells in oxidative stress-induced cells.

(E) Giorgi G, Marcantonio P, Bersani F, Gavoçi E, Del Re B. Effect of extremely low frequency magnetic field exposure on DNA transposition in relation to frequency, wave shape and exposure time. Int J Radiat Biol. 87(6):601-608, 2011. (VT, AE, GT, WS)

PURPOSE: To examine the effect of extremely low frequency magnetic field (ELF-MF) exposure on transposon (Tn) mobility in relation to the exposure time, the frequency and the wave shape of the field applied. **MATERIALS AND METHODS:** Two Escherichia coli model systems were used: (1) Cells unable to express β-galactosidase (LacZ(-)), containing a mini-transposon Tn10 element able to give ability to express β-galactosidase (LacZ(+)) upon its transposition; therefore in these cells transposition activity can be evaluated by analysing LacZ(+) clones; (2) cells carrying Fertility plasmid (F(+)), and a Tn5 element located on the chromosome; therefore in these cells transposition activity can be estimated by a bacterial conjugation assay. Cells were exposed to sinusoidal (SiMF) or pulsed-square wave (PMF) magnetic fields of various frequencies (20, 50, 75 Hz) and for different exposure times (15 and 90 min). **RESULTS:** Both mini-Tn10 and Tn5 transposition decreased under SiMF and increased under PMF, as compared to sham exposure control. No significant difference was found between frequencies and between exposure times. **CONCLUSIONS:** ELF-MF exposure affects transposition activity and the effects critically depend on the wave shape of the field, but not on the frequency and the exposure time, at least in the range observed.

(NE) Giorgi G, Lecciso M, Capri M, Lukas Yani S, Virelli A, Bersani F, Del Re B. An evaluation of genotoxicity in human neuronal-type cells subjected to oxidative stress under an extremely low frequency pulsed magnetic field. Mutat Res Genet Toxicol Environ Mutagen. 775-776:31-37, 2014. (VT, AE, GT)

The possible genotoxicity of extremely low frequency magnetic field (ELF-MF) exposure is still a controversial topic. The most of the reported data suggests that it alone does not affect DNA integrity, but several recent reports have suggested that sinusoidal ELF-MF may increase the effect of known genotoxic agents. Only a few studies deal with non sinusoidal ELF-MF, including pulsed magnetic field (PMF), which are produced by several devices. The aim of this study is to investigate whether PMF exposure can interfere with DNA damage and repair in the presence of a genotoxic oxidative agent in neuronal type cells. To this purpose gamma-H2AX

foci formation, which is a sensitive marker of DNA double strand breaks (DSB), was investigated at different points of time (1, 24, 48, 72h) after the H₂O₂ treatment (300 μM for 1h) under PMF exposure (1 mT, 50 Hz) in human neuroblastoma BE(2)C cells. Moreover, cytotoxicity evaluation, by MTT assay and cell cycle analysis, was performed at various points of time after the treatment. Taken together, results suggest that PMF exposure does not interfere with genotoxicity and cytotoxicity induced by oxidative stress.

(E) Giorgi G, Pirazzini C, Bacalini MG, Giuliani C, Garagnani P, Capri M, Bersani F, Del Re B. Assessing the combined effect of extremely low-frequency magnetic field exposure and oxidative stress on LINE-1 promoter methylation in human neural cells. Radiat Environ Biophys. 56(2):193-200, 2017. (VT, AE, GT, EP)

Extremely low frequency magnetic fields (ELF-MF) have been classified as "possibly carcinogenic", but their genotoxic effects are still unclear. Recent findings indicate that epigenetic mechanisms contribute to the genome dysfunction and it is well known that they are affected by environmental factors. To our knowledge, to date the question of whether exposure to ELF-MF can influence epigenetic modifications has been poorly addressed. In this paper, we investigated whether exposure to ELF-MF alone and in combination with oxidative stress (OS) can affect DNA methylation, which is one of the most often studied epigenetic modification. To this end, we analyzed the DNA methylation levels of the 5'untranslated region (5'UTR) of long interspersed nuclear element-1s (LINE-1 or L1), which are commonly used to evaluate the global genome methylation level. Human neural cells (BE(2)C) were exposed for 24 and 48 h to extremely low frequency pulsed magnetic field (PMF; 50 Hz, 1 mT) in combination with OS. The methylation levels of CpGs located in L1 5'UTR region were measured by MassARRAY EpiTYPER. The results indicate that exposures to the single agents PMF and OS induced weak decreases and increases of DNA methylation levels at different CpGs. However, the combined exposure to PMF and OS lead to significant decrease of DNA methylation levels at different CpG sites. Most of the changes were transient, suggesting that cells can restore homeostatic DNA methylation patterns. The results are discussed and future research directions outlined.

Giorgi G, Del Re B Epigenetic dysregulation in various types of cells exposed to extremely low-frequency magnetic fields. Cell Tissue Res 2021 Jul 21. doi: 10.1007/s00441-021-03489-6. Online ahead of print. (Review)

Epigenetic mechanisms regulate gene expression, without changing the DNA sequence, and establish cell-type-specific temporal and spatial expression patterns. Alterations of epigenetic marks have been observed in several pathological conditions, including cancer and neurological disorders. Emerging evidence indicates that a variety of environmental factors may cause epigenetic alterations and eventually influence disease risks. Humans are increasingly exposed to extremely low-frequency magnetic fields (ELF-MFs), which in 2002 were classified as possible carcinogens by the International Agency for Research on Cancer. This review summarizes the current knowledge of the link between the exposure to ELF-MFs and epigenetic alterations in various cell types. In spite of the limited number of publications, available evidence indicates that ELF-MF exposure can be associated with epigenetic changes, including DNA methylation,

modifications of histones and microRNA expression. Further research is needed to investigate the molecular mechanisms underlying the observed phenomena.

(E) Groiss S, Lammegger R, Brislinger D. Anti-Oxidative and Immune Regulatory Responses of THP-1 and PBMC to Pulsed EMF Are Field-Strength Dependent. *Int J Environ Res Public Health* 18(18):9519m 2021. (VT, AE, GE, CS)

Innate immune cells react to electromagnetic fields (EMF) by generating reactive oxygen species (ROS), crucial intracellular messengers. Discrepancies in applied parameters of EMF studies, e.g., flux densities, complicate direct comparison of downstream anti-oxidative responses and immune regulatory signaling. We therefore compared the impact of different EMF flux densities in human leukemic THP1 cells and peripheral blood mononuclear cells (PBMC) of healthy donors to additionally consider a potential disparate receptivity based on medical origin. ROS levels increased in THP1 cells stimulated with lipopolysaccharide (LPS) after one hour of EMF exposure. Moreover, weak EMF mitigated the depletion of the reducing agent NAD(P)H in THP1. Neither of these effects occurred in PBMC. Landscaping transcriptional responses to varied EMF revealed elevation of the anti-oxidative enzymes PRDX6 (2-fold) and DHCR24 (6-fold) in THP1, implying involvement in lipid metabolism. Furthermore, our study confirmed anti-inflammatory effects of EMF by 6-fold increased expression of IL10. Strikingly, THP1 responded to weak EMF, while PBMC were primarily affected by strong EMF, yet with severe cellular stress and enhanced rates of apoptosis, indicated by HSP70 and caspase 3 (CASP3). Taken together, our results emphasize an altered susceptibility of immune cells of different origin and associate EMF-related effects with anti-inflammatory signaling and lipid metabolism.

(E) Han Q, Chen R, Wang F, Chen S, Sun X, Guan X, Yang Y, Peng B, Pan X, Li J, Yi W, Li P, Zhang H, Feng D, Chen A, Li X, Li S, Yin Z. Pre-exposure to 50 Hz-electromagnetic fields enhanced the antiproliferative efficacy of 5-fluorouracil in breast cancer MCF-7 cells. *PLoS One* 13(4):e0192888, 2018. (VT, AE, GE, IX)

Resistance to 5-fluorouracil (5-FU) and its induced immune suppression have prevented its extensive application in the clinical treatment of breast cancer. In this study, the combined effect of 50 Hz-EMFs and 5-FU in the treatment of breast cancer was explored. MCF-7 and MCF10A cells were pre-exposed to 50 Hz-EMFs for 0, 2, 4, 8 and 12 h and then treated with different concentrations of 5-FU for 24 h; cell viability was analyzed by MTT assay and flow cytometry. After pre-exposure to 50 Hz-EMFs for 12 h, apoptosis and cell cycle distribution in MCF-7 and MCF10A cells were detected via flow cytometry and DNA synthesis was measured by EdU incorporation assay. Apoptosis-related and cell cycle-related gene and protein expression levels were monitored by qPCR and western blotting. Pre-exposure to 50 Hz-EMFs for 12 h enhanced the antiproliferative effect of 5-FU in breast cancer cell line MCF-7 in a dose-dependent manner but not in normal human breast epithelial cell line MCF10A. Exposure to 50 Hz-EMFs had no effect on apoptosis and P53 expression of MCF-7 and MCF10A cells, whereas it promoted DNA synthesis, induced entry of MCF-7 cells into the S phase of cell cycle, and upregulated the expression levels of cell cycle-related proteins Cyclin D1 and Cyclin E. Considering the pharmacological mechanisms of 5-FU in specifically disrupting DNA synthesis, this enhanced

inhibitory effect might have resulted from the specific sensitivity of MCF7 cells in active S phase to 5-FU. Our findings demonstrate the enhanced cytotoxic activity of 5-FU on MCF7 cells through promoting entry into the S phase of the cell cycle via exposure to 50 Hz-EMFs, which provides a novel method of cancer treatment based on the combinatorial use of 50 Hz-EMFs and chemotherapy.

(E) He Z, Selvamurugan N, Warshaw J, Partridge NC. Pulsed electromagnetic fields inhibit human osteoclast formation and gene expression via osteoblasts. Bone 2018 Jan;106:194-203. (VT, LE, GE)

Pulsed electromagnetic fields (PEMFs) can be effective in promoting the healing of delayed union or nonunion fractures. We previously reported that PEMF (Spinal-Stim® by Orthofix, Inc., Lewisville, TX) stimulated proliferation, differentiation and mineralization of rat calvarial osteoblastic cells in culture. In the present work we investigated the effects of PEMF (Physio-Stim® by Orthofix, Inc., Lewisville, TX) on human bone marrow macrophages (hBMMs) differentiated to osteoclasts. PEMF had striking inhibitory effects on formation of osteoclasts from hBMMs from both younger and older women. There were significantly greater changes in gene expression as ascertained by RNAseq from cells from older women. Interestingly, all of the genes identified by RNAseq were upregulated, and all were genes of mesenchymal or osteoblastic cells and included members of the TGF- β signaling pathway and many extracellular matrix proteins, as well as RANKL and osteoprotegerin, indicating the mixed nature of these cultures. From these results, we suggest that PEMF can inhibit osteoclast formation via action on osteoblasts. Thus, PEMF may be very effective for bone mass maintenance in subjects with osteoporosis.

(E) Heidari S, Abdi S, Karizi SZ Evaluation of BCL2 and Its Regulatory MIRS, MIR-15-B and MIR-16 Expression Changes Under the Exposure of Extremely Low-Frequency Electromagnetic Fields on Human Gastric Cancer Cell Line. Radiat Prot Dosimetry 197(2):93-100, 2021. (VT, AE, GE)

In this research, changes in the expression of B-cell lymphoma 2 (BCL2), miR-15-b and miR-16 in human adenocarcinoma gastric cancer cell line (AGS) following the exposure to magnetic flux densities (MFDs) of 0.2 and 2 mT continuously and discontinuously (1.5 h on/1.5 h off) for 18 h were investigated. Changes in the cell viability were evaluated by the MTT assay. Real-time PCR was used to evaluate the expression changes of BCL2, miR-15-b and miR-16. The results showed that extremely low frequency electromagnetic field (ELF-EMF) could significantly reduce the viability of AGS cells in the continuous MFD of 2 mT. The BCL2 expression was significantly decreased following the exposure to continuous MFDs of 0.2 and 2 mT and discontinuous MFD of 2 mT. The expressions of miR-15-b and miR-16 were significantly increased in continuous and discontinuous MFD of 2 mT. According to the results, weak and moderate extremely low-frequency electromagnetic fields can change the expressions of BCL2,

(E) Helekar SA, Hambarde S, Ijare OB, Pichumani K, Baskin DS, Sharpe MA. Selective induction of rapid cytotoxic effect in glioblastoma cells by oscillating magnetic fields. J Cancer Res Clin Oncol 2021 Dec;147(12):3577-3589. (VT, AE, GE, CS)

Purpose: The mechanisms underlying anticancer effects of electromagnetic fields are poorly understood. An alternating electric field-generating therapeutic device called Optune™ device has been approved for the treatment of glioblastoma (GBM). We have developed a new device that generates oscillating magnetic fields (OMF) by rapid rotation of strong permanent magnets in specially designed patterns of frequency and timing and have used it to treat an end-stage recurrent GBM patient under an expanded access/compassionate use treatment protocol. Here, we ask whether OMF causes selective cytotoxic effects in GBM and whether it is through generation of reactive oxygen species (ROS). **Methods:** We stimulated patient derived GBM cells, lung cancer cells, normal human cortical neurons, astrocytes, and bronchial epithelial cells using OMF generators (oncoscillators) of our Oncomagnetic Device and compared the results to those obtained under unstimulated or sham-stimulated control conditions. Quantitative fluorescence microscopy was used to assess cell morphology, viability, and ROS production mechanisms. **Results:** We find that OMF induces highly selective cell death of patient derived GBM cells associated with activation of caspase 3, while leaving normal tissue cells undamaged. The cytotoxic effect of OMF is also seen in pulmonary cancer cells. The underlying mechanism is a marked increase in ROS in the mitochondria, possibly in part through perturbation of the electron flow in the respiratory chain. **Conclusion:** Rotating magnetic fields produced by a new noninvasive device selectively kill cultured human glioblastoma and non-small cell lung cancer cells by raising intracellular reactive oxygen species, but not normal human tissue cells.

(E) Heredia-Rojas JA, Rodríguez de la Fuente AO, Alcocer González JM, Rodríguez-Flores LE, Rodríguez-Padilla C, Santoyo-Stephano MA, Castañeda-Garza E, Taméz-Guerra RS. Effect of 60 Hz magnetic fields on the activation of hsp70 promoter in cultured INER-37 and RMA E7 cells. In Vitro Cell Dev Biol Anim. 46(9):758-63, 2010. (VT, AE, GE, CS)

It has been reported that 50-60 Hz magnetic fields (MF) with flux densities ranging from microtesla to millitesla are able to induce heat shock factor or heat shock proteins in various cells. In this study, we investigated the effect of 60 Hz sinusoidal MF at 8 and 80 μ T on the expression of the luciferase gene contained in a plasmid labeled as electromagnetic field-plasmid (pEMF). This gene construct contains the specific sequences previously described for the induction of hsp70 expression by MF, as well as the reporter for the luciferase gene. The pEMF vector was transfected into INER-37 and RMA E7 cell lines that were later exposed to either MF or thermal shock (TS). Cells that received the MF or TS treatments and their controls were processed according to the luciferase assay system for evaluate luciferase activity. An increased luciferase gene expression was observed in INER-37 cells exposed to MF and TS compared with controls ($p < 0.05$), but MF exposure had no effect on the RMA E7 cell line.

(E) Hirai T, Taniura H, Goto Y, Ogura M, Sng JCG, Yoneda Y. Stimulation of ubiquitin-proteasome pathway through the expression of amidohydrolase for N-terminal asparagine (Ntan1) in cultured rat hippocampal neurons exposed to static magnetism. J Neurochem 96(6):1519-1530, 2006. (VT, AE, GE)

In order to elucidate mechanisms underlying modulation by static magnetism of the cellular functionality and/or integrity in the brain, we screened genes responsive to brief magnetism in cultured rat hippocampal neurons using differential display analysis. We have for the first time cloned and identified Ntan1 (amidohydrolase for N-terminal asparagine) as a magnetism responsive gene in rat brain. Ntan1 is an essential component of a protein degradation signal, which is a destabilizing N-terminal residue of a protein, in the N-end rule. In situ hybridization histochemistry revealed abundant expression of Ntan1 mRNA in hippocampal neurons in vivo. Northern blot analysis showed that Ntan1 mRNA was increased about three-fold after 3 h in response to brief magnetism. Brief magnetism also increased the transcriptional activity of Ntan1 promoter by luciferase reporter assay. Brief magnetism induced degradation of microtubule-associated protein 2 (MAP2) without affecting cell morphology and viability, which was prevented by a selective inhibitor of 26S proteasome in hippocampal neurons. Overexpression of Ntan1 using recombinant Ntan1 adenovirus vector resulted in a marked decrease in the MAP2 protein expression in hippocampal neurons. Our results suggest that brief magnetism leads to the induction of Ntan1 responsible for MAP2 protein degradation through ubiquitin-proteasome pathway in rat hippocampal neurons.

(E) Hong R, Zhang Y, Liu Y, Weng EQ. [Effects of extremely low frequency electromagnetic fields on DNA of testicular cells and sperm chromatin structure in mice] Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 23(6):414-417, 2005. (VO, LE, GT, RP)
[Article in Chinese]

OBJECTIVE: To study the effects of 50 Hz electromagnetic fields (EMFs) on DNA of testicular cells and sperm chromatin structure in mice. **METHODS:** Mice were exposed to 50 Hz, 0.2 mT or 6.4 mT electromagnetic fields for 4 weeks. DNA strand breakage in testicular cells was detected by single-cell gel electrophoresis assay. Sperm chromatin structure was analyzed by sperm chromatin structure assay with flow cytometry. **RESULTS:** After 50 Hz, 0.2 mT or 6.4 mT EMFs exposure, the percentage of cells with DNA migration in total testicular cells increased from the control level of 25.64% to 37.83% and 39.38% respectively. The relative length of comet tail and the percentage of DNA in comet tail respectively increased from the control levels of 13.06% +/- 12.38% and 1.52% +/- 3.25% to 17.86% +/- 14.60% and 2.32% +/- 4.26% after 0.2 mT exposure and to 17.88% +/- 13.71% and 2.35% +/- 3.87% after 6.4 mT exposure ($P < 0.05$). Exposure to EMFs had not induced significant changes in S.D.alphaT and XalphaT, but COMPalphaT (cells outside the main population of alpha t), the percentage of sperms with abnormal chromatin structure, increased in the two exposed groups. **CONCLUSION:** 50 Hz EMFs may have the potential to induce DNA strand breakage in testicular cells and sperm chromatin condensation in mice.

(NE) Huwiler SG, Beyer C, Fröhlich J, Hennecke H, Egli T, Schürmann D, Rehrauer H, Fischer HM. Genome-wide transcription analysis of Escherichia coli in response to extremely low-frequency magnetic fields. Bioelectromagnetics. 33(6):488-496, 2012. (VT, AE, GE)

The widespread use of electricity raises the question of whether or not 50 Hz (power line frequency in Europe) magnetic fields (MFs) affect organisms. We investigated the transcription of *Escherichia coli* K-12 MG1655 in response to extremely low-frequency (ELF) MFs. Fields generated by three signal types (sinusoidal continuous, sinusoidal intermittent, and power line intermittent; all at 50 Hz, 1 mT) were applied and gene expression was monitored at the transcript level using an Affymetrix whole-genome microarray. Bacterial cells were grown continuously in a chemostat (dilution rate $D = 0.4 \text{ h}^{-1}$) fed with glucose-limited minimal medium and exposed to 50 Hz MFs with a homogenous flux density of 1 mT. For all three types of MFs investigated, neither bacterial growth (determined using optical density) nor culturable counts were affected. Likewise, no statistically significant change (fold-change > 2 , $P \leq 0.01$) in the expression of 4,358 genes and 714 intergenic regions represented on the gene chip was detected after MF exposure for 2.5 h (1.4 generations) or 15 h (8.7 generations). Moreover, short-term exposure (8 min) to the sinusoidal continuous and power line intermittent signal neither affected bacterial growth nor showed evidence for reliable changes in transcription. In conclusion, our experiments did not indicate that the different tested MFs (50 Hz, 1 mT) affected the transcription of *E. coli*.

(E) Ilijin L, Mrdaković M, Todorović D, Vlahović M, Grčić A, Filipović A, Perić-Mataruga V. Biological effects of chronic exposure of *Blattica dubia* (Blattodea: Blaberidae) nymphs to static and extremely low frequency magnetic fields. *An Acad Bras Cienc* 2021 Jun 4;93(2):e20190118. (VO, LE, GE)

In this paper, we analyzed the effects of chronic exposure (5 months) to static magnetic field (110 mT; SMF) and extremely low frequency magnetic field (ELF MF; 10 mT, 50 Hz) on *Blattica dubia* nymphs. We have examined acetylcholinesterase (AChE) activity and heat shock protein 70 (HSP70) level, two sensitive biomarkers of stress in terrestrial insects. Relative growth rate (RGR), as a life history trait, was estimated. AChE activity was determined spectrophotometrically and HSP70 levels were quantified using indirect non-competitive ELISA and Western blotting. Calculated RGR was significantly changed upon exposure to both types of ambient MFs. The effects of chronic exposure of *B. dubia* nymphs to SMF and ELF MF (50 Hz) were observed as decreased activity of AChE. The increased level of HSP70 was present only after exposure to SMF. The strength of ELF MF was most likely below the energy level needed to induce the expression of this stress protein. Different patterns of the expression of two HSP70 isoforms, where isoform 2 was sensitive only to SMF, are most likely a possibly switch-off in the expression of constitutive and/or inducible HSP70 isoforms.

(E) Ivancsits S, Diem E, Pilger A, Rudiger HW, Jahn O. Induction of DNA strand breaks by intermittent exposure to extremely-low-frequency electromagnetic fields in human diploid fibroblasts. *Mutat Res.* 519(1-2):1-13, 2002. (VT, AE, GT, WS)

Results of epidemiological research show low association of electromagnetic field (EMF) with increased risk of cancerous diseases and missing dose-effect relations. An important component in assessing potential cancer risk is knowledge concerning any genotoxic effects of extremely-low-frequency-EMF (ELF-EMF). Human diploid fibroblasts were exposed to continuous or intermittent ELF-EMF (50Hz, sinusoidal, 24h, 1000microT).

For evaluation of genotoxic effects in form of DNA single- (SSB) and double-strand breaks (DSB), the alkaline and the neutral comet assay were used. In contrast to continuous ELF-EMF exposure, the application of intermittent fields reproducibly resulted in a significant increase of DNA strand break levels, mainly DSBs, as compared to non-exposed controls. The conditions of intermittence showed an impact on the induction of DNA strand breaks, producing the highest levels at 5min field-on/10min field-off. We also found individual differences in response to ELF-EMF as well as an evident exposure-response relationship between magnetic flux density and DNA migration in the comet assay. Our data strongly indicate a genotoxic potential of intermittent EMF. This points to the need of further studies in vivo and consideration about environmental threshold values for ELF exposure.

(E) Ivancsits S, Diem E, Jahn O, Rüdiger HW. Intermittent extremely low frequency electromagnetic fields cause DNA damage in a dose-dependent way. Int Arch Occup Environ Health. 76(6):431-436, 2003a. (VT, AE, GT)

Objectives: Epidemiological studies have reported an association between exposure to extremely low frequency electromagnetic fields (ELF-EMFs) and increased risk of cancerous diseases, albeit without dose-effect relationships. The validity of such findings can be corroborated only by demonstration of dose-dependent DNA-damaging effects of ELF-EMFs in cells of human origin in vitro. **Methods:** DNA damage was determined by alkaline and neutral comet assay. **Results:** ELF-EMF exposure (50 Hz, sinusoidal, 1-24 h, 20-1,000 μ T, 5 min on/10 min off) induced dose-dependent and time-dependent DNA single-strand and double-strand breaks. Effects occurred at a magnetic flux density as low as 35 μ T, being well below proposed International Commission of Non-Ionising Radiation Protection (ICNIRP) guidelines. After termination of exposure the induced comet tail factors returned to normal within 9 h. **Conclusion:** The induced DNA damage is not based on thermal effects and arouses concern about environmental threshold limit values for ELF exposure.

(E) Ivancsits S, Diem E, Jahn O, Rudiger HW. Age-related effects on induction of DNA strand breaks by intermittent exposure to electromagnetic fields. Mech Ageing Dev. 124(7):847-850, 2003b. (VT, AE, GT)

Several studies indicating a decline of DNA repair efficiency with age raise the question, if senescence per se leads to a higher susceptibility to DNA damage upon environmental exposures. Cultured fibroblasts of six healthy donors of different age exposed to intermittent ELF-EMF (50 Hz sinus, 1 mT) for 1-24 h exhibited different basal DNA strand break levels correlating with age. The cells revealed a maximum response at 15-19 h of exposure. This response was clearly more pronounced in cells from older donors, which could point to an age-related decrease of DNA repair efficiency of ELF-EMF induced DNA strand breaks.

(E) Ivancsits S, Pilger A, Diem E, Jahn O, Rudiger HW. Cell type-specific genotoxic effects of intermittent extremely low-frequency electromagnetic fields. Mutat Res.

583(2):184-188, 2005. (VT, AE, GT, CS)

The issue of adverse health effects of extremely low-frequency electromagnetic fields (ELF-EMFs) is highly controversial. Contradictory results regarding the genotoxic potential of ELF-EMF have been reported in the literature. To test whether this controversy might reflect differences between the cellular targets examined we exposed cultured cells derived from different tissues to an intermittent ELF-EMF (50 Hz sinusoidal, 1 mT) for 1-24h. The alkaline and neutral comet assays were used to assess ELF-EMF-induced DNA strand breaks. We could identify three responder (human fibroblasts, human melanocytes, rat granulosa cells) and three non-responder cell types (human lymphocytes, human monocytes, human skeletal muscle cells), which points to the significance of the cell system used when investigating genotoxic effects of ELF-EMF.

(E)Jajte J, Zmyslony M, Palus J, Dziubaltowska E, Rajkowska E. Protective effect of melatonin against in vitro iron ions and 7 mT 50 Hz magnetic field-induced DNA damage in rat lymphocytes. Mutat Res. 483(1-2):57-64, 2001. (VT, AE, GT, OX)

We have previously shown that simultaneous exposure of rat lymphocytes to iron ions and 50Hz magnetic field (MF) caused an increase in the number of cells with DNA strand breaks. Although the mechanism of MF-induced DNA damage is not known, we suppose that it involves free radicals. In the present study, to confirm our hypothesis, we have examined the effect of melatonin, an established free radicals scavenger, on DNA damage in rat peripheral blood lymphocytes exposed in vitro to iron ions and 50Hz MF. The alkaline comet assay was chosen for the assessment of DNA damage. During preincubation, part of the cell samples were supplemented with melatonin (0.5 or 1.0mM). The experiments were performed on the cell samples incubated for 3h in Helmholtz coils at 7mT 50Hz MF. During MF exposure, some samples were treated with ferrous chloride (FeCl₂, 10microg/ml), while the rest served as controls. A significant increase in the number of cells with DNA damage was found only after simultaneous exposure of lymphocytes to FeCl₂ and 7mT 50Hz MF, compared to the control samples or those incubated with FeCl₂ alone. However, when the cells were treated with melatonin and then exposed to iron ions and 50Hz MF, the number of damaged cells was significantly reduced, and the effect depended on the concentration of melatonin. The reduction reached about 50% at 0.5mM and about 100% at 1.0mM. Our results indicate that melatonin provides protection against DNA damage in rat lymphocytes exposed in vitro to iron ions and 50Hz MF (7mT). Therefore, it can be suggested that free radicals may be involved in 50Hz magnetic field and iron ions-induced DNA damage in rat blood lymphocytes. The future experimental studies, in vitro and in vivo, should provide an answer to the question concerning the role of melatonin in the free radical processes in the power frequency magnetic field.

(E) Jedrzejczak-Silicka M, Kordas M, Konopacki M, Rakoczy R. Modulation of Cellular Response to Different Parameters of the Rotating Magnetic Field (RMF)-An In Vitro Wound Healing Study. Int J Mol Sci 2021 May 28;22(11):5785. (VT, AE, GE, CS)

Since the effect of MFs (magnetic fields) on various biological systems has been studied, different results have been obtained from an insignificant effect of weak MFs on the disruption of the circadian clock system. On the other hand, magnetic fields, electromagnetic fields, or electric fields are used in medicine. The presented study was conducted to determine whether a low-frequency RMF (rotating magnetic field) with different field parameters could evoke the cellular response in vitro and is possible to modulate the cellular response. The cellular metabolic activity, ROS and Ca²⁺ concentration levels, wound healing assay, and gene expression analyses were conducted to evaluate the effect of RMF. It was shown that different values of magnetic induction (*B*) and frequency (*f*) of RMF evoke a different response of cells, e.g., increase in the general metabolic activity may be associated with the increasing of ROS levels. The lower intracellular Ca²⁺ concentration (for 50 Hz) evoked the inability of cells to wound closure. It can be stated that the subtle balance in the ROS level is crucial in the wound for the effective healing process, and it is possible to modulate the cellular response to the RMF in the context of an in vitro wound healing.

(E) Jeong H, Jo Y, Yoon M, Hong S. Thymidine decreases the DNA damage and apoptosis caused by tumor-treating fields in cancer cell lines. Genes Genomics 2021 Sep;43(9):995-1001. (VT, AE, GT, GE)

Background: Tumor-treating fields (TTFields) is an emerging non-invasive cancer-treatment modality using alternating electric fields with low intensities and an intermediate range of frequency. TTFields affects an extensive range of charged and polarizable cellular factors known to be involved in cell division. However, it causes side-effects, such as DNA damage and apoptosis, in healthy cells. **Objective:** To investigate whether thymidine can have an effect on the DNA damage and apoptosis, we arrested the cell cycle of human glioblastoma cells (U373) at G1/S phase by using thymidine and then exposed these cells to TTFields. **Methods:** Cancer cell lines and normal cell (HaCaT) were arrested by thymidine double block method. Cells were seeded into the gap of between the insulated wires. The exposed in alternative electric fields at 120 kHz, 1.2 V/cm. They were counted the cell numbers and analyzed for cancer malignant such as colony formation, Annexin V/PI staining, γ H2AX and RT-PCR. **Results:** The colony-forming ability and DNA damage of the control cells without thymidine treatment were significantly decreased, and the expression levels of BRCA1, PCNA, CDC25C, and MAD2 were distinctly increased. Interestingly, however, cells treated with thymidine did not change the colony formation, apoptosis, DNA damage, or gene expression pattern. **Conclusions:** These results demonstrated that thymidine can inhibit the TTFields-caused DNA damage and apoptosis, suggesting that combining TTFields and conventional treatments, such as chemotherapy, may enhance prognosis and decrease side effects compared with those of TTFields or conventional treatments alone.

(NE) Jin H, Yoon HE, Lee JS, Kim JK, Myung SH, Lee YS. Effects on g2/m phase cell cycle distribution and aneuploidy formation of exposure to a 60 Hz electromagnetic field in

combination with ionizing radiation or hydrogen peroxide in L132 nontumorigenic human lung epithelial cells. Korean J Physiol Pharmacol. 19(2):119-124, 2015. (VT, AE, GT, IX)

The aim of the present study was to assess whether exposure to the combination of an extremely low frequency magnetic field (ELF-MF; 60 Hz, 1 mT or 2 mT) with a stress factor, such as ionizing radiation (IR) or H₂O₂, results in genomic instability in non-tumorigenic human lung epithelial L132 cells. To this end, the percentages of G2/M-arrested cells and aneuploid cells were examined. Exposure to 0.5 Gy IR or 0.05 mM H₂O₂ for 9 h resulted in the highest levels of aneuploidy; however, no cells were observed in the subG1 phase, which indicated the absence of apoptotic cell death. Exposure to an ELF-MF alone (1 mT or 2 mT) did not affect the percentages of G2/M-arrested cells, aneuploid cells, or the populations of cells in the subG1 phase. Moreover, when cells were exposed to a 1 mT or 2 mT ELF-MF in combination with IR (0.5 Gy) or H₂O₂ (0.05 mM), the ELF-MF did not further increase the percentages of G2/M-arrested cells or aneuploid cells. These results suggest that ELF-MFs alone do not induce either G2/M arrest or aneuploidy, even when administered in combination with different stressors.

(NE) Jin YB, Kang GY, Lee JS, Choi JI, Lee JW, Hong SC, Myung SH, Lee YS. Effects on micronuclei formation of 60-Hz electromagnetic field exposure with ionizing radiation, hydrogen peroxide, or c-Myc overexpression. Int J Radiat Biol. 88(4):374-380, 2012. (V, AE, GT, IX) (noncancerous cells)

PURPOSE: Epidemiological studies have demonstrated a possible correlation between exposure to extremely low-frequency magnetic fields (ELF-MF) and cancer. However, this correlation has yet to be definitively confirmed by epidemiological studies. The principal objective of this study was to assess the effects of 60 Hz magnetic fields in a normal cell line system, and particularly in combination with various external factors, via micronucleus (MN) assays. **MATERIALS AND METHODS:** Mouse embryonic fibroblast NIH3T3 cells and human lung fibroblast WI-38 cells were exposed for 4 h to a 60 Hz, 1 mT uniform magnetic field with or without ionizing radiation (IR, 2 Gy), H₂O₂ (100 μM) and cellular myelocytomatosis oncogene (c-Myc) activation. **RESULTS:** The results obtained showed no significant differences between the cells exposed to ELF-MF alone and the unexposed cells. Moreover, no synergistic effects were observed when ELF-MF was combined with IR, H₂O₂, and c-Myc activation. **CONCLUSIONS:** Our results demonstrate that ELF-MF did not enhance MN frequency by IR, H₂O₂ and c-Myc activation.

(NE) Jin YB, Choi SH, Lee JS, Kim JK, Lee JW, Hong SC, Myung SH, Lee YS. Absence of DNA damage after 60-Hz electromagnetic field exposure combined with ionizing radiation, hydrogen peroxide, or c-Myc overexpression. Radiat Environ Biophys. 53(1):93-101. 2014. (VT, AE, GT, IX)

The principal objective of this study was to assess the DNA damage in a normal cell line system after exposure to 60 Hz of extremely low frequency magnetic field (ELF-MF) and particularly in combination with various external factors, via comet assays. NIH3T3 mouse fibroblast cells, WI-38 human lung fibroblast cells, L132 human lung epithelial cells, and MCF10A human mammary gland epithelial cells were exposed for 4 or 16 h to a 60-Hz, 1 mT uniform magnetic

field in the presence or absence of ionizing radiation (IR, 1 Gy), H₂O₂ (50 μM), or c-Myc oncogenic activation. The results obtained showed no significant differences between the cells exposed to ELF-MF alone and the unexposed cells. Moreover, no synergistic or additive effects were observed after 4 or 16 h of pre-exposure to 1 mT ELF-MF or simultaneous exposure to ELF-MF combined with IR, H₂O₂, or c-Myc activation.

(E) Jin Y, Guo W, Hu X, Liu M, Xu X, Hu F, Lan Y, Lv C, Fang Y, Liu M, Shi T, Ma S, Fang Z, Huang J. Static magnetic field regulates Arabidopsis root growth via auxin signaling. Sci Rep. 9(1):14384, 2019. (VO, LE, GE)

Static magnetic field (SMF) plays important roles in biological processes of many living organisms. In plants, however, biological significance of SMF and molecular mechanisms underlying SMF action remain largely unknown. To address these questions, we treated Arabidopsis young seedlings with different SMF intensities and directions. Magnetic direction from the north to south pole was adjusted in parallel (N0) with, opposite (N180) and perpendicular to the gravity vector. We discovered that root growth is significantly enhanced by 600 mT treatments except for N180, but not by any 300 mT treatments. N0 treatments lead to more active cell division of the meristem, and higher auxin content that is regulated by coordinated expression of PIN3 and AUX1 in root tips. Consistently, N0-promoted root growth disappears in pin3 and aux1 mutants. Transcriptomic and gene ontology analyses revealed that in roots 85% of the total genes significantly down-regulated by N0 compared to untreated are enriched in plastid biological processes, such as metabolism and chloroplast development. Lastly, no difference in root length is observed between N0-treated and untreated roots of the double cryptochrome mutant cry1 cry2. Taken together, our data suggest that SMF-regulated root growth is mediated by CRY and auxin signaling pathways in Arabidopsis.

(E) Jouni FJ, Abdolmaleki P, Ghanati F. Oxidative stress in broad bean (Vicia faba L.) induced by static magnetic field under natural radioactivity. Mutat Res. 741(1-2):116-121, 2012. (VT, LE, GT, OX, IX)

The investigation was performed to evaluate the influence of the static magnetic field on oxidative stress in Vicia faba cultivated in soil from high background natural radioactivity in Iran. Soil samples were collected from Ramsar, Iran where the annual radiation absorbed dose from background radiation is substantially higher than 20 mSv/year. The soil samples were then divided into 2 separate groups including high and low natural radioactivity. The plants were continuously exposed to static magnetic field of 15 mT for 8 days, each 8h/day. The results showed that in the plants cultivated in soils with high background natural radioactivity and low background natural radioactivity the activity of antioxidant enzymes as well as flavonoid content were lower than those of the control. Treatment of plants with static magnetic field showed similar results in terms of lowering of antioxidant defense system and increase of peroxidation of membrane lipids. Accumulation of ROS also resulted in chromosomal aberration and DNA damage. This phenomenon was more pronounced when a combination of natural radiation and treatment with static magnetic field was applied. The results suggest that exposure to static magnetic field causes accumulation of reactive oxygen species in V. faba and natural radioactivity of soil exaggerates oxidative stress.

(E) Kazemi M, Sahraei H, Aliyari H, Tekieh E, Saberi M, Tavacoli H, Meftahi GH, Ghanaati H, Salehi M, Hajnasrollah M. Effects of the Extremely Low Frequency Electromagnetic Fields on NMDA-Receptor Gene Expression and Visual Working Memory in Male Rhesus Macaques. Basic Clin Neurosci. 9(3):167-176, 2018. (AS, CE, GE)

INTRODUCTION: The present research aimed to examine Visual Working Memory (VWM) test scores, as well as hormonal, genomic, and brain anatomic changes in the male rhesus macaques exposed to Extremely Low Frequency Magnetic Field (ELF-MF). **METHODS:** Four monkeys were exposed to two different ELF-MF frequencies: 1 Hz (control) and 12 Hz (experiment) with 0.7 μ T (magnitude) 4 h/d for 30 consecutive days. Before and after the exposure, VWM test was conducted using a coated device on a movable stand. About 10 mL of the animals' blood was obtained from their femoral vein and used to evaluate their melatonin concentration. Blood lymphocytes were used for assaying the expressions of N-Methyl-D-aspartate NMDA-receptor genes expression before and after ELF exposure. Anatomical changes of hippocampus size were also assessed using MRI images. **RESULTS:** Results indicated that VWM scores in primates exposed to 12 Hz frequency ELF increased significantly. Plasma melatonin level was also increased in these animals. However, these variables did not change in the animals exposed to 1 Hz ELF. At last, expression of the NMDA receptors increased at exposure to 12 Hz frequency. However, hippocampal volume did not increase significantly in the animals exposed to both frequencies. **CONCLUSION:** In short, these results indicate that ELF (12 Hz) may have a beneficial value for memory enhancement (indicated by the increase in VWM scores). This may be due to an increase in plasma melatonin and or expression of NMDA glutamate receptors. However, direct involvement of the hippocampus in this process needs more research.

(E) Kesari KK, Luukkonen J, Juutilainen J, Naarala J. Genomic instability induced by 50 Hz magnetic fields is a dynamically evolving process not blocked by antioxidant treatment. Mutat Res Genet Toxicol Environ Mutagen 794:46-51, 2015. (VT, AE, GT, OX)

Increased level of micronuclei was observed in SH-SY5Y cells in a previous study at 8 and 15 days after exposure to extremely low frequency (ELF) magnetic fields (MF), indicating possible induction of genomic instability in the progeny of the exposed cells. The aim of this study was to further explore the induction of genomic instability by ELF MFs by increasing the follow-up time up to 45 days after exposure. Human SH-SY5Y neuroblastoma cells were exposed to a 50Hz, 100 μ T MF for 24h with or without co-exposure to menadione (MQ), a chemical agent that increases cellular superoxide production. Micronuclei, reactive oxygen species (ROS) and lipid peroxidation (LPO) were measured at 15, 30 and 45 days after exposure. To study the possible causal role of ROS in the delayed effects of MF, the antioxidant N-acetylcysteine (NAC) was administered before MF exposure. Consistently with the previous study, the level of micronuclei was statistically significantly elevated 15 days after exposure. A similar effect was observed at 30 days, but not at 45 days after exposure. The level of LPO was statically significantly decreased 30 and 45 days after exposure. Consistently with our previous findings, the MF effect did not depend on co-exposure to MQ. Treatment with NAC effectively decreased cellular ROS

level and suppressed the effect of MQ on ROS, but it did not block the MF effect, indicating that increase in ROS is not needed as a causal link between MF exposure and induction of delayed effects. The results presented here are consistent with genomic instability that persists in the progeny of MF-exposed cells up to at least 30 days after exposure. Changes in LPO observed at 30 and 45 days after exposure indicates that the MF-initiated process may continue up to at least 45 days after exposure.

(E) Kesari KK, Juutilainen J, Luukkonen J, Naarala J. Induction of micronuclei and superoxide production in neuroblastoma and glioma cell lines exposed to weak 50 Hz magnetic fields. J R Soc Interface. 13(114):20150995, 2016. (VT, AE, GT, LI)

Extremely low-frequency (ELF) magnetic fields (MF) have been associated with adverse health effects in epidemiological studies. However, there is no known mechanism for biological effects of weak environmental MFs. Previous studies indicate MF effects on DNA integrity and reactive oxygen species, but such evidence is limited to MFs higher (greater than or equal to 100 μ T) than those generally found in the environment. Effects of 10 and 30 μ T fields were studied in SH-SY5Y and C6 cells exposed to 50-Hz MFs for 24 h. Based on earlier findings, menadione (MQ) was used as a cofactor. Responses to MF were observed in both cell lines, but the effects differed between the cell lines. Micronuclei were significantly increased in SH-SY5Y cells at 30 μ T. This effect was largest at the highest MQ dose used. Increased cytosolic and mitochondrial superoxide levels were observed in C6 cells. The effects on superoxide levels were independent of MQ, enabling further mechanistic studies without co-exposure to MQ. The micronucleus and mitochondrial superoxide data were consistent with a conventional rising exposure-response relationship. For cytosolic superoxide, the effect size was unexpectedly large at 10 μ T. The results indicate that the threshold for biological effects of ELF MFs is 10 μ T or less.

(E) Khalil AM, Qassem W. Cytogenetic effects of pulsing electromagnetic fields on human lymphocytes in vitro: Chromosome aberrations, sister-chromatid exchanges and cell kinetics. Mutat Res 247:141–146, 1991. (VT, AE, GT)

Exposure of human lymphocyte cultures to a pulsing electromagnetic field (PEMF; 50 Hz, 1.05 mT) for various durations (24, 48 and 72 h) resulted in a statistically significant suppression of mitotic activity and a higher incidence of chromosomal aberrations. Furthermore, the shorter exposure times (24 and 48 h) did not cause a significant delay in cell turnover (cell proliferation index) or an increase in the baseline frequency of sister-chromatid exchanges (SCE). However, cultures continuously exposed to PEMF for 72 h exhibited significant reduction of the cell proliferation index (CPI) and an elevation of SCE rate. These results suggest that exposure to PEMF may induce a type of DNA lesions that lead to chromosomal aberrations and cell death but not to SCE, except probably at longer exposure times.

(E) Ki GE, Kim YM, Lim HM, Lee EC, Choi YK, Seo YK. Extremely low-frequency electromagnetic fields increase the expression of anagen-related molecules in human dermal papilla cells via GSK-3 β /ERK/Akt signaling pathway. Int J Mol Sci 21(3):784, 2020. (VT, LE, GE)

Despite advances in medical treatments, the proportion of the population suffering from alopecia is increasing, thereby creating a need for new treatments to control hair loss and prevent balding. Human hair follicle dermal papilla cells (hDPCs), a type of specialized fibroblast in the hair bulb, play an essential role in controlling hair growth and in conditions like androgenic alopecia. This study aimed to evaluate the intensity-dependent effect of extremely low-frequency electromagnetic fields (ELF-EMFs) on the expression of anagen-related molecules in hDPCs in vitro. We examined the effect of ELF-EMF on hDPCs to determine whether activation of the GSK-3 β /ERK/Akt signaling pathway improved hDPC activation and proliferation; hDPCs were exposed to ELF-EMFs at a frequency of 70 Hz and at intensities ranging from 5 to 100 G, over four days. Various PEMF intensities significantly increased the expression of anagen-related molecules, including collagen IV, laminin, ALP, and versican. In particular, an intensity of 10 G is most potent for promoting the proliferation of hDPC and expression of anagen-related molecules. Moreover, 10 G ELF-EMF significantly increased β -catenin and Wnt3 α expression and GSK-3 β /ERK/Akt phosphorylation. Our results confirmed that ELF-EMFs enhance hDPC activation and proliferation via the GSK-3 β /ERK/Akt signaling pathway, suggesting a potential treatment strategy for alopecia.

(E) Kim HJ, Jung J, Park JH, Kim JH, Ko KN, Kim CW. Extremely low-frequency electromagnetic fields induce neural differentiation in bone marrow derived mesenchymal stem cells. Exp Biol Med (Maywood). 238(8):923-931, 2013. (VT, AE, GT) (medical application)

Extremely low-frequency electromagnetic fields (ELF-EMF) affect numerous biological functions such as gene expression, cell fate determination and even cell differentiation. To investigate the correlation between ELF-EMF exposure and differentiation, bone marrow derived mesenchymal stem cells (BM-MSCs) were subjected to a 50-Hz electromagnetic field during in vitro expansion. The influence of ELF-EMF on BM-MSCs was analysed by a range of different analytical methods to understand its role in the enhancement of neural differentiation. ELF-EMF exposure significantly decreased the rate of proliferation, which in turn caused an increase in neuronal differentiation. The ELF-EMF-treated cells showed increased levels of neuronal differentiation marker (MAP2), while early neuronal marker (Nestin) was down-regulated. In addition, eight differentially expressed proteins were detected in two-dimensional electrophoresis maps, and were identified using ESI-Q-TOF LC/MS/MS. Among them, ferritin light chain, thioredoxin-dependent peroxide reductase, and tubulin β -6 chain were up-regulated in the ELF-EMF-stimulated group. Ferritin and thioredoxin-dependent peroxide reductase are involved in a wide variety of functions, including Ca(2+)

regulation, which is a critical component of neurodegeneration. We also observed that the intracellular Ca(2+) content was significantly elevated after ELF-EMF exposure, which strengthens the modulatory role of ferritin and thioredoxin-dependent peroxide reductase, during differentiation. Notably, western blot analysis indicated significantly increased expression of the ferritin light chain in the ELF-EMF-stimulated group (0.60 vs. 1.08; $P < 0.01$). These proteins may help understand the effect of ELF-EMF stimulation on BM-MSCs during neural differentiation and its potential use as a clinically therapeutic option for treating neurodegenerative diseases.

(E) Kim J, Ha CS, Lee HJ, Song K. Repetitive exposure to a 60-Hz time-varying magnetic field induces DNA double-strand breaks and apoptosis in human cells. Biochem Biophys Res Commun. 400(4):739-744, 2010. (VT, LE, GT, GE)

We investigated the effects of extremely low frequency time-varying magnetic fields (MFs) on human normal and cancer cells. Whereas a single exposure to a 60-Hz time-varying MF of 6 mT for 30min showed no effect, repetitive exposure decreased cell viability. This decrease was accompanied by phosphorylation of γ -H2AX, a common DNA double-strand break (DSB) marker, and checkpoint kinase 2 (Chk2), which is critical to the DNA damage checkpoint pathway. In addition, repetitive exposure to a time-varying MF of 6 mT for 30 min every 24 h for 3 days led to p38 activation and induction of apoptosis in cancer and normal cells. Therefore, these results demonstrate that repetitive exposure to MF with extremely low frequency can induce DNA DSBs and apoptosis through p38 activation. These results also suggest the need for further evaluation of the effects of repetitive exposure to environmental time-varying MFs on human health.

(E) Kim J, Yoon Y, Yun S, Park GS, Lee HJ, Song K. Time-varying magnetic fields of 60 Hz at 7 mT induce DNA double-strand breaks and activate DNA damage checkpoints without apoptosis. Bioelectromagnetics. 33(5):383-393, 2012. (VT, AE, GT)

The potential genotoxic effect of a time-varying magnetic field (MF) on human cells was investigated. Upon continuous exposure of human primary fibroblast and cervical cancer cells to a 60 Hz MF at 7 mT for 10-60 min, no significant change in cell viability was observed. However, deoxyribonucleic acid (DNA) double-strand breaks (DSBs) were detected, and the DNA damage checkpoint pathway was activated in these cells without programmed cell death (called apoptosis). The exposure of human cells to a 60 Hz MF did not induce intracellular reactive oxygen species (ROS) production, suggesting that the observed DNA DSBs are not directly caused by ROS. We also compared the position and time dependency of DNA DSBs with numerical simulation of MFs. The Lorentz force and eddy currents in these experiments were numerically calculated to investigate the influence of each factor on DNA DSBs. The DNA DSBs mainly occurred at the central region, where the MF was strongest, after a 30-min exposure. After 90 min, however, the amount of DNA DSBs increased rapidly in the outer regions, where the eddy current and Lorentz force were strong.

(E) Kimsa-Dudek M, Synowiec-Wojtarowicz A, Derewniuk M, Gawron S, Paul-Samojedny M, Kruszniewska-Rajs C, Pawłowska-Góral K. Impact of fluoride and a static magnetic

field on the gene expression that is associated with the antioxidant defense system of human fibroblasts. Chem Biol Interact. 287:13-19, 2018. (VT, AE, GE, IX)

Fluoride cytotoxicity has been associated with apoptosis, oxidative stress, general changes in DNA and RNA and protein biosynthesis, whereas the results of studies on the effect of SMF on antioxidant activity of cells are contradictory. Therefore, the aim of our study was to evaluate the simultaneous exposure of human cells to fluoride SMF that are generated by permanent magnets on the expression profile of the genes that are associated with the antioxidant defense system. Control fibroblasts and fibroblasts that had been treated with fluoride were subjected to the influence of SMF with a moderate induction. In order to achieve our aims, we applied modern molecular biology techniques such as the oligonucleotide microarray. Among the antioxidant defense genes, five (SOD1, PLK3, CLN8, XPA, HAO1), whose expression was significantly altered by the action of fluoride ions and the exposure to SMF were normalized their expression was identified. We showed that fluoride ions cause oxidative stress, whereas exposure to SMF with a moderate induction can suppress their effects by normalizing the expression of the genes that are altered by fluoride. Our research may explain the molecular mechanisms of the influence of fluoride and SMF that are generated by permanent magnets on cells.

(E) Kimsa-Dudek M, Synowiec-Wojtarowicz A, Krawczyk A, Kruszniewska-Rajs C, Gawron S, Paul-Samojedny M, Gola J. Anti-apoptotic effect of a static magnetic field in human cells that had been treated with sodium fluoride. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2020 Jun 25:1-8.(VT, AE, GE, IX)

Static magnetic field (SMF) is widely used in industry, in consumer devices and diagnostic medical equipment, hence the widespread exposure to SMF in the natural environment and in people occupationally exposed to it. In environment and in some workplaces, there is a risk of exposure also to various chemicals. Environmental factors can affect the cellular processes which can be the cause of the development of various pathological conditions. Therefore, the aim of this study was to assess the effect of SMF on the expression of the apoptosis-related genes in human fibroblast cultures that had been co-treated with fluoride ions. The control and NaF-treated cells were subjected to the influence of SMF with a moderate induction. The flow-cytometric analysis showed that the fluoride ions reduced the number of viable cells and induced early apoptosis. However, exposure to the SMF reduced the number of dead cells that had been treated with fluoride ions. Moreover, specific genes that were involved in apoptosis exhibited a differential expression in the NaF-treated cells and exposure to the SMF yielded a modulation of their transcriptional activity. Our results suggest some beneficial properties of using a moderate-intensity static magnetic field to reduce the adverse effects of fluoride.

(E) Kimsa-Dudek M, Synowiec-Wojtarowicz A, Krawczyk A, Kosowska A, Kimsa-Furdzik M, Francuz T. The Apoptotic Effect of Caffeic or Chlorogenic Acid on the C32 Cells That Have Simultaneously Been Exposed to a Static Magnetic Field. Int J Mol Sci 2022 Mar 31;23(7):3859. (VT, AE, GE, GT)

The induction of apoptosis is one of the main goals of the designed anti-cancer therapies. In recent years, increased attention has been paid to the physical factors such as magnetic fields and to the natural bioactive compounds and the possibilities using them in medicine. Hence, the aim

of this study was to evaluate the anti-tumor effect of caffeic or chlorogenic acid in combination with a moderate-strength static magnetic field on C32 melanoma cells by assessing the effect of both factors on the apoptotic process. The apoptosis of the C32 cells was evaluated using a flow cytometry analysis. The expression of the apoptosis-associated genes was determined using the RT-qPCR technique. The caspase activity and the concentration of the oxidative damage markers were also measured. It was found that phenolic acids and a static magnetic field trigger the apoptosis of the C32 cells and also affect the expression of the genes encoding the apoptosis regulatory proteins. In conclusion, our study indicated that both of the phenolic acids and a static magnetic field can be used supportively in the treatment of melanoma and that caffeic acid is more pro-apoptotic than chlorogenic acid.

(E) Kimura T, Takahashi K, Suzuki Y, Konishi Y, Ota Y, Mori C, Ikenaga T, Takanami T, Saito R, Ichiishi E, Awaji S, Watanabe K, Higashitani A. The effect of high strength static magnetic fields and ionizing radiation on gene expression and DNA damage in *Caenorhabditis elegans* Bioelectromagnetics. 29(8):605-614, 2008. (VO, AE, GE)

Magnetic resonance imaging with high static magnetic fields (SMFs) has become widely used for medical imaging purposes because SMFs cause fewer genotoxic side effects than ionizing radiation (IR). However, the effect of exposure to high SMFs on global transcription is little understood. We demonstrate that genes involved in motor activity, actin binding, cell adhesion, and cuticles are transiently and specifically induced following exposure to 3 or 5 T SMF in the experimental model metazoan *Caenorhabditis elegans*. In addition, transient induction of hsp12 family genes was observed after SMF exposure. The small-heat shock protein gene hsp16 was also induced but to a much lesser extent, and the LacZ-stained population of hsp-16.1::lacZ transgenic worms did not significantly increase after exposure to SMFs with or without a second stressor, mild heat shock. Several genes encoding apoptotic cell-death activators and secreted surface proteins were upregulated after IR, but were not induced by SMFs. Real-time quantitative RT-PCR analyses for 12 of these genes confirmed these expression differences between worms exposed to SMFs and IR. In contrast to IR, exposure to high SMFs did not induce DNA double-strand breaks or germline cell apoptosis during meiosis. These results suggest that the response of *C. elegans* to high SMFs is unique and capable of adjustment during long exposure, and that this treatment may be less hazardous than other therapeutic tools.

(E) Kindzelskii AL, Petty HR. Extremely low frequency pulsed DC electric fields promote neutrophil extension, metabolic resonance and DNA damage when phasematched with metabolic oscillators. Biochim Biophys Acta. 1495(1):90-111, 2000. (VT, AE, GT, OX)

Application of extremely low frequency pulsed DC electric fields that are frequency- and phase-matched with endogenous metabolic oscillations leads to greatly exaggerated neutrophil extension and metabolic resonance wherein oscillatory NAD(P)H amplitudes are increased. In the presence of a resonant field, migrating cell length grows from 10 to approximately 40 microm, as does the overall length of microfilament assemblies. In contrast, cells stop locomotion and become spherical when exposed to phase-mismatched

fields. Although cellular effects were not found to be dependent on electrode type and buffer, they were sensitive to temporal constraints (phase and pulse length) and cell surface charge. We suggest an electromechanical coupling hypothesis wherein applied electric fields and cytoskeletal polymerization forces act together to overcome the surface/cortical tension of neutrophils, thus promoting net cytoskeletal assembly and heightened metabolic amplitudes. Metabolic resonance enhances reactive oxygen metabolic production by neutrophils. Furthermore, cellular DNA damage was observed after prolonged metabolic resonance using both single cell gel electrophoresis ('comet' assay) and 3'-OH DNA labeling using terminal deoxynucleotidyl transferase. These results provide insights into transmembrane signal processing and cell interactions with weak electric fields.

(NE) Kirschenlohr H, Ellis P, Hesketh R, Metcalfe J. Gene expression profiles in white blood cells of volunteers exposed to a 50 Hz electromagnetic field. Radiat Res. 178(3): 138-149, 2012. (HU, AE, GE)

Consistent and independently replicated laboratory evidence to support a causative relationship between environmental exposure to extremely low-frequency electromagnetic fields (EMFs) at power line frequencies and the associated increase in risk of childhood leukemia has not been obtained. In particular, although gene expression responses have been reported in a wide variety of cells, none has emerged as robust, widely replicated effects. DNA microarrays facilitate comprehensive searches for changes in gene expression without a requirement to select candidate responsive genes. To determine if gene expression changes occur in white blood cells of volunteers exposed to an ELF-EMF, each of 17 pairs of male volunteers age 20-30 was subjected either to a 50 Hz EMF exposure of $62.0 \pm 7.1 \mu\text{T}$ for 2 h or to a sham exposure ($0.21 \pm 0.05 \mu\text{T}$) at the same time (11:00 a.m. to 13:00 p.m.). The alternative regime for each volunteer was repeated on the following day and the two-day sequence was repeated 6 days later, with the exception that a null exposure ($0.085 \pm 0.01 \mu\text{T}$) replaced the sham exposure. Five blood samples (10 ml) were collected at 2 h intervals from 9:00 to 17:00 with five additional samples during the exposure and sham or null exposure periods on each study day. RNA samples were pooled for the same time on each study day for the group of 17 volunteers that were subjected to the ELF-EMF exposure/sham or null exposure sequence and were analyzed on Illumina microarrays. Time courses for 16 mammalian genes previously reported to be responsive to ELF-EMF exposure, including immediate early genes, stress response, cell proliferation and apoptotic genes were examined in detail. No genes or gene sets showed consistent response profiles to repeated ELF-EMF exposures. A stress response was detected as a transient increase in plasma cortisol at the onset of either exposure or sham exposure on the first study day. The cortisol response diminished progressively on subsequent exposures or sham exposures, and was attributable to mild stress associated with the experimental protocol.

(E) Kozirowska A, Depciuch J, Bialek J, Woś I, Koziol K, Sadło S, Piechowicz B. Electromagnetic field of extremely low frequency has an impact on selected chemical components of the honeybee. Pol J Vet Sci 2020 Dec;23(4):537-544. (VO, AE, GT)

The electromagnetic field (EMF) is an environmental factor affecting living organisms. The aim of this study was to demonstrate the effect of an extremely low frequency electromagnetic field (ELF-EMF) on selected chemical components of the honeybee (*Apis mellifera* L.) using Fourier Transform Infrared (FTIR) spectroscopy. The FTIR method provides information on the chemical structure of compounds through identification and analysis of functional groups. The honeybees were treated with EMF at a frequency of 50 Hz and magnetic induction of 1.6 mT for 2, 6, 12, 24 and 48 hours. Analysis of FTIR spectra showed that EMF exposure longer than 2 hours induced changes in the structure of chemical compounds, especially in the IR region corresponding to DNA, RNA, phospholipids and protein vibrations, compared to control samples (bees not EMF treated). The results confirm the effect of EMF on bees depending on the duration of exposure.

(E) Kozłowska W, Drzewiecka EM, Paukzto L, Zmijewska A, Wydorski PJ, Jastrzebski JP, Franczak A. Exposure to the electromagnetic field alters the transcriptomic profile in the porcine endometrium during the peri-implantation period. J Physiol Pharmacol 2021 Dec;72(6). doi: 10.26402/jpp.2021.6.04. (VT, AE, GE)

A low-frequency electromagnetic field (EMF) is an environmental pollutant that may influence female reproduction. This research was undertaken to test the hypothesis that EMF causes alterations in the transcriptomic profile of the endometrium. This study investigated the in vitro effects of EMF treatment (50 Hz, 2 h) on global transcriptome alterations in the endometrium isolated from pigs during the peri-implantation period. The control endometrium was not treated with EMF. The EMF treatment altered the expression of 1561 transcriptionally active regions (TARs) in the endometrium. In the group of 461 evaluated DEGs, 156 were up-regulated (34%), 305 were down-regulated (66%) and 341 (74%) had known biological functions. A total of 210 long noncoding RNAs (lncRNAs) with changes in expression profiles, and 146 predicted RNA editing sites were also evaluated. Exposure to EMF changes the expression of genes encoding proteins that are involved in proliferation and metabolism in endometrial tissue. These results provide useful inputs for further research into the impact of EMF on molecular changes in the uterus during the peri-implantation period and, consequently, pregnancy outcome.

(E) Koyama S, Sakurai T, Nakahara T, Miyakoshi J. Extremely low frequency (ELF) magnetic fields enhance chemically induced formation of apurinic/aprimidinic (AP) sites in A172 cells. Int J Radiat Biol. 84(1):53-59, 2008. (VT, AE, GT, IX)

PURPOSE: To detect the effects of extremely low frequency (ELF) magnetic fields, the number of apurinic/aprimidinic (AP) sites in human glioma A172 cells was measured following exposure to ELF magnetic fields. **MATERIALS AND METHODS:** The cells were exposed to an ELF magnetic field alone, to genotoxic agents (methyl methane sulfonate (MMS) and hydrogen peroxide (H₂O₂)) alone, or to an ELF magnetic field with the genotoxic agents. After exposure, DNA was extracted, and the number of AP sites was measured. **RESULTS:** There was no difference in the number of AP sites between cells exposed to an ELF magnetic field and sham controls. With MMS or H₂O₂ alone, the number of AP sites increased with longer

treatment times. Exposure to an ELF magnetic field in combination with the genotoxic agents increased AP-site levels compared with the genotoxic agents alone. **CONCLUSIONS:** Our results suggest that the number of AP sites induced by MMS or H₂O₂ is enhanced by exposure to ELF magnetic fields at 5 millitesla (mT). This may occur because such exposure can enhance the activity or lengthen the lifetime of radical pairs.

(E) Kubinyi G, Zeitler Z, Thuróczy G, Juhász P, Bakos J, Sinay H, László J. Effects of homogeneous and inhomogeneous static magnetic fields combined with gamma radiation on DNA and DNA repair. *Bioelectromagnetics*. 31(6):488-494, 2010. (VT, AE, GT, IX)

The aim of this study was to reveal whether static magnetic fields (SMFs) influence the repair of radiation-damaged DNA on leukocytes or has any effect on DNA. After 4 Gy of (60)Co-gamma irradiation, some of the samples were exposed to inhomogeneous SMFs with a lateral magnetic flux density gradient of 47.7, 1.2, or 0.3 T/m by 10 mm lateral periodicity, while other samples were exposed to homogeneous SMF of 159.2 +/- 13.4 mT magnetic flux density for a time period of 0.5 min, 1, 2, 4, 6, 18, 20, or 24 h. Another set of samples was exposed to the aforementioned SMFs before gamma irradiation. The following three groups were examined: (i) exposed to SMF only, (ii) exposed to SMF following irradiation by (60)Co-gamma, and (iii) exposed to SMF before (60)Co-gamma irradiation. The analysis of the DNA damage was made by single-cell gel electrophoresis technique (comet assay). Statistically significant differences were found at 1 h (iSMF), 4 h (hSMF), and 18 h (hSMF) if samples were exposed to only SMF, compared to control. When the SMF exposure followed the (60)Co-gamma irradiation, statistically significant differences were found at 1 h (iSMF) and 4 h (hSMF). If exposure to SMF preceded (60)Co-gamma irradiation, no statistically significant difference was found compared to 4 Gy gamma-irradiated group.

(E) Kumari K, Koivisto H, Viluksela M, Paldanius KMA, Marttinen M, Hiltunen M, Naarala J, Tanila H, Juutilainen J. Behavioral testing of mice exposed to intermediate frequency magnetic fields indicates mild memory impairment. *PLoS One*. 2017 Dec 4;12(12):e0188880. (VO, LE, GE)

Human exposure to intermediate frequency magnetic fields (MF) is increasing due to applications like electronic article surveillance systems and induction heating cooking hobs. However, limited data is available on their possible health effects. The present study assessed behavioral and histopathological consequences of exposing mice to 7.5 kHz MF at 12 or 120 μ T for 5 weeks. No effects were observed on body weight, spontaneous activity, motor coordination, level of anxiety or aggression. In the Morris swim task, mice in the 120 μ T group showed less steep learning curve than the other groups, but did not differ from controls in their search bias in the probe test. The passive avoidance task indicated a clear impairment of memory over 48 h in the 120 μ T group. No effects on astroglial activation or neurogenesis were observed in the hippocampus. The mRNA expression of brain-derived neurotrophic factor did not change but expression of the proinflammatory cytokine tumor necrosis factor alpha mRNA was significantly increased in the 120 μ T group. These findings suggest that 7.5 kHz MF exposure may lead to

mild learning and memory impairment, possibly through an inflammatory reaction in the hippocampus.

(NE) Lacy-Hulbert, A, Wilkins, R.C., Hesketh, T.R., Metcalfe, J.C. (1995). No effect of 60 Hz electromagnetic fields on MYC or beta-actin expression in human leukemic cells. Rad. Res. 144:9-17. (VT, AE, GE)

Epidemiological studies have shown weak correlations between exposure to extremely low-frequency electromagnetic fields (ELF EMFs) and the incidence of several cancers, particularly childhood leukemias, although negative studies have also been reported. These observations have prompted a broad range of in vitro cellular studies in which effects of ELF EMFs have been observed. However, no reported response has been replicated widely in independent laboratories. One potentially important response is the rapid activation of proto-oncogenes and other genes in human leukemic (HL60) cells and a wide variety of other eukaryotic cells, because of the role of these genes in cell proliferation. We describe quantitative Northern analysis of MYC and beta-actin mRNAs from HL60 cells exposed to fields under conditions very similar to those reported previously to activate these genes, namely 60 Hz sinusoidal magnetic fields of 0.57, 5.7 or 57 microT for 20 min. In addition we have used a new design of field-exposure system and introduced a number of other modifications to the protocol to optimize any response. We have also developed a novel method providing enhanced accuracy for the quantitative measurement of mRNA. No significant effect of ELF EMFs on gene expression was observed using any of these systems and analytical methods.

(E) Lagroye I, Poncy JL. The effect of 50 Hz electromagnetic fields on the formation of micronuclei in rodent cell lines exposed to gamma radiation. Int J Radiat Biol. 72(2):249-254, 1997.(VT, AE, GT, IX)

Low frequency electromagnetic fields (EMF) do not produce enough energy to damage DNA, in contrast to ionizing radiations. Any relationship between increased incidence of cancer and EMF must therefore be explained by a promoting effect on cellular transformation by ionizing radiation. The aim of this study was to investigate using the cytokinesis-blocked micronucleus assay a possible amplification of the genotoxic effects of ionizing radiations in cells exposed to combined static and power-frequency electromagnetic fields. Rat tracheal epithelial cell lines were first exposed in vitro to ⁶⁰Co gamma rays (0, 2 and 6 Gy) and cells were then cultured for 24 h in a homogeneous sinusoidal 50 Hz magnetic field (flux density: 100 microTrms) combined with an artificial geomagnetic-like field created by the use of horizontal and vertical pairs of Helmholtz coils. Control cells were cultured in an adjacent incubator where the background EMF was about 0.1 microTrms. Under our in vitro experimental conditions, EMF appeared to have no significant direct effect on micronucleus induction in rat tracheal cell lines. However, an increased frequency of binucleated cells with micronuclei was observed in cells exposed to 6 Gy of gamma rays and EMF, compared with gamma irradiation alone. This could enhance radiation-induced genomic alterations and increase the probability of neoplastic transformation.

(E)Lai H, Singh NP. Acute exposure to a 60 Hz magnetic field increases DNA strand breaks in rat brain cells. Bioelectromagnetics. 18(2):156-165, 1997a. (VO, AE, GT)

Acute (2 h) exposure of rats to a 60 Hz magnetic field (flux densities 0.1, 0.25, and 0.5 mT) caused a dose-dependent increase in DNA strand breaks in brain cells of the animals (assayed by a microgel electrophoresis method at 4 h postexposure). An increase in single-strand DNA breaks was observed after exposure to magnetic fields of 0.1, 0.25, and 0.5 mT, whereas an increase in double-strand DNA breaks was observed at 0.25 and 0.5 mT. Because DNA strand breaks may affect cellular functions, lead to carcinogenesis and cell death, and be related to onset of neurodegenerative diseases, our data may have important implications for the possible health effects of exposure to 60 Hz magnetic fields.

(E) Lai H, Singh NP. Melatonin and N-tert-butyl-alpha-phenylnitronone block 60-Hz magnetic field-induced DNA single and double strand breaks in rat brain cells. J Pineal Res. 22(3):152-162, 1997b. (VO, AE, GT, OX)

In previous research, we have found an increase in DNA single- and double-strand breaks in brain cells of rats after acute exposure (two hours) to a sinusoidal 60-Hz magnetic field. The present experiment was carried out to investigate whether treatment with melatonin and the spin-trap compound N-tert-butyl-alpha-phenylnitronone (PBN) could block the effect of magnetic fields on brain cell DNA. Rats were injected with melatonin (1 mg/kg, sc) or PBN (100 mg/kg, ip) immediately before and after two hours of exposure to a 60-Hz magnetic field at an intensity of 0.5 mT. We found that both drug treatments blocked the magnetic field-induced DNA single- and double-strand breaks in brain cells, as assayed by a microgel electrophoresis method. Since melatonin and PBN are efficient free radical scavengers, these data suggest that free radicals may play a role in magnetic field-induced DNA damage.

(E) Lai H, Singh NP. Magnetic-field-induced DNA strand breaks in brain cells of the rat. Environ Health Perspect. 112(6):687-694, 2004. (VO, AE, GT, OX)

In previous research, we found that rats acutely (2 hr) exposed to a 60-Hz sinusoidal magnetic field at intensities of 0.1-0.5 millitesla (mT) showed increases in DNA single and double-strand breaks in their brain cells. Further research showed that these effects could be blocked by pretreating the rats with the free radical scavengers melatonin and N-tert-butyl-alpha-phenylnitronone, suggesting the involvement of free radicals. In the present study, effects of magnetic field exposure on brain cell DNA in the rat were further investigated. Exposure to a 60-Hz magnetic field at 0.01 mT for 24 hr caused a significant increase in DNA single- and double-strand breaks. Prolonging the exposure to 48 hr caused a larger increase. This indicates that the effect is cumulative. In addition, treatment with Trolox (a vitamin E analog) or 7-nitroindazole (a nitric oxide synthase inhibitor) blocked magnetic-field-induced DNA strand breaks. These data further support a role of free radicals on the effects of magnetic fields. Treatment with the iron chelator deferiprone also blocked the effects of magnetic fields on brain cell DNA, suggesting the involvement of iron. Acute magnetic field exposure increased apoptosis and necrosis of

brain cells in the rat. We hypothesize that exposure to a 60-Hz magnetic field initiates an iron-mediated process (e.g., the Fenton reaction) that increases free radical formation in brain cells, leading to DNA strand breaks and cell death. This hypothesis could have an important implication for the possible health effects associated with exposure to extremely low-frequency magnetic fields in the public and occupational environments.

(E) Laramee CB, Frisch P, McLeod K, Li GC. Elevation of heat shock gene expression from static magnetic field exposure in vitro. Bioelectromagnetics. 35(6):406-413, 2014. (VT, AE, GE)

Previously, we found that extremely low frequency (ELF) electric fields were able to elicit an approximate 3.5-fold increase in heat shock gene expression, a response which may have applicability to cancer therapy. Based on recent studies demonstrating the ability of magnetic fields to influence gene expression, we hypothesized that low level static magnetic fields may be able to affect heat shock gene expression while avoiding some of the clinical difficulties that arise with electric fields. Transfected rat primary cells in monolayer were exposed to magnetic fields of 1 to 440 mT for 16, 24, or 48 h starting at 24 and 48 h post transfection. Heat shock protein (HSP70) expression, as indicated by a promoter linked luciferase reporter, was followed for up to 96 h and showed a dependence on flux density, exposure duration, and start time post transfection. A nonlinear response was observed for increasing flux density with a maximum of a 3.5-fold increase in expression for 48 h of exposure starting 48 h after transfection. These results demonstrate an enhancement of gene expression similar in magnitude to that observed with external electric field exposure, while eliminating many of the clinical complications.

(E) Laubitz D, Jankowska A, Sikora A, Woliński J, Zabielski R, Grzesiuk E. Gut myoelectrical activity induces heat shock response in Escherichia coli and Caco-2 cells. Exp Physiol 91(5):867-875, 2006. (VT, AE, GE)

The heat shock response is associated with the intracellular expression of a number of highly conserved heat shock proteins (Hsps). According to their molecular size, Hsps have been divided into several groups, which are strongly conserved and show high homology between the species, e.g., Hsp70, MW 70 kDa (Lindquist & Craig, 1998; Morimoto, 1998; Jolly & Morimoto, 2000; Zyllicz et al. 2001). In all organisms the Hsp expression under stress conditions is regulated at transcriptional level, e.g., in humans by the heat shock transcription factor Hsf1 (Morimoto, 1998; Wu, 1995), while in Escherichia coli by replacement of the sigma factor sigma(70) in RNA polymerase by the sigma factor sigma(32) (Gross, 1987). The Hsps allow cell survival under stress conditions by renaturing of denatured proteins, protecting of stress-labile proteins, preventing protein aggregation (chaperone functions), and by degradation of damaged proteins (protease activities) (Lindquist & Craig, 1988; Morimoto, 1998; Jolly & Morimoto, 2000). They have also many housekeeping functions under non-stressful conditions during the cell cycle, growth, development, and differentiation (Morimoto, 1998). Among a number of plausible inducing factors already studied, extremely low artificial electromagnetic fields have been shown to induce stress response in various cells, such as expression of sigma(32) mRNA (Cairo et al. 1998) and induction of DnaJ and DnaK proteins in Escherichia coli (Chow & Tung, 2000); expression of hsp-16 gene in Caenorhabditis elegans (Miyakawa et al., 2001); induction

of heat shock transcription factor Hsf1 and Hsp70, Hsp90 and Hsp27 in human cells (Lin et al. 1997; Lin et al. 1998; Goodman & Blank, 1998; Pipkin et al. 1999). Nevertheless, the role of endogenous electromagnetic fields, i.e., generated by electrically active cells within a body remains controversial. Heat shock proteins (Hsps) protect cells against various environmental and endogenous stressors. Cytoprotection caused by Hsps involves tolerance induced by one agent against other, more severe agents. We have found that exposure of prokaryotic (*Escherichia coli*) and eukaryotic (Caco-2) cells to an electrical field (EF) connected with a myoelectrical migrating complex (MMC) generated by the small intestine smooth muscle induces the heat shock response. Using Western blot analysis, we have detected an elevated level of sigma factor 32 in *E. coli* cells exposed to MMC-related EF, and confocal microscopy indicated an increased level of the inducible form of Hsp70 protein in EF-stimulated Caco-2 cells. Additionally, we have found that this induced level of Hsp70 protected the Caco-2 cells against apoptosis caused by camptothecin. Our observations suggest that the myoelectrical activity of the gut may induce heat shock mechanisms in the cells of gut epithelium as well as in gastrointestinal micro-organisms.

(E) Lee C-H, Hung Y-C, Huang GS. Static magnetic field accelerates aging and development in nematode. *Commun Integr Biol* 3(6):528-529 (2010). (VO, AE, GE)

Electro-magnetic fields are everywhere in our life. The strength and duration of human exposure is proportional to the degree of industrialization. The possible health hazard has been investigated for decades. *C. elegans* (nematode) has been a sensitive tool to study aging and development. The current study investigated the possible effects of static magnetic fields (SMFs) on the developmental and aging processes of *C. elegans*. Nematodes were grown in the presence of SMFs of strengths varying from 0 to 200 mT. Treatment with a 200 mT SMF reduced the development times from L2 to young adult by approximately 20%. After SMF treatment, the average lifespan was reduced from 31 days to 25 days for wild-type nematodes. The upregulation of genes associated with development and aging was verified by quantitative real-time RT-PCR. Nematodes carrying mutation in these genes also exhibited resistance to the SMFs treatment. Apparently, induction of gene expression is selective and dose-dependent. SMFs accelerate nematode development and shorten nematode lifespan through pathways associated with *let-7*, *clk-1*, *unc-3* and *age-1*

(E) Lee HC, Hong MN, Jung SH, Kim BC, Suh YJ, Ko YG, Lee YS, Lee BY, Cho YG, Myung SH, Lee JS. Effect of extremely low frequency magnetic fields on cell proliferation and gene expression. *Bioelectromagnetics*. 36(7):506-516, 2016. (VT, AE, GE, CS)

Owing to concerns regarding possible effects of extremely low frequency magnetic fields (ELF-MF) on human health, many studies have been conducted to elucidate whether ELF-MF can induce modifications in biological processes. Despite this, controversies regarding effects of ELF-MF are still rife. In this study, we investigated biological effects of ELF-MF on MCF10A, MCF7, Jurkat, and NIH3T3 cell lines. ELF-MF with a magnetic flux density of 1 mT at 60 Hz was employed to stimulate cells for 4 or 16 h, after which the effects of ELF-MF on cell proliferation, cell death, cell viability, and DNA synthesis rates were assessed. Whereas Jurkat

and NIH3T3 cells showed no consistent variation in cell number, cell viability, and DNA synthesis rate, MCF10A and MCF7 cells showed consistent and significant decreases in cell number, cell viability, and DNA synthesis rates. However, there was no effect of ELF-MF on cell death in any of tested cell lines. Next, to investigate the effect of ELF-MF on gene expression, we exposed MCF7 cells to 2 mT at 60 Hz for 16 h and examined transcriptional responses by using gene expression array. We found a gene, PMAIP1, that exhibited statistically significant variation using two-fold cut-off criteria and certified its expression change by using semi-quantitative and quantitative reverse transcription polymerase chain reaction. From these results, we concluded that ELF-MF could induce the delay of cell cycle progression in MCF7 and MCF10A cells in a cell context-specific manner and could up-regulate PMAIP1 in MCF7 cells.

(E) Lee JW, Kim MS, Kim YJ, Choi YJ, Lee Y, Chung HW. Genotoxic effects of 3 T magnetic resonance imaging in cultured human lymphocytes. Bioelectromagnetics. 32(7):535-542, 2011. (VT, AE, GT)

The clinical and preclinical use of high-field intensity (HF, 3 T and above) magnetic resonance imaging (MRI) scanners have significantly increased in the past few years. However, potential health risks are implied in the MRI and especially HF MRI environment due to high-static magnetic fields, fast gradient magnetic fields, and strong radiofrequency electromagnetic fields. In this study, the genotoxic potential of 3 T clinical MRI scans in cultured human lymphocytes in vitro was investigated by analyzing chromosome aberrations (CA), micronuclei (MN), and single-cell gel electrophoresis. Human lymphocytes were exposed to electromagnetic fields generated during MRI scanning (clinical routine brain examination protocols: three-channel head coil) for 22, 45, 67, and 89 min. We observed a significant increase in the frequency of single-strand DNA breaks following exposure to a 3 T MRI. In addition, the frequency of both CAs and MN in exposed cells increased in a time-dependent manner. The frequencies of MN in lymphocytes exposed to complex electromagnetic fields for 0, 22, 45, 67, and 89 min were 9.67, 11.67, 14.67, 18.00, and 20.33 per 1000 cells, respectively. Similarly, the frequencies of CAs in lymphocytes exposed for 0, 45, 67, and 89 min were 1.33, 2.33, 3.67, and 4.67 per 200 cells, respectively. These results suggest that exposure to 3 T MRI induces genotoxic effects in human lymphocytes.

Lee SK, Park S, Gimm YM, Kim YW. Extremely low frequency magnetic fields induce spermatogenic germ cell apoptosis: possible mechanism. Biomed Res Int. 2014:567183, 2014. (Review)

The energy generated by an extremely low frequency electromagnetic field (ELF-EMF) is too weak to directly induce genotoxicity. However, it is reported that an extremely low frequency magnetic field (ELF-MF) is related to DNA strand breakage and apoptosis. The testes that conduct spermatogenesis through a dynamic cellular process involving meiosis and mitosis seem vulnerable to external stress such as heat, MF exposure, and chemical or physical agents. Nevertheless the results regarding adverse effects of ELF-EMF on human or animal reproductive functions are inconclusive. According to the guideline of the International Commission on Non-

Ionizing Radiation Protection (ICNIRP; 2010) for limiting exposure to time-varying MF (1 Hz to 100 kHz), overall conclusion of epidemiologic studies has not consistently shown an association between human adverse reproductive outcomes and maternal or paternal exposure to low frequency fields. In animal studies there is no compelling evidence of causal relationship between prenatal development and ELF-MF exposure. However there is increasing evidence that EL-EMF exposure is involved with germ cell apoptosis in testes. Biophysical mechanism by which ELF-MF induces germ cell apoptosis has not been established. This review proposes the possible mechanism of germ cell apoptosis in testes induced by ELF-MF.

(E) Leone L, Fusco S, Mastrodonato A, Piacentini R, Barbati SA, Zaffina S, Pani G, Podda MV, Grassi C. Epigenetic modulation of adult hippocampal neurogenesis by extremely low-frequency electromagnetic fields. Mol Neurobiol. 49(3):1472-1486, 2014. (VT, AE, GE) (medical application)

Throughout life, adult neurogenesis generates new neurons in the dentate gyrus of hippocampus that have a critical role in memory formation. Strategies able to stimulate this endogenous process have raised considerable interest because of their potential use to treat neurological disorders entailing cognitive impairment. We previously reported that mice exposed to extremely low-frequency electromagnetic fields (ELFEFs) showed increased hippocampal neurogenesis. Here, we demonstrate that the ELFEF-dependent enhancement of hippocampal neurogenesis improves spatial learning and memory. To gain insights on the molecular mechanisms underlying ELFEFs' effects, we extended our studies to an in vitro model of neural stem cells (NSCs) isolated from the hippocampi of newborn mice. We found that ELFEFs enhanced proliferation and neuronal differentiation of hippocampal NSCs by regulation of epigenetic mechanisms leading to pro-neuronal gene expression. Upon ELFEF stimulation of NSCs, we observed a significant enhancement of expression of the pro-proliferative gene hairy enhancer of split 1 and the neuronal determination genes NeuroD1 and Neurogenin1. These events were preceded by increased acetylation of H3K9 and binding of the phosphorylated transcription factor cAMP response element-binding protein (CREB) on the regulatory sequence of these genes. Such ELFEF-dependent epigenetic modifications were prevented by the Ca_v1-channel blocker nifedipine, and were associated with increased occupancy of CREB-binding protein (CBP) to the same loci within the analyzed promoters. Our results unravel the molecular mechanisms underlying the ELFEFs' ability to improve endogenous neurogenesis, pointing to histone acetylation-related chromatin remodeling as a critical determinant. These findings could pave the way to the development of novel therapeutic approaches in regenerative medicine.

(E) Li SH, Chow KC. Magnetic field exposure induces DNA degradation. Biochem Biophys Res Commun. 280(5):1385-1388, 2001. (VT, AE, GT, OX)

In our earlier experiments, we discovered that magnetic field exposure could bring both stabilizing and destabilizing effects to the DNA of Escherichia coli, depending on our parameters of assessment, and both of these effects were associated with the induced synthesis of the heat shock proteins Hsp70/Hsp40 (DnaK/DnaJ). These contradicting results prompted us to explore in this study the effect of magnetic field exposure on the DNA stability in vivo when the heat shock response of the cell was suppressed. By using

plasmid pUC18 in E. coli as the indicator, we found that without the protection of the heat shock response, magnetic field exposure indeed induced DNA degradation and this deleterious effect could be diminished by the presence of an antioxidant, Trolox C. In our in vitro test, we also showed that the magnetic field could potentiate the activity of oxidant radicals

(NE) Li L, Xiong DF, Liu JW, Li ZX, Zeng GC, Li HL. A cross-sectional study on oxidative stress in workers exposed to extremely low frequency electromagnetic fields. Int J Radiat Biol. 91(5):420-425, 2015. (HU, LE, GT)

Purpose: To investigate whether extremely low frequency electromagnetic field (ELF-EMF) exposure could induce oxidative stress in workers performing tour-inspection near transformers and distribution power lines. **Materials and methods:** Occupational short-term 'spot' measurements were performed. In total, 310 inspection workers exposed to ELF-EMF were selected as the exposure group and 300 logistical staff as the control group. Plasma total antioxidant capacity (T-AOC) and glutathione peroxidase (GPx) activity were tested by the colorimetric method. Superoxide dismutase (SOD) activity was tested using the xanthine oxidase method. Plasma malondialdehyde (MDA) concentration was determined with a thiobarbituric acid assay. The micronucleus cell frequency (MCF) and Micronuclei frequency (MN) were also tested for genotoxic assessment. **Results:** No significant changes of enzyme activities or MDA concentration were found. Neither the frequency of micronucleus lymphocytes nor micronuclei frequency changes were statistically significant. **Conclusion:** Continual ELF-EMF exposure might not induce oxidative stress in workers from a power supply bureau..

(E) Li SS, Zhang ZY, Yang CJ, Lian HY, Cai P. Gene expression and reproductive abilities of male Drosophila melanogaster subjected to ELF-EMF exposure. Mutat Res Genet Toxicol Environ Mutagen . 758(1-2):95-103, 2013. (VO, AE, LE, GE, RP)

Extremely low frequency electromagnetic field (ELF-EMF) exposure is attracting increased attention as a possible disease-inducing factor. The in vivo effects of short-term and long-term ELF-EMF exposure on male *Drosophila melanogaster* were studied using transcriptomic analysis for preliminary screening and QRT-PCR for further verification. Transcriptomic analysis indicated that 439 genes were up-regulated and 874 genes were down-regulated following short-term exposures and that 514 genes were up-regulated and 1206 genes were down-regulated following long-term exposures (expression >2- or <0.5-fold, respectively). In addition, there are 238 up-regulated genes and 598 down-regulated genes in the intersection of short-term and long-term exposure (expression >2- or <0.5-fold). The DEGs (differentially expressed genes) in *D. melanogaster* following short-term exposures were involved in metabolic processes, cytoskeletal organization, mitotic spindle organization, cell death, protein modification and proteolysis. Long-term exposure let to changes in expression of genes involved in metabolic processes, response to stress, mitotic spindle organization, aging, cell death and cellular respiration. In the intersection of short-term and long-term exposure, a series of DEGs were related to apoptosis, aging, immunological stress and reproduction. To check the ELF-EMF effects on reproduction, some experiments on male reproduction ability were performed. Their results indicated that short-term ELF-EMF exposure may decrease the reproductive ability of males, but long-term

exposures had no effect on reproductive ability. Down-regulation of ark gene in the exposed males suggests that the decrease in reproductive capacity may be induced by the effects of ELF-EMF exposure on spermatogenesis through the caspase pathway. QRT-PCR analysis confirmed that jra, ark and decay genes were down regulated in males exposed for 1 Generation (1G) and 72 h, which suggests that apoptosis may be inhibited in vivo. ELF-EMF exposure may have accelerated cell senescence, as suggested by the down-regulation of both cat and jra genes and the up-regulation of hsp22 gene. Up-regulation of totA and hsp22 genes during exposure suggests that exposed flies might induce an in vivo immune response to counter the adverse effects encountered during ELF-EMF exposure. Down-regulation of cat genes suggests that the partial oxidative protection system might be restrained, especially during short-term exposures. This study demonstrates the bioeffects of ELF-EMF exposure and provides evidence for understanding the in vivo mechanisms of ELF-EMF exposure on male *D. melanogaster*.

(E) Li Y, Liu X, Liu K, Miao W, Zhou C, Li Y, Wu H. Extremely low-frequency magnetic fields induce developmental toxicity and apoptosis in zebrafish (*Danio rerio*) embryos. Biol Trace Elem Res. 162(1-3):324-332, 2014.(VO, AE, GE)

Extremely low-frequency (ELF) magnetic field (MF), as a widespread ecological factor, has an influence on all living beings. In the present study, biological effects of ELF-MF on the development of zebrafish (*Danio rerio*) embryos were investigated. Fertilized embryos were divided into seven groups as control, sham, and five experimental groups. Embryos of experimental groups were continuously exposed to 50-Hz sinusoidal MF with intensities of 30, 100, 200, 400, and 800 μ T for 96 h. The sham group was treated as the experimental groups, but without any ELF-MF exposure. The control group was not subjected to anything. The results showed that ELF-MF exposure caused delayed hatching and decreased heart rate at the early developmental stages of zebrafish embryos, whereas no significant differences in embryo mortality and abnormality were observed. Moreover, acridine orange staining assays showed notable signals of apoptosis mainly in the ventral fin and spinal column. The transcription of apoptosis-related genes (caspase-3, caspase-9) was significantly upregulated in ELF-MF-exposed embryos. In conclusion, the overall results demonstrated that ELF-MF exposure has detrimental effects on the embryonic development of zebrafish by affecting the hatching, decreasing the heart rate, and inducing apoptosis, although such effects were not mortal threat. The results also indicate that zebrafish embryos can serve as a reliable model to investigate the biological effect of ELF-MF.

(E) Li Y, Yan X, Liu J, Li L, Hu X, Sun H, Tian J. Pulsed electromagnetic field enhances brain-derived neurotrophic factor expression through L-type voltage-gated calcium channel- and Erk-dependent signaling pathways in neonatal rat dorsal root ganglion neurons. Neurochem Int. 75:96-104, 2014. (CS, AE, GE)

(E) Li Y, Wang X, Yao L (2015). Directional migration and transcriptional analysis of oligodendrocyte precursors subjected to stimulation of electrical signal. Am J Physiol Cell Physiol 309(8):C532-540. (VT, AE, GE)

Loss of oligodendrocytes as the result of central nervous system disease causes demyelination that impairs axon function. Effective directional migration of endogenous or grafted

oligodendrocyte precursor cells (OPCs) to a lesion is crucial in the neural remyelination process. In this study, the migration of OPCs in electric fields (EFs) was investigated. We found that OPCs migrated anodally in applied EFs, and the directedness and displacement of anodal migration increased significantly when the EF strength increased from 50 to 200 mV/mm. However, EFs did not significantly affect the cell migration speed. The transcriptome of OPCs subjected to EF stimulation (100 and 200 mV/mm) was analyzed using RNA sequencing (RNA-Seq), and results were verified by the reverse transcription quantitative polymerase chain reaction. A Kyoto Encyclopedia of Genes and Genomes pathway analysis revealed that the mitogen-activated protein kinase pathway that signals cell migration was significantly upregulated in cells treated with an EF of 200 mV/mm compared with control cells. Gene ontology enrichment analysis showed the downregulation of differentially expressed genes in chemotaxis. This study suggests that an applied EF is an effective cue to guiding OPC migration in neural regeneration and that transcriptional analysis contributes to the understanding of the mechanism of EF-guided cell migration.

(E) Li Y, Zhang Y, Wang W, Zhang Y, Yu Y, Lai-Ying Cheing G, Pan W. Effects of pulsed electromagnetic fields on learning and memory abilities of STZ-induced dementia rats. Electromagn Biol Med 38(2):123-130. 2019. (VO, LE, GE)

Introduction: Recent studies have shown that pulsed electromagnetic field (EMF) has therapeutic potential for dementia, but the associated neurobiological effects are unclear. This study aimed to determine the effects of pulsed EMF on Streptozotocin (STZ)-induced dementia rats.

Methods: Forty Sprague-Dawley rats were randomly allocated to one of the four groups: (i) control, (ii) normal saline injection (sham group), (iii) STZ injection (STZ group) and (iv) STZ injection with pulsed EMF exposure (PEMF, 10 mT at 20 Hz) (STZ + MF group). Morris water maze was used to assess the learning and memory abilities. Insulin growth factors 1 and 2 (IGF-1 and IGF-2) gene expression were determined by quantitative PCR. Results: The results showed that the mean escape latency in STZ-induced dementia rats was reduced by 66% under the exposure of pulsed EMF. Compared with the STZ group, the swimming distance and the time for first crossing the platform decreased by 55 and 41.6% in STZ + MF group, respectively. Furthermore, the IGF-2 gene expression significantly increased compared to that of the STZ group. Conclusions: Our findings indicate that the pulsed EMF exposure can improve the ability of learning and memory in STZ-induced dementia rats and this effect may be related to the process of IGF signal transduction, suggesting a potential role for the pulsed EMF for the amelioration of cognition impairment.

(E) Lian H-Y, Lin K-W, Yang C, Cai P. Generation and propagation of yeast prion [URE3] are elevated under electromagnetic field. Cell Stress Chaperones 23(4):581-594, 2018. (VO, AE, GE, OX)

In this study, we studied the effect of 2.0 GHz radio frequency electromagnetic field (RF-EMF) and 50 Hz extremely low frequency electromagnetic field (ELF-EMF) exposure on prion generation and propagation using two budding yeast strains, NT64C and SB34, as model organisms. Under exposure to RF-EMF or ELF-EMF, the de novo generation and propagation of yeast prions [URE3] were elevated in both strains. The elevation increased over time, and the

effects of ELF-EMF occurred in a dose-dependent manner. The transcription and expression levels of the molecular chaperones Hsp104, Hsp70-Ssa1/2, and Hsp40-Ydj1 were not statistically significantly changed after exposure. Furthermore, the levels of ROS, as well as the activities of superoxide dismutase (SOD) and catalase (CAT), were significantly elevated after short-term, but not long-term exposure. This work demonstrated for the first time that EMF exposure could elevate the de novo generation and propagation of yeast prions and supports the hypothesis that ROS may play a role in the effects of EMF on protein misfolding. The effects of EMF on protein folding and ROS levels may mediate the broad effects of EMF on cell function.

(E) A R Liboff, T Williams Jr, D M Strong, R Wistar Jr Time-varying magnetic fields: effect on DNA synthesis. Science 223(4638):818-820, 1984. (VT)

Human fibroblasts have exhibited enhanced DNA synthesis when exposed to sinusoidally varying magnetic fields for a wide range of frequencies (15 hertz to 4 kilohertz) and amplitudes (2.3×10^{-6} to 5.6×10^{-4} tesla). This effect, which is at maximum during the middle of the S phase of the cell cycle, appears to be independent of the time derivative of the magnetic field, suggesting an underlying mechanism other than Faraday's law. The threshold is estimated to be between 0.5×10^{-5} and 2.5×10^{-5} tesla per second. These results bring into question the allegedly specific magnetic wave shapes now used in therapeutic devices for bone nonunion. The range of magnetic field amplitudes tested encompass the geomagnetic field, suggesting the possibility of mutagenic interactions directly arising from short-term changes in the earth's field.

(E) Lin H, M Head, M Blank, L Han, M Jin, R Goodman Myc-mediated transactivation of HSP70 expression following exposure to magnetic fields. J Cell Biochem 69(2):181-188, 1998. (VT, AE, GE)

We investigated c-myc protein-binding sites on the HSP70 promoter as modulators of the induction of HSP70 gene expression in response to magnetic field stimulation (8 microT at 60Hz) and whether the presence of c-myc protein potentiates transactivation of HSP70 expression. A 320 base pair region in the HSP70 promoter (+1 to -320) was analyzed. This region contains two c-myc-protein binding sites with consensus sequences located at -230 and -160 nucleotide positions (relative to the transcription initiation site) and overlapping with the region reported for the regulation of HSP70 gene expression by c-myc protein. This promoter region is upstream of other regulatory sequences, including the heat shock element (HSE), AP-2, and serum response element (SRE). Transfectants containing both c-myc protein-binding sites, HSP-MYC A and HSP-MYC B, and exposed to magnetic fields showed a 3.0-fold increase in expression of CAT activity as compared with sham-exposed control transfectants. Transfectants containing one c-myc binding site, HSP-MYC A, and exposed to magnetic fields showed a 2.3-fold increase in CAT expression. Transfectants in which both HSP-MYC A and HSP-MYC B binding sites were deleted showed no magnetic field sensitivity; values were virtually identical with sham-exposed controls. If the c-myc expression vector was not co-transfected with the constructs containing myc-binding sites, there was no difference in the expression of CAT activity between magnetically stimulated and sham-exposed controls, although both responded to heat shock. These data suggest that endogenous elevated levels of myc protein contribute to the induction of HSP70 in response to magnetic field stimulation.

(E) Lin H, L Han, M Blank, M Head, R Goodman Magnetic field activation of protein-DNA binding. J Cell Biochem 70(3):297-303, 1998. (VT, AE, GE)

The mechanisms involved in sensing, signaling, and coordinating changes resulting from magnetic field-induced stress show substantial similarities to those of heat shock, e.g., magnetic field-induced heat shock 70 gene (HSP70) expression involves heat shock factor (HSF) activation and heat shock element binding. However, an additional requirement for transactivation of HSP70 expression by magnetic fields is the binding of Myc protein, indicating that additional elements and/or pathways are involved in the induction of HSP70 expression by magnetic fields. To investigate the possible participation of additional genetic elements in magnetic field-induced HSP70 expression, we examined both magnetic field exposure and heat shock on protein-DNA binding of the transcription factors HSF, AP-1, AP-2, and SP-1 in four human cell lines. The binding sites for these transcription factors are present in the HSP70 promoter. AP-1 binding activity, normally not increased by heat shock, was increased by magnetic fields; heat shock induced an increase only in HSF binding. Although intersecting and converging signaling pathways could account for the multiplicity of elements involved in magnetic field-induced HSP70 transcription, direct interaction of magnetic fields with DNA is also a possible mechanism. Because magnetic fields penetrate the cell, they could well react with conducting electrons present in the stacked bases of the DNA.

(E)Lin KW, Yang CJ, Lian HY, Cai P. Exposure of ELF-EMF and RF-EMF increase the rate of glucose transport and TCA cycle in budding yeast. Front Microbiol. 7:1378, 2016.(VO, AE, GE)

In this study, we investigated the transcriptional response to 50 Hz extremely low frequency electromagnetic field (ELF-EMF) and 2.0 GHz radio frequency electromagnetic field (RF-EMF) exposure by Illumina sequencing technology using budding yeast as the model organism. The transcription levels of 28 genes were upregulated and those of four genes were downregulated under ELF-EMF exposure, while the transcription levels of 29 genes were upregulated and those of 24 genes were downregulated under RF-EMF exposure. After validation by reverse transcription quantitative polymerase chain reaction (RT-qPCR), a concordant direction of change both in differential gene expression (DGE) and RT-qPCR was demonstrated for nine genes under ELF-EMF exposure and for 10 genes under RF-EMF exposure. The RT-qPCR results revealed that ELF-EMF and RF-EMF exposure can upregulate the expression of genes involved in glucose transportation and the tricarboxylic acid (TCA) cycle, but not the glycolysis pathway. Energy metabolism is closely related with the cell response to environmental stress including EMF exposure. Our findings may throw light on the mechanism underlying the biological effects of EMF

(E) Liu Y, Liu WB, Liu KJ, Ao L, Zhong JL, Cao J, Liu JY. Effect of 50 Hz extremely low-frequency electromagnetic fields on the DNA methylation and DNA methyltransferases in mouse spermatocyte-derived cell line GC-2. Biomed Res Int. 2015:237183, 2015. (VT, AE, GT, EP)

Previous studies have shown that the male reproductive system is one of the most sensitive organs to electromagnetic radiation. However, the biological effects and molecular mechanism are largely unclear. Our study was designed to elucidate the epigenetic effects of 50 Hz ELF-EMF in vitro. Mouse spermatocyte-derived GC-2 cell line was exposed to 50 Hz ELF-EMF (5 min on and 10 min off) at magnetic field intensity of 1 mT, 2 mT, and 3 mT with an intermittent exposure for 72 h. We found that 50 Hz ELF-EMF exposure decreased genome-wide methylation at 1 mT, but global methylation was higher at 3 mT compared with the controls. The expression of DNMT1 and DNMT3b was decreased at 1 mT, and 50 Hz ELF-EMF can increase the expression of DNMT1 and DNMT3b of GC-2 cells at 3 mT. However, 50 Hz ELF-EMF had little influence on the expression of DNMT3a. Then, we established DNA methylation and gene expression profiling and validated some genes with aberrant DNA methylation and expression at different intensity of 50 Hz ELF-EMF. These results suggest that the alterations of genome-wide methylation and DNMTs expression may play an important role in the biological effects of 50 Hz ELF-EMF exposure.

(NE) Lopucki M, Schmerold I, Dadak A, Wiktor H, Niedermuller H, Kankofer M. Low dose magnetic fields do not cause oxidative DNA damage in human placental cotyledons in vitro. *Virchows Arch.* 446(6):634-639, 2005. (VT, AE, GT, OX)

The biological impact of low dose magnetic fields generated by electric appliances present in the human environment is still uncertain. In this study, human placentas served as a model tissue for the evaluation of the potential effect of oscillating low intensity magnetic fields on the concentration of 8-hydroxy-2'-deoxyguanosine (8-OH-dG) in cellular DNA. Cotyledons were dissected from placentas obtained immediately after physiological labours and exposed to magnetic fields (groups MF A, 2 mT, 50 Hz and MF B, 5 mT, 50 Hz) or sham exposed (group C) during an in vitro perfusion of 3 h. Cellular DNA was isolated, hydrolyzed and analyzed by HPLC. Native nucleosides were monitored at 254 nm and 8-OH-dG by electrochemical detection. Results were expressed as μmol 8-OH-dG/mol deoxyguanosine (dG). The concentrations of 8-OH-dG in group C, MF A and MF B were 28.45 ± 15.27 $\mu\text{mol/mol}$ dG, 62.80 ± 31.91 $\mu\text{mol/mol}$ dG, and 27.49 ± 14.23 $\mu\text{mol/mol}$ dG, respectively, demonstrating no significant difference between the groups. The results suggest that placental tissues possess a capacity to protect DNA against oxidative alterations by magnetic field of intensities previously shown to produce radical mediated DNA damage in rat brain cells in vivo and imbalances in electrolyte release of cotyledons under in vitro conditions.

(E) Lourencini da Silva R, Albano F, Lopes dos Santos LR, Tavares AD Jr, Felzenszwalb I. The effect of electromagnetic field exposure on the formation of DNA lesions. *Redox Rep.* 5(5):299-301, 2000. (VT, AE, GT, OX)

In an attempt to determine whether electromagnetic field (EMF) exposure might lead to DNA damage, we exposed SnCl₂-treated pBR322 plasmids to EMF and analysed the resulting conformational changes using agarose gel electrophoresis. An EMF-dependent potentiation of DNA scission (i.e. the appearance of relaxed plasmids) was observed. In confirmation of this, plasmids pre-exposed to EMF also were less capable of

transforming *Escherichia coli*. The results indicate that EMF, in the presence of a transition metal, is capable of causing DNA damage. These observations support the idea that EMF, probably through secondary generation of reactive oxygen species, can be clastogenic and provide a possible explanation for the observed correlation between EMF exposure and the frequency of certain types of cancers in humans.

(NE) Luceri C, De Filippo C, Giovannelli L, Blangiardo M, Cavalieri D, Aglietti F, Pampaloni M, Andreuccetti D, Pieri L, Bambi F, Biggeri A, Dolara P. Extremely low-frequency electromagnetic fields do not affect DNA damage and gene expression profiles of yeast and human lymphocytes. *Radiat Res.* 164(3):277-285, 2005. (VT, AE, LI, GT, GE)

We studied the effects of extremely low-frequency (50 Hz) electromagnetic fields (EMFs) on peripheral human blood lymphocytes and DBY747 *Saccharomyces cerevisiae*. Graded exposure to 50 Hz magnetic flux density was obtained with a Helmholtz coil system set at 1, 10 or 100 microT for 18 h. The effects of EMFs on DNA damage were studied with the single-cell gel electrophoresis assay (comet assay) in lymphocytes. Gene expression profiles of EMF-exposed human and yeast cells were evaluated with DNA microarrays containing 13,971 and 6,212 oligonucleotides, respectively. After exposure to the EMF, we did not observe an increase in the amount of strand breaks or oxidated DNA bases relative to controls or a variation in gene expression profiles. The results suggest that extremely low-frequency EMFs do not induce DNA damage or affect gene expression in these two different eukaryotic cell systems.

(E) Luo Q, Yang J, Zeng Q-L, Zhu X-M, Qian Y-L, Huang H-F. 50-Hertz electromagnetic fields induce gammaH2AX foci formation in mouse preimplantation embryos in vitro. *Biol Reprod* 75(5):673-680, 2006. (VT, AE, GT)

Effects of electromagnetic fields (EMFs) on DNA damage in mammals are still controversial. In the present study, the effects of EMFs on DNA damage in preimplantation mouse embryos in vitro were investigated by using gammaH2AX foci formation, a new sensitive indicator for detecting DNA double-strand breaks (DSBs). The data obtained demonstrated that EMFs decreased the cleavage rate of preimplantation mouse embryos. This decreasing effect of EMFs was related to the DNA-damaging effect indicated by the induction of gammaH2AX foci formation in preimplantation mouse embryos. The inducing effects of EMFs on gammaH2AX foci formation could be inhibited by the treatment of noise MFs or wortmannin, a phosphatidylinositol 3-kinase (PI3K) family inhibitor. Furthermore, the data obtained also showed that EMFs could activate the DNA damage-repair mechanism by recruiting repair factor Rad50 to the damaged DNA sites to repair the corresponding DNA damage. These findings suggest that EMFs could cause DNA damage in preimplantation embryos in vitro and that the adverse effects of EMFs on development might at least partly act through DNA damage. The DNA damage induced by EMFs could be at least partly repaired by the natural activation of DNA damage-repair mechanism or prevented by the simultaneous treatment of noise magnetic fields.

(E) Lupke M, Frahm J, Lantow M, Maercker C, Remondini D, Bersani F, Simkó M. Gene expression analysis of ELF-MF exposed human monocytes indicating the involvement of the alternative activation pathway. *Biochim Biophys Acta*. 1763(4):402-12, 2006. (VT, AE, GE)

This study focused on the cell activating capacity of extremely low frequency magnetic fields (ELF-MF) on human umbilical cord blood-derived monocytes. Our results confirm the previous findings of cell activating capacity of ELF-MF (1.0 mT) in human monocytes, which was detected as an increased ROS release. Furthermore, gene expression profiling (whole-genome cDNA array Human Unigene RZPD-2) was performed to achieve a comprehensive view of involved genes during the cell activation process after 45 min ELF-MF exposure. Our results indicate the alteration of 986 genes involved in metabolism, cellular physiological processes, signal transduction and immune response. Significant regulations could be analyzed for 5 genes (expression >2- or <0.5-fold): IL15RA (Interleukin 15 receptor, alpha chain), EPS15R (Epidermal growth factor receptor pathway substrate 15 - like 1), DNMT3A (Hypothetical protein MGC16121), DNMT3A (DNA (cytosine-5) methyltransferase 3 alpha), and one gene with no match to known genes, DKFZP586J1624. Real-time RT-PCR analysis of the kinetic of the expression of IL15RA, and IL10RA during 45 min ELF-MF exposure indicates the regulation of cell activation via the alternative pathway, whereas the delayed gene expression of FOS, IL2RA and the melatonin synthesizing enzyme HIOMT suggests the suppression of inflammatory processes. Accordingly, we suggest that ELF-MF activates human monocytes via the alternative pathway.

(E) Luukkonen J, Liimatainen A, Höytö A, Juutilainen J, Naarala J. Pre-exposure to 50 Hz magnetic fields modifies menadione-induced genotoxic effects in human SH-SY5Y neuroblastoma cells. *PLoS One*. 6(3):e18021, 2011. (VT, AE, GT, IX)

BACKGROUND: Extremely low frequency (ELF) magnetic fields (MF) are generated by power lines and various electric appliances. They have been classified as possibly carcinogenic by the International Agency for Research on Cancer, but a mechanistic explanation for carcinogenic effects is lacking. A previous study in our laboratory showed that pre-exposure to ELF MF altered cancer-relevant cellular responses (cell cycle arrest, apoptosis) to menadione-induced DNA damage, but it did not include endpoints measuring actual genetic damage. In the present study, we examined whether pre-exposure to ELF MF affects chemically induced DNA damage level, DNA repair rate, or micronucleus frequency in human SH-SY5Y neuroblastoma cells. **METHODOLOGY/PRINCIPAL FINDINGS:** Exposure to 50 Hz MF was conducted at 100 μ T for 24 hours, followed by chemical exposure for 3 hours. The chemicals used for inducing DNA damage and subsequent micronucleus formation were menadione and methyl methanesulphonate (MMS). Pre-treatment with MF enhanced menadione-induced DNA damage, DNA repair rate, and micronucleus formation in human SH-SY5Y neuroblastoma cells. Although the results with MMS indicated similar effects, the differences were not statistically significant. No effects were observed after MF exposure alone. **CONCLUSIONS:** The results confirm our previous findings showing that pre-exposure to MFs as low as 100 μ T alters cellular responses to menadione, and show that increased genotoxicity results from such interaction. The present findings also indicate that complementary data at several chronological points may be

critical for understanding the MF effects on DNA damage, repair, and post-repair integrity of the genome.

(E) Luukkonen J, Liimatainen A, Juutilainen J, Naarala J. Induction of genomic instability, oxidative processes, and mitochondrial activity by 50Hz magnetic fields in human SH-SY5Y neuroblastoma cells. *Mutat Res.* 760:33-41, 2014. (VT, AE, GT, OX, IX)

Epidemiological studies have suggested that exposure to 50 Hz magnetic fields (MF) increases the risk of childhood leukemia, but there is no mechanistic explanation for carcinogenic effects. In two previous studies we have observed that a 24-h pre-exposure to MF alters cellular responses to menadione-induced DNA damage. The aim of this study was to investigate the cellular changes that must occur already during the first 24h of exposure to MF, and to explore whether the MF-induced changes in DNA damage response can lead to genomic instability in the progeny of the exposed cells. In order to answer these questions, human SH-SY5Y neuroblastoma cells were exposed to a 50-Hz, 100- μ T MF for 24h, followed by 3-h exposure to menadione. The main finding was that MF exposure was associated with increased level of micronuclei, used as an indicator of induced genomic instability, at 8 and 15d after the exposures. Other delayed effects in MF-exposed cells included increased mitochondrial activity at 8d, and increased reactive oxygen species (ROS) production and lipid peroxidation at 15d after the exposures. Oxidative processes (ROS production, reduced glutathione level, and mitochondrial superoxide level) were affected by MF immediately after the exposure. In conclusion, the present results suggest that MF exposure disturbs oxidative balance immediately after the exposure, which might explain our previous findings on MF altered cellular responses to menadione-induced DNA damage. Persistently elevated levels of micronuclei were found in the progeny of MF-exposed cells, indicating induction of genomic instability.

(E) Luukkonen J, Höytö A, Sokka M, Liimatainen A, Syväoja J, Juutilainen J, Naarala J. Modification of p21 level and cell cycle distribution by 50 Hz magnetic fields in human SH-SY5Y neuroblastoma cells. *Int J Radiat Biol.* 93(2):240-248, 2017. (VT, AE, GT, IX)

PURPOSE: In our previous studies, exposure to extremely low frequency (ELF) magnetic fields (MF) altered responses to DNA damage caused by menadione. The aim of this study was to evaluate possible ELF MF induced changes in proteins involved in DNA damage responses and in cell cycle distribution. **MATERIALS AND METHODS:** Based on our previous studies, the exposure protocol included pre-exposure of human SH-SY5Y neuroblastoma cells to a 50 Hz, 100 μ T MF for 24 h prior to a 3-h menadione treatment. As DNA damage responses are relatively fast processes, a 1-h menadione treatment was also included in the experiments. The menadione concentrations used were 1, 10, 15, 20, and 25 μ M. Immunoblotting was used to assess the levels of DNA damage response-related proteins (γ -H2AX, Chk1, phospho-Chk1, p21, p27, and p53), while the level of DNA damage was assessed by the alkaline Comet assay. Cell cycle distribution was assayed by SYTOX Green staining followed by flow cytometry analysis. **RESULTS:** The main findings in MF-exposed cells were decreased p21 protein level after the 1-h menadione treatment, as well as increased proportion of cells in the G1 phase and decreased proportion of S phase cells after the 3-h menadione treatment. These effects were detectable also in the absence of menadione. **CONCLUSIONS:** The results indicate that MF exposure can

alter the G1 checkpoint response and that the p21 protein may be involved in early responses to MF exposure.

(NE) Lv Y, Chen S, Zhu B, Xu H, Xu S, Liu W, Shen Y, Zeng Q. Exposure to 50 Hz Extremely-Low-Frequency Magnetic Fields Induces No DNA Damage in Cells by Gamma H2AX Technology. Biomed Res Int. 2021 Feb 15;2021:8510315. doi: 10.1155/2021/8510315. (VT, AE, GT)

The current results for extremely-low-frequency magnetic fields (ELF-MF) on DNA damage are still debated. A sensitive indicator and systematic research are needed to assess the effects of ELF-MF. In this study, we used γ H2AX as an early and sensitive molecular marker to evaluate the DNA damage effects of ELF-MF in vitro. Human amnion epithelial cells (FLs), human skin fibroblast cells (HSFs), and human umbilical vein endothelial cells (HUVECs) were exposed to 50 Hz ELF-MF at 0.4, 1, and 2 mT for 15 min, 1 h, and 24 h, respectively. After exposure, cells were subjected to γ H2AX immunofluorescence and western blot. The results showed no significant difference in the average number of foci per cell, the percentage of γ H2AX foci-positive cells, or the expression of γ H2AX between the sham and 50 Hz ELF-MF exposure groups ($P > 0.05$). In conclusion, 50 Hz ELF-MF did not induce DNA damage in FLs, HSFs, or HUVECs, which was independent of the intensity or duration of the exposure.

(E) Ma Q, Deng P, Zhu G, Liu C, Zhang L, Zhou Z, Luo X, Li M, Zhong M, Yu Z, Chen C, Zhang Y. Extremely low-frequency electromagnetic fields affect transcript levels of neuronal differentiation-related genes in embryonic neural stem cells. PLoS One. 9(3):e90041, 2014. (VT, AE, GE, DE)

Previous studies have reported that extremely low-frequency electromagnetic fields (ELF-EMF) can affect the processes of brain development, but the underlying mechanism is largely unknown. The proliferation and differentiation of embryonic neural stem cells (eNSCs) is essential for brain development during the gestation period. To date, there is no report about the effects of ELF-EMF on eNSCs. In this paper, we studied the effects of ELF-EMF on the proliferation and differentiation of eNSCs. Primary cultured eNSCs were treated with 50 Hz ELF-EMF; various magnetic intensities and exposure times were applied. Our data showed that there was no significant change in cell proliferation, which was evaluated by cell viability (CCK-8 assay), DNA synthesis (Edu incorporation), average diameter of neurospheres, cell cycle distribution (flow cytometry) and transcript levels of cell cycle related genes (P53, P21 and GADD45 detected by real-time PCR). When eNSCs were induced to differentiation, real-time PCR results showed a down-regulation of Sox2 and up-regulation of Math1, Math3, Ngn1 and Tuj1 mRNA levels after 50 Hz ELF-EMF exposure (2 mT for 3 days), but the percentages of neurons (Tuj1 positive cells) and astrocytes (GFAP positive cells) were not altered when detected by immunofluorescence assay. Although cell proliferation and the percentages of neurons and astrocytes differentiated from eNSCs were not affected by 50 Hz ELF-EMF, the expression of genes regulating neuronal differentiation was altered. In conclusion, our results support that 50 Hz ELF-EMF induce molecular changes during eNSCs differentiation, which might be compensated by post-transcriptional mechanisms to support cellular homeostasis.

***(E) Qinlong Ma¹, Chunhai Chen^{1,2}, Ping Deng¹, Gang Zhu¹, Min Lin¹, Lei Zhang^{1,2}, Shangcheng Xu^{1,2}, Mindi He^{1,2}, Yonghui Lu^{1,2}, Weixia Duan¹, Huifeng Pi¹, Zhengwang Cao¹, Liping Pei^{1,2}, Min Li¹, Chuan Liu¹, Yanwen Zhang^{1,2}, Min Zhong^{1,2}, Zhou Zhou^{1,2}, Zhengping Yu^{1,2} Extremely Low-Frequency Electromagnetic Fields Promote In Vitro Neuronal Differentiation and Neurite Outgrowth of Embryonic Neural Stem Cells via Up-Regulating TRPC1. PLoS One 2016 Mar 7;11(3):e0150923. (VT, LE, GE)**

Exposure to extremely low-frequency electromagnetic fields (ELF-EMFs) can enhance hippocampal neurogenesis in adult mice. However, little is focused on the effects of ELF-EMFs on embryonic neurogenesis. Here, we studied the potential effects of ELF-EMFs on embryonic neural stem cells (eNSCs). We exposed eNSCs to ELF-EMF (50 Hz, 1 mT) for 1, 2, and 3 days with 4 hours per day. We found that eNSC proliferation and maintenance were significantly enhanced after ELF-EMF exposure in proliferation medium. ELF-EMF exposure increased the ratio of differentiated neurons and promoted the neurite outgrowth of eNSC-derived neurons without influencing astrocytes differentiation and the cell apoptosis. In addition, the expression of the proneural genes, NeuroD and Ngn1, which are crucial for neuronal differentiation and neurite outgrowth, was increased after ELF-EMF exposure. Moreover, the expression of transient receptor potential canonical 1 (TRPC1) was significantly up-regulated accompanied by increased the peak amplitude of intracellular calcium level induced by ELF-EMF. Furthermore, silencing TRPC1 expression eliminated the up-regulation of the proneural genes and the promotion of neuronal differentiation and neurite outgrowth induced by ELF-EMF. These results suggest that ELF-EMF exposure promotes the neuronal differentiation and neurite outgrowth of eNSCs via up-regulation the expression of TRPC1 and proneural genes (NeuroD and Ngn1). These findings also provide new insights in understanding the effects of ELF-EMF exposure on embryonic brain development.

(E) Mahaki H, Jabarivasal N, Sardarian K, Zamani A. The effects of extremely low-frequency electromagnetic fields on c-Maf, STAT6, and ROR α expressions in spleen and thymus of rat. Electromagn Biol Med. 38(2):177-183, 2019.(VO, LE, GE)

The study investigated the effect of extremely low-frequency electromagnetic fields (ELF-EMFs) exposure at different magnetic flux densities on genes expression of transcription factor Maf (c-Maf), signal transducer and activator of transcription 6 (STAT6), and retinoid-related orphan receptor alpha (ROR α) in the spleen and thymus of rats. Eighty adult male rats were separated into four ELF-EMFs exposed and were exposed to magnetic flux densities of 1, 100, 500, and 2000 μ T at a frequency of 50 Hz for 2 h daily for up to 60 d. All rats were intraperitoneally immunized on d 31, 44, and 58 of exposure. The experimental results showed that the expression levels of c-Maf, STAT6, and ROR α in the thymus were not significantly changed at different magnetic flux densities. The expression levels of ROR α and c-Maf were significantly downregulated at the densities of 1 and 100 μ T, while the expression of STAT6 was only significantly decreased at the density of 100 μ T. In conclusion, low magnetic flux densities of ELF-EMFs may reduce the expression levels of c-Maf, STAT6, and ROR α genes in the spleen.

(E) Mahdavinejad L, Alahgholi-Hajibehzad M, Eftekharian MM, Zaerieghane Z, Salehi I, Hajilooi M, Mahaki H, Zamani A. Extremely Low Frequency Electromagnetic Fields Decrease Serum Levels of Interleukin-17, Transforming Growth Factor- β and Downregulate Foxp3 Expression in the Spleen. J Interferon Cytokine Res 2018 Oct;38(10):457-462. (VT, LE, LI, GE)

The study aimed to determine effect of extremely low frequency (50 Hz) electromagnetic fields (ELF-EMFs) exposure on serum levels of interleukin-17 (IL-17) and transforming growth factor- β (TGF- β) as signature cytokines of Th17 and regulatory T (Treg) cells, respectively. Retinoid-related orphan receptor γ T and transcription factor forkhead box P3 (Foxp3) expression levels as lineage defining of Th17 and Treg cells were also assessed in the spleen and thymus. Eighty male rats were separated into 4 exposed groups (1, 100, 500, and 2,000 μ T magnetic flux intensities) and a control. All rats were immunized by human serum albumin after 1 month of the exposure and the experiment was continued in the same manner for 1 month more. The results demonstrated that the weight of thymuses was significantly declined at intensity of 2,000 μ T. At the preimmunization phase, the serum levels of IL-17 and TGF- β were significantly decreased at intensities of 1 and 100 μ T. The expression of Foxp3 was also downregulated at intensities of 1 and 100 μ T. In conclusion, low intensities of ELF-EMF may reduce the serum levels of IL-17 and TGF- β and downregulate the expression of Foxp3 in spleen.

(E) Mahmoudinasab H, Saadat M. Short-term Exposure to 50-Hz Electromagnetic field and alterations in *NQO1* and *NQO2* expression in MCF-7 Cells. Open Access Maced J Med Sci. 4(4):548-550, 2016. (VT, AE, GE)

Aim: Extremely low-frequency electromagnetic fields (ELF-EMFs) have some genotoxic effects and it may alter the mRNA levels of antioxidant genes. The NAD(P)H: quinone oxidoreductase-1 (*NQO1*) and *NQO2* are ubiquitously expressed. Considering that there is no published data on the effect(s) of ELF-EMF (50-Hz) exposure and expression levels of *NQO1* and *NQO2* in the human MCF-7 cells, the present study was carried out. **Methods:** The ELF-EMF (0.25 and 0.50 mT) exposure patterns were: 5 min field-on/5 min field-off, 15 min field-on/15 min field-off, and 30 min field-on continuously. In all exposure conditions, total exposure time were 30 minutes. The RNA extraction was done at two times; immediately post exposure and two hours post exposure. The effect of ELF-EMF on gene expression was assessed by real-time PCR. **Results:** The *NQO1* mRNA level (at 0h) decreased in the cells exposed to 5 min field-on/5 min field-off condition at 0.25 mT EMF when compared with the unexposed cells. The *NQO2* mRNA level (at 0h and 2h) increased in the cells exposed to 5 min field-on/5 min field-off condition at 0.50 mT EMF when compared with the unexposed cells. **Conclusions:** Alterations in the *NQO1* and *NQO2* mRNA levels seem at the "5 min field-on/5 min field-off" condition.

(E) Mahmoudinasab H, Saadat M. Electromagnetic Field Could Protect SH-SY5Y Cells Against Cisplatin Cytotoxicity, But Not MCF-7 Cells. DNA Cell Biol 37(4):330-335, 2018a. (VT, AE, GE, CS, IX)

Cisplatin [cis-dichlorodiammine platinum (II), CDDP], morphine (Mor), and electromagnetic field (EMF) induced oxidative stress. In this study, we tried to increase the cytotoxicity of CDDP

in combination with Mor and/or EMF in MCF-7 and SH-SY5Y cells. Furthermore, we evaluate the expression levels of 11 antioxidant genes in both cell lines. We designed four treatments: CDDP alone, "CDDP+Mor," "CDDP+EMF," and "CDDP+Mor+EMF." Serial dilutions of CDDP, Mor (5.0 μ M), and EMF (50 Hz, 0.50 mT, "15 min field-on/15 min field-off") were used for estimation of relative IC₅₀ values. The mRNA expression levels of antioxidant genes were determined by real-time PCR. The IC₅₀ value of CDDP in "CDDP+Mor+EMF" treatment was significantly higher than CDDP alone and "CDDP+Mor" treatments in both cell lines. Whereas the expression levels of antioxidant genes in the four treatments showed similar patterns in MCF-7 cells, in SH-SY5Y cells, most of the antioxidant genes showed an upregulation with "CDDP+EMF" and "CDDP+Mor+EMF" treatments. Moreover, significant differences in the number of upregulated genes were observed between different treatments in SH-SY5Y cells. The molecular mechanism of CDDP-reduced cytotoxicity in our designed combinations is probably different in MCF-7 and SH-SY5Y cells. CDDP in combination with EMF could protect SH-SY5Y cells from the cytotoxicity, whereas it has no significant change in MCF-7 cells.

(E) Mahmoudinasab H, Saadat M. Expressions of some antioxidant genes in SH-SY5Y cells treated with β -lapachone, morphine and electromagnetic field. Mol Biol Rep. 45(3):379-387, 2018b.(VT, AE, GE, IX)

β -Lapachone (β -Lap), morphine (Mor), and electromagnetic field (EMF) generate reactive oxygen species. The goal of the present study was to examine the effects of Mor and EMF, in combination with β -Lap on the cell growth inhibition and expression of several antioxidant genes. The 0.50 mT intensity of 50 Hz EMF and two exposure conditions ("15 min field-on/15 min field-off" and "30 min field-on continuously") on SH-SY5Y cells were used. The effects of Mor and EMF, in combination with β -Lap on cell growth inhibition and the expression levels of several antioxidant genes (NQO1, NQO2, SOD1, SOD2, CAT, GSTO1, GSTM2, GSTM3, GSTP1, MGST1, MGST3) in SH-SY5Y cells were measured. The relative mRNA levels were calculated according to the [Formula: see text]. Whereas NQO1 mRNA level decreased in the "15 min field-on/15 min field-off" condition, the expression level of NQO2 was increased. Both NQO1 and NQO2 expressions increased in Mor treated cells. IC₅₀ values of β -Lap in combination with Mor, EMF, and "Mor + EMF" were higher than cells treated only with β -Lap. The NQO1 expression level in the cells treated with β -Lap was higher than the other treatments, indicating that β -Lap induces the expression of NQO1. Moreover, multiple linear regression analysis indicated that NQO1 mRNA levels were associated positively with β -Lap and negatively with EMF. At least in part, the mRNA levels of NQO1 were associated with IC₅₀ values of β -Lap in designed treatments. There is a negative association between mRNA levels of NQO1 and IC₅₀ values of β -Lap but not NQO2.

(E) Mahmoudinasab H, Sanie-Jahromi F, Saadat M. Effects of extremely low-frequency electromagnetic field on expression levels of some antioxidant genes in human MCF-7 cells. Mol Biol Res Commun. 5(2):77-85, 2016. (VT, AE, GE, WS)

In the past three decades, study on the biological effects of extremely low-frequency electromagnetic fields (ELF-EMFs) has been of interest to scientists. Although the exact mechanism of its effect is not fully understood, free radical processes has been proposed as a possible mechanism. This study was designed to evaluate the effect of 50-Hz EMFs on the mRNA levels of seven antioxidant genes (*CAT*, *SOD1*, *SOD2*, *GSTO1*, *GSTM3*, *MSGT1*, and *MSGT3*) in human MCF-7 cells. The EMF exposure patterns were: 1) 5 min field-on/5 min field-off, 2) 15 min field-on/15 min field-off, 3) 30 min field-on continuously. In all three exposure conditions we tried to have total exposure time of 30 minutes. Control cultures were located in the exposure apparatus when the power was off. The experiments were done at two field intensities; 0.25 mT and 0.50 mT. The RNA extraction was done at two times; immediately post exposure and two hours post exposure. The mRNA levels were determined using quantitative real-time polymerase chain reaction. MTT assay for three exposure conditions in the two field intensities represented no cytotoxic effect on MCF-7 cells. Statistical comparison showed a significant difference between 0.25 mT and 0.50 mT intensities for "the 15 min field-on/15 min field-off condition" (Fisher's exact test, $P=0.041$), indicating that at 0.50 mT intensity field, the number of down-regulated and/or up-regulated genes increased compared with the other ones. However, there is no statistical significant difference between the field intensities for the two others EMF exposure conditions.

(E) Mairs RJ, Hughes K, Fitzsimmons S, Prise KM, Livingstone A, Wilson L, Baig N, Clark AM, Timpson A, Patel G, Folkard M, Angerson WJ, Boyd M. Microsatellite analysis for determination of the mutagenicity of extremely low-frequency electromagnetic fields and ionising radiation in vitro. *Mutat Res.* 626(1-2):34-41, 2007. (VT, AE, GT, IX)

Extremely low-frequency electromagnetic fields (ELF-EMF) have been reported to induce lesions in DNA and to enhance the mutagenicity of ionising radiation. However, the significance of these findings is uncertain because the determination of the carcinogenic potential of EMFs has largely been based on investigations of large chromosomal aberrations. Using a more sensitive method of detecting DNA damage involving microsatellite sequences, we observed that exposure of UVW human glioma cells to ELF-EMF alone at a field strength of 1 mT (50 Hz) for 12 h gave rise to 0.011 mutations/locus/cell. This was equivalent to a 3.75-fold increase in mutation induction compared with unexposed controls. Furthermore, ELF-EMF increased the mutagenic capacity of 0.3 and 3 Gy gamma-irradiation by factors of 2.6 and 2.75, respectively. These results suggest not only that ELF-EMF is mutagenic as a single agent but also that it can potentiate the mutagenicity of ionising radiation. Treatment with 0.3 Gy induced more than 10 times more mutations per unit dose than irradiation with 3 Gy, indicating hypermutability at low dose.

(E) Malagoli D, Lusvardi M, Gobba F, Ottaviani E. 50 Hz magnetic fields activate mussel immunocyte p38 MAP kinase and induce HSP70 and 90. *Comp Biochem Physiol C Toxicol Pharmacol* 137(1):75-79, 2004. (VO, LE, GE)

Fifty hertz magnetic fields (MFs) induced the expression of heat shock proteins (HSPs) 70 and 90 in immunocytes of the mussel *Mytilus galloprovincialis*. Animals exposed at 300 microT for three different times (30; 2 x 30; 3 x 30 min), did not show differences in the HSP densitometric values in comparison with non-exposed mussels. At 400 microT, exposed animals showed a time-dependent increase in HSP expression as revealed by Western blot. After exposure to 600 microT, the HSP densitometric values were significantly higher than controls but not related to exposure duration. The induction of HSPs is concomitant with the activation of p38 MAP kinase signalling pathway. The present findings suggest the possibility to modulate the expression of HSPs by an appropriate time-intensity magnetic field exposure.

***(E) Mansoury F, Babaei N, Abdi S, Entezari M, Doosti A. Changes in NOTCH1 gene and its regulatory circRNA, hsa_circ_0005986 expression pattern in human gastric adenocarcinoma and human normal fibroblast cell line following the exposure to extremely low frequency magnetic field.** *Electromagn Biol Med.* 2021 Feb 23:1-9. doi: 10.1080/15368378.2021.1891092. Online ahead of print. PMID: 33620018 **(VT, AE, GE)**

The effect of an extremely low-frequency magnetic field (ELF-MFs) on the expression levels of NOTCH1 and its regulatory circular RNA (circ-RNA) in gastric cancer has not yet investigated. This study aimed to find the expression changes of NOTCH1 and its regulatory circ-RNA, hsa_circ_0005986, in human gastric adenocarcinoma cell line (AGS) and human normal fibroblast (Hu02) cells following the exposure to discontinuously magnetic flux densities (MFDs) of 0.25, 0.5, 1 and 2 millitesla (mT) for 18h in comparison to unexposed cells. In addition, the effect of various MFDs on viability of tumor and normal cells was investigated. The cell viability was evaluated by MTT assay. The relative expression of NOTCH1 and hsa_circ_0005986 mRNAs was analyzed by quantitative Real-time PCR. The viability of tumor cells was decreased under the exposure of MFs, while the normal cells viability was increased. NOTCH1 was significantly down-regulated in AGS cells and up-regulated in Hu02 cells at all MFDs. The expression changes of NOTCH1 in tumor and normal cells was depended to the MFD of MFs. According to our results, the tumor and normal cells show different behavior at the molecular level in various MFDs in terms of NOTCH1 and hsa_circ_0005986 expression level. Decrease in tumor cell survival following the exposure to ELF-MFs may be the result of decreased in the expression level of NOTCH1 and its Reg-circ-RNA. These magnetic field reducing effects on cancer cell survival through the change on the expression of genes involved in the proliferation and progression of cancer can be a new key in cancer treatment.

(E) Manzella N, Bracci M, Ciarapica V, Staffolani S, Strafella E, Rapisarda V, Valentino M, Amati M, Copertaro A, Santarelli L. Circadian gene expression and extremely low frequency magnetic fields: an in vitro study. *Bioelectromagnetics.* 36(4):294-301, 2015. **(VT, AE, GE)**

It is well known that circadian clocks are mainly regulated by light targeting signaling pathways in the hypothalamic suprachiasmatic nucleus. However, an entrainment mediated by non-photic

sensory stimuli was also suggested for peripheral clocks. Exposure to extremely low frequency (ELF) electromagnetic fields might affect circadian rhythmicity. The goal of this research was to investigate effects of ELF magnetic fields (ELF-MF) on circadian clock genes in a human fibroblast cell line. We found that an ELF-MF (0.1 mT, 50 Hz) exposure was capable of entraining expression of clock genes BMAL1, PER2, PER3, CRY1, and CRY2. Moreover, ELF-MF treatment induced an alteration in circadian clock gene expression previously entrained by serum shock stimulation. These results support the hypothesis that ELF-MF may be able to drive circadian physiologic processes by modulating peripheral clock gene expression.

(E) Mariucci G, Villarini M, Moretti M, Taha E, Conte C, Minelli A, Aristei C, Ambrosini MV. Brain DNA damage and 70-kDa heat shock protein expression in CD1 mice exposed to extremely low frequency magnetic fields. Int J Radiat Biol. 86(8):701-710, 2010. (VO, LE, GT)

PURPOSE: The question of whether exposure to extremely low frequency magnetic fields (ELF-MF), may contribute to cerebral cancer and neurodegeneration is of current interest. In this study we investigated whether exposure to ELF-MF (50 Hz-1 mT) harms cerebral DNA and induces expression of 70-kDa heat shock protein (hsp70). **MATERIALS AND METHODS:** CD1 mice were exposed to a MF (50 Hz-1 mT) for 1 or 7 days (15 h/day) and sacrificed either at the end of exposure or after 24 h. Unexposed and sham-exposed mice were used as controls. Mouse brains were dissected into cerebral cortex-striatum, hippocampus and cerebellum to evaluate primary DNA damage and hsp70 gene expression. Food intake, weight gain, and motor activity were also evaluated. **RESULTS:** An increase in primary DNA damage was detected in all cerebral areas of the exposed mice sacrificed at the end of exposure, as compared to controls. DNA damage, as can be evaluated by the comet assay, appeared to be repaired in mice sacrificed 24 h after a 7-day exposure. Neither a short (15 h) nor long (7 days) MF-exposure induced hsp70 expression, metabolic and behavioural changes. **CONCLUSIONS:** These results indicate that in vivo ELF-MF induce reversible brain DNA damage while they do not elicit the stress response.

(E) Markkanen A, Juutilainen J, Naarala J. Pre-exposure to 50 Hz magnetic fields modifies menadione-induced DNA damage response in murine L929 cells. Int J Radiat Biol. 84(9):742-751, 2008. (VT, AE, GT, IX)

PURPOSE: Effects on DNA damage response were investigated in murine L929 cells exposed to 50 Hz magnetic fields (MF) with or without ultraviolet B (UVB, wavelength 280-320 nm) radiation or menadione (MQ). **MATERIALS AND METHODS:** Cells were exposed to MF at 100 or 300 microT combined with MQ (150 microM, 1 hour) or UVB radiation (160 J/m²) using various exposure schedules. The samples were stained with propidium iodide (PI) and analysed by flow cytometer for cell cycle stages. Apoptotic cells were defined as sub G(1) events. **RESULTS:** In cells first exposed to 100 microT MF for 24 h, the response to subsequent MQ treatment was significantly altered so that the proportion of sub G(1) cells was decreased and the proportion of cells in the G(2)/M phase was increased. When a 300 microT MF was used, also the proportion of cells in the G(1) phase was decreased. MF exposures after MQ treatment did not alter responses to MQ. No effects were found from MF exposure alone or from

MF combined with UVB radiation. **CONCLUSIONS:** The results strengthen previous findings suggesting that pre-exposure to MF can alter cellular responses to other agents, and indicate that MF as low as 100 microT has measurable impacts on cancer-relevant cellular processes such as DNA-damage.

(E) Martini F, Pellati A, Mazzoni E, Salati S, Caruso G, Contartese D, De Mattei M. Bone Morphogenetic Protein-2 Signaling in the Osteogenic Differentiation of Human Bone Marrow Mesenchymal Stem Cells Induced by Pulsed Electromagnetic Fields. Int J Mol Sci 21(6):2104, 2020. (VT, AE, IX, GE)

Pulsed electromagnetic fields (PEMFs) are clinically used with beneficial effects in the treatment of bone fracture healing. This is due to PEMF ability to favor the osteogenic differentiation of mesenchymal stem cells (MSCs). Previous studies suggest that PEMFs enhance the osteogenic activity of bone morphogenetic protein-2 (BMP2) which is used in various therapeutic interventions. This study investigated the molecular events associated to the synergistic activity of PEMFs and BMP2 on osteogenic differentiation. To this aim, human MSCs (hMSCs) were exposed to PEMFs (75 Hz, 1.5 mT) in combination with BMP2, upon detection of the minimal dose able to induce differentiation. Changes in the expression of BMP signaling pathway genes including receptors and ligands, as well as in the phosphorylation of BMP downstream signaling proteins, such as SMAD1/5/8 and MAPK, were analyzed. Results showed the synergistic activity of PEMFs and BMP2 on osteogenic differentiation transcription factors and markers. The PEMF effects were associated to the increase in BMP2, BMP6, and BMP type I receptor gene expression, as well as SMAD1/5/8 and p38 MAPK activation. These results increase knowledge concerning the molecular events involved in PEMF stimulation showing that PEMFs favor hMSCs osteogenic differentiation by the modulation of BMP signaling components.

(E) Mastrodonato A, Barbati SA, Leone L, Colussi C, Gironi K, Rinaudo M, Piacentini R, Denny CA, Grassi C. Olfactory memory is enhanced in mice exposed to extremely low-frequency electromagnetic fields via Wnt/ β -catenin dependent modulation of subventricular zone neurogenesis. Sci Rep 8(1):262, 2018. (VO, LE, GE)

Exposure to extremely low-frequency electromagnetic fields (ELFEF) influences the expression of key target genes controlling adult neurogenesis and modulates hippocampus-dependent memory. Here, we assayed whether ELFEF stimulation affects olfactory memory by modulating neurogenesis in the subventricular zone (SVZ) of the lateral ventricle, and investigated the underlying molecular mechanisms. We found that 30 days after the completion of an ELFEF stimulation protocol (1 mT; 50 Hz; 3.5 h/day for 12 days), mice showed enhanced olfactory memory and increased SVZ neurogenesis. These effects were associated with upregulated expression of mRNAs encoding for key regulators of adult neurogenesis and were mainly dependent on the activation of the Wnt pathway. Indeed, ELFEF stimulation increased Wnt3 mRNA expression and nuclear localization of its downstream target β -catenin. Conversely, inhibition of Wnt3 by Dkk-1 prevented ELFEF-induced upregulation of neurogenic genes and abolished ELFEF's effects on olfactory memory. Collectively, our findings suggest that ELFEF stimulation increases olfactory memory via enhanced Wnt/ β -catenin signaling in the SVZ and point to ELFEF as a promising tool for enhancing SVZ neurogenesis and olfactory function.

(E) Mayer-Wagner S, Hammerschmid F, Blum H, Krebs S, Redeker JI, Holzapfel BM, Volkmar Jansson V, Müller PE. Effects of single and combined low frequency electromagnetic fields and simulated microgravity on gene expression of human mesenchymal stem cells during chondrogenesis. Arch Med Sci 2018 Apr;14(3):608-616. (VT, LE, GE, IX, WS)

Introduction: Low frequency electromagnetic fields (LF-EMF) and simulated microgravity (SMG) have been observed to affect chondrogenesis. A controlled bioreactor system was developed to apply LF-EMF and SMG singly or combined during chondrogenic differentiation of human mesenchymal stem cells (hMSCs) in 3D culture. Material and methods: An external motor gear SMG bioreactor was combined with magnetic Helmholtz coils for EMF (5 mT; 15 Hz). Pellets of hMSCs (\pm TGF- β 3) were cultured (P5) under SMG, LF-EMF, LF-EMF/SMG and control (1 g) conditions for 3 weeks. Sections were stained with safranin-O and collagen type II. Gene expression was evaluated by microarray and real-time polymerase chain reaction analysis. Results: Simulated microgravity application significantly changed gene expression; specifically, COLXA1 but also COL2A1, which represents the chondrogenic potential, were reduced ($p < 0.05$). Low frequency electromagnetic fields application showed no gene expression changes on a microarray basis. LF-EMF/SMG application obtained significant different expression values from cultures obtained under SMG conditions with a re-increase of COL2A1, therefore rescuing the chondrogenic potential, which had been lowered by SMG. Conclusions: Simulated microgravity lowered hypertrophy but also the chondrogenic potential of hMSCs. Combined LF-EMF/SMG provided a rescue effect of the chondrogenic potential of hMSCs although no LF-EMF effect was observed under optimal conditions. The study provides new insights into how LF-EMF and SMG affect chondrogenesis of hMSCs and how they generate interdependent effects.

(NE) McNamee JP, Bellier PV, McLean JR, Marro L, Gajda GB, Thansandote A. DNA damage and apoptosis in the immature mouse cerebellum after acute exposure to a 1 mT, 60 Hz magnetic field. Mutat Res. 513(1-2):121-133, 2002. (VO, AE, GT)

Several recent studies have reported that whole-body exposure of rodents to power frequency magnetic fields (MFs) can result in DNA single- and double-strand breaks in the brains of these animals. The current study was undertaken to investigate whether an acute 2h exposure of a 1 mT, 60 Hz MF could elicit DNA damage, and subsequently apoptosis, in the brains of immature (10-day-old) mice. DNA damage was quantitated at 0, 2, 4, and 24h after exposure using the alkaline comet assay. Apoptosis was quantitated in the external granule cell layer (EGCL) of the immature mouse cerebellum at 0 and 24h after exposure to MF by the TdT-mediated dUTP nick-end labeling (TUNEL) assay. Four parameters (tail ratio, tail moment, comet length and tail length) were used to assess DNA damage for each comet. While increased DNA damage was detected by tail ratio at 2h after MF exposure, no supporting evidence of increased DNA damage was detected by the other parameters. In addition, no similar differences were observed using these parameters at any of the other post-exposure times. No increase in apoptosis was observed in the EGCL of MF-exposed mice, when compared to sham mice. Taken

together, these results do not support the hypothesis that acute MF exposure causes DNA damage in the cerebellums of immature mice.

(NE) McNamee JP, Bellier PV, Chauhan V, Gajda GB, Lemay E, Thansandote A. Evaluating DNA damage in rodent brain after acute 60 Hz magnetic-field exposure. Radiat Res. 164(6):791-797, 2005. (VO, AE, GT)

In recent years, numerous studies have reported a weak association between 60 Hz magnetic-field exposure and the incidence of certain cancers. To date, no mechanism to explain these findings has been identified. The objective of the current study was to investigate whether acute magnetic-field exposure could elicit DNA damage within brain cells from both whole brain and cerebellar homogenates from adult rats, adult mice and immature mice. Rodents were exposed to a 60 Hz magnetic field (0, 0.1, 1 or 2 mT) for 2 h. Then, at 0, 2 and 4 h after exposure, animals were killed humanely, their brains were rapidly removed and homogenized, and cells were cast into agarose gels for processing by the alkaline comet assay. Four parameters (tail ratio, tail moment, comet length and tail length) were used to assess DNA damage for each comet. For each species, a significant increase in DNA damage was detected by each of the four parameters in the positive control (2 Gy X rays) relative to the concurrent nonirradiated negative and sham controls. However, none of the four parameters detected a significant increase in DNA damage in brain cell homogenates from any magnetic-field exposure (0- 2 mT) at any time after exposure. The dose-response and time-course data from the multiple animal groups tested in this study provide no evidence of magnetic-field-induced DNA damage.

(E) Mercado-Sáenz S, Burgos-Molina AM, López-Díaz B, Sendra-Portero F, Ruiz-Gómez MJ¹. Effect of sinusoidal and pulsed magnetic field exposure on the chronological aging and cellular stability of *S. cerevisiae*. Int J Radiat Biol. 95(11):1588-1596, 2019. (VO, LE, GT, WS)

Purpose: The aim of this study is to investigate the effects of low frequency and intensity sinusoidal magnetic field (SMF) and pulsed magnetic field (PMF) exposure on the chronological aging and cellular stability of *Saccharomyces cerevisiae*. **Materials and methods:** The *S. cerevisiae* wild type strain (WS8105-1C) was exposed to SMF (2.45 mT, 50 Hz, continuous) and PMF (1.5 mT, 25 Hz, 8 h/day). Chronological aging was evaluated during 40 days. Survival was assayed by clonogenic assay and drop test. Cellular stability was studied by spontaneous mutation count and the index of respiratory competence (IRC). **Results:** We found that exposure to PMF produces an acceleration of cellular chronological aging, not observed in the groups treated with SMF. A decrease in the spontaneous frequency of mitochondrial mutation during aging was observed in PMF-treated samples. However, no alterations in the IRC during aging were found for both, SMF and PMF, treatments. **Conclusions:** Exposure to PMF produces the acceleration of aging and an alteration in cellular stability.

(E) Mie M, Endoh T, Yanagida Y, Kobatake E, Aizawa M. Induction of neural differentiation by electrically stimulated gene expression of NeuroD2. J Biotechnol 100(3):231-238, 2003. (VT, AE, GE)

Regulation of cell differentiation is an important assignment for cellular engineering. One of the techniques for regulation is gene transfection into undifferentiated cells. Transient expression of NeuroD2, one of neural bHLH transcription factors, converted mouse N1E-115 neuroblastoma cells into differentiated neurons. The regulation of neural bHLH expression should be a novel strategy for cell differentiation. In this study, we tried to regulate neural differentiation by NeuroD2 gene inserted under the control of heat shock protein-70 (HSP) promoter, which can be activated by electrical stimulation. Mouse neuroblastoma cell line, N1E-115, was stably transfected with expression vector containing mouse NeuroD2 cDNA under HSP promoter. Transfected cells were cultured on the electrode surface and applied electrical stimulation. After stimulation, NeuroD2 expression was induced, and transfected cells adopt a neuronal morphology at 3 days after stimulation. These results suggest that neural differentiation can be induced by electrically stimulated gene expression of NeuroD2.

(E) Miller SL, Coughlin DG, Waldorff EI, Ryaby JT, Lotz JC. Pulsed electromagnetic field (PEMF) treatment reduces expression of genes associated with disc degeneration in human intervertebral disc cells. Spine J 2016 Jun;16(6):770-6. (VT, LE, GE)

Background context: Pulsed electromagnetic field (PEMF) therapies have been applied to stimulate bone healing and to reduce the symptoms of arthritis, but the effects of PEMF on intervertebral disc (IVD) biology is unknown. Purpose: The purpose of this study was to determine how PEMF affects gene expression of IVD cells in normal and inflammatory environments. Study design/setting: This was an in vitro human cell culture and microarray gene expression study. Methods: Human annulus fibrosus (AF) and nucleus pulposus (NP) cells were separately encapsulated in alginate beads and exposed to interleukin 1 α (IL-1 α) (10 ng/mL) to stimulate the inflammatory environment associated with IVD degeneration and/or stimulated by PEMF for 4 hours daily for up to 7 days. RNA was isolated from each treatment group and analyzed via microarray to assess IL-1 α - and PEMF-induced changes in gene expression. Results: Although PEMF treatment did not completely inhibit the effects of IL-1 α , PEMF treatment lessened the IL-1 α -induced upregulation of genes expressed in degenerated IVDs. Consistent with our previous results, after 4 days, PEMF tended to reduce IL-1 α -associated gene expression of IL-6 (25%, p=.07) in NP cells and MMP13 (26%, p=.10) in AF cells. Additionally, PEMF treatment significantly diminished IL-1 α -induced gene expression of IL-17A (33%, p=.01) and MMP2 (24%, p=.006) in NP cells and NF κ B (11%, p=.04) in AF cells. Conclusions: These results demonstrate that IVD cells are responsive to PEMF and motivate future studies to determine whether PEMF may be helpful for patients with IVD degeneration.

(NE) Miller SC, J Haberer, U Venkatachalam, M J Furniss. NF-kappaB or AP-1-dependent reporter gene expression is not altered in human U937 cells exposed to power-line frequency magnetic fields. Radiat Res 151(3):310-318, 1999. (VT, AE, GE)

A number of studies have reported that human leukemia cells respond to exposure to power-line frequency electromagnetic fields (EMFs), providing evidence for an EMF-induced signaling pathway involving activation of protein tyrosine kinases (PTKs), phospholipase-Cy and protein kinase C (PKC). Because activation of PKC is also important in the signaling pathways that regulate the transcription factors NF-kappaB and AP-1, we evaluated the effect of exposure to a 60 Hz EMF on NF-kappaB or AP-1-dependent reporter gene expression in cells of the human promonocytic U937 leukemia cell line. Reporter genes were electroporated into U937 cells and activation of the NF-kappaB or AP-1 signaling pathway was evaluated by measuring chloramphenicol acetyltransferase (CAT) protein by CAT ELISA. In contrast to the effects of well-understood chemical or biological agents, the exposure to magnetic-field intensities of 0.08, 0.1, 1.0 or 1.3 mT had no effect on the NF-kappaB or AP-1 signaling pathways.

(E) Miyakawa T, S Yamada, S Harada, T Ishimori, H Yamamoto, R Hosono Exposure of *Caenorhabditis elegans* to extremely low frequency high magnetic fields induces stress responses. *Bioelectromagnetics* 22(5):333-339, 2001. (VO, AE, GE)

Responses of the small heat shock protein gene, hsp-16, were examined in transgenic *Caenorhabditis elegans* exposed to electromagnetic fields. Expression of the hsp-16-lacZ gene was enhanced when transgenic animals were exposed to magnetic fields up to 0.5 T at 60 Hz. The hsp-16 promoter was more efficiently expressed at the embryonic than at the post-embryonic stage irrespective of exposure. Promoter activity was more sensitive to the stimulus in the intestine at the post-embryonic stage. Evidence is presented that the induction occurs at the transcriptional step of hsp-16.

(NE) Miyakoshi J, Ohtsu S, Shibata T, Takebe H Exposure to magnetic field (5 mT at 60 Hz) does not affect cell growth and c-myc gene expression. *J Radiat Res.* 37(3):185-191, 1996a.(VT, AE, GT)

We designed and manufactured equipment for long-term and low-density (0 to 9 mT) exposures of cultured cells to extremely low frequency magnetic fields (ELF-MF), and examined the effects of ELF-MF on cell growth and c-myc mRNA expression in Chinese hamster ovary (CHO) cells. The ELF-MF equipment consists of a CO₂ incubator with a built-in magnet generator using Helmholtz coils being 250 mm in inner diameter, 160 mm in distance and 128 turns, a slide regulator and a thermocontroller. No significant difference in the growth rate and the c-myc expression of CHO cells was observed with 5 mT ELF-MF exposure, sham-exposure and incubation in a conventional incubator.

(E) Miyakoshi J, Yamagishi N, Ohtsu S, Mohri K, Takebe H. Increase in hypoxanthine-guanine phosphoribosyl transferase gene mutations by exposure to high-density 50-Hz magnetic fields. *Mutat Res.* 349(1):109-114, 1996b. (VT, AE, GT, IX)

Exposure to extremely low frequency magnetic field (ELFMF) of 50 Hz and 400 mT induced mutations in the hypoxanthine-guanine phosphoribosyl transferase gene of human melanoma MeWo cells. The mutant frequency was enhanced both by increasing

the exposure period and the induced current intensity. Mutations induced by X-rays were enhanced by ELFMF exposure. No significant increase in mutant frequency occurred when DNA replication was inhibited during ELFMF exposure. DNA replication error is suspected of causing the mutations produced by ELFMF exposure.

(E) Miyakoshi J, Kitagawa K, Takebe H. Mutation induction by high-density, 50-Hz magnetic fields in human MeWo cells exposed in the DNA synthesis phase. Int J Radiat Biol. 71(1):75-79, 1997. (VT, AE, GT)

Exposure of cultured human MeWo cells to high-density (400 mT at 50 Hz) extremely low frequency magnetic fields (ELF-MF) induced mutations in the hypoxanthine-guanine phosphoribosyl transferase gene. Mutation induced by the ELF-MF increased during the DNA-synthesis phase in synchronously growing cells. DNA replication errors and/or disturbance of the mismatch repair systems caused by exposure to ELF-MF may be involved in the mutagenic effect.

(E) Miyakoshi J, Mori Y, Yamagishi N, Yagi K, Takebe H. Suppression of high-density magnetic field (400 mT at 50 Hz)-induced mutations by wild-type p53 expression in human osteosarcoma cells. Biochem Biophys Res Commun 243(2):579-84, 1998.(VT, AE, GT)

Exposure of cultured human osteosarcoma cells (Saos-LP-12) to high-density (400 mT at 50 Hz) extremely low frequency magnetic fields (ELFMF) induced mutations in the hypoxanthine-guanine phosphoribosyl transferase gene. Saos-LP-12 cells, which are isolated from parental Saos-2 cells and have a deletion in the coding region of the p53 gene, are introduced to the wild-type (wt) p53 expression plasmid (pOPRSVp53). The mutation in Saos-LP-12 cells was suppressed by expression of the introduced wt p53 gene during 400 mT ELFMF exposure. No marked difference in the mutation spectrum was observed among the treatments of ELFMF [p53 (-)], ELFMF [p53 (+)], and sham exposures. Our findings suggest that wt p53 has a function in suppression of DNA replication errors and/or in maintenance of genomic stability after high-density ELFMF exposure.

(E) Miyakoshi J, Koji Y, Wakasa T, Takebe H. Long-term exposure to a magnetic field (5 mT at 60 Hz) increases X-ray-induced mutations. J Radiat Res. 40(1):13-21, 1999. (VT, LE, GT, IX)

Exposure to extremely low frequency magnetic field (ELFMF) at 400 mT has been shown to induce mutations (Mutat. Res., 349: 109-114, 1996; Int. J. Radiat. Biol., 71: 75-79, 1997; and Biochem. Biophys. Res. Commun., 243: 579-584, 1998). However, whether ELFMF at low flux densities (under 1 mT) induces mutations is debatable. We investigated the effect of long-term exposure to 5 mT ELFMF at 60 Hz on mutant frequency. Chinese hamster ovary K1 (CHO-K1) cells were exposed or sham-exposed to 5 mT ELFMF for up to 6 weeks with or without X-irradiation (3 Gy), and the mutant frequency of the hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene was analyzed. Long-term exposure to 5 mT ELFMF did not increase mutations, suggesting a threshold for mutation induction greater than 115 mA/m² or a magnetic density of 5 mT. However, enhancement of the X-ray-induced mutation rate was observed after

treatment with X-irradiation followed by long-term exposure to 5 mT ELFMF. At little as a 1-week exposure to ELFMF after X-irradiation enhanced the mutation rate. We also found that 400 mT exposure enhanced the mutation rate induced by X-irradiation (Mutat. Res., 349: 109-114, 1996). These results suggest that exposure to more than 5 mT ELFMF may promote X-ray-induced mutations.

(E)Miyakoshi J, Yoshida M, Shibuya K, Hiraoka M. Exposure to strong magnetic fields at power frequency potentiates X-ray-induced DNA strand breaks. J Radiat Res (Tokyo). 41(3):293-302, 2000. (VT, AE, GT, IX)

We examined the effect of an extremely low-frequency magnetic field (ELFMF) at 5, 50 and 400 mT on DNA strand breaks in human glioma MO54 cells. A DNA damage analysis was performed using the method of alkaline comet assay. The cells were exposed to X-rays alone (5 Gy), ELFMF alone, or X-rays followed by ELFMF at 4 degrees C or on ice. No significant difference in the tail moment was observed between control and ELFMF exposures up to 400 mT. X-ray irradiation increased DNA strand breaks. When cells were exposed to X-rays followed by ELFMF at 50 and 400 mT, the tail moment increased significantly compared with that for X-rays alone. When the exposure of cells was performed at 37 degrees C, no significant change was observed between X-rays alone and X-rays plus 400 mT. We previously observed that exposure to 400 mT ELFMF for 2 h increased X-ray-induced mutations (Miyakoshi et al, Mutat. Res., 349: 109-114, 1996). Additionally, an increase in the mutation by exposure to the ELFMF was observed in cells during DNA-synthesizing phase (Miyakoshi et al., Int. J. Radiat. Biol., 71: 75-79, 1997). From these results, it appears that exposure to the high density ELFMF at more than 50 mT may potentiate X-ray-induced DNA strand breaks.

Miyakoshi J. Effects of static magnetic fields at the cellular level. Prog Biophys Mol Biol. 2005 Feb-Apr;87(2-3):213-23. (Review)

There have been few studies on the effects of static magnetic fields at the cellular level, compared to those of extremely low frequency magnetic fields. Past studies have shown that a static magnetic field alone does not have a lethal effect on the basic properties of cell growth and survival under normal culture conditions, regardless of the magnetic density. Most but not all studies have also suggested that a static magnetic field has no effect on changes in cell growth rate. It has also been shown that cell cycle distribution is not influenced by extremely strong static magnetic fields (up to a maximum of 10 T). A further area of interest is whether static magnetic fields cause DNA damage, which can be evaluated by determination of the frequency of micronucleus formation. The presence or absence of such micronuclei can confirm whether a particular treatment damages cellular DNA. This method has been used to confirm that a static magnetic field alone has no such effect. However, the frequency of micronucleus formation increases significantly when certain treatments (e.g., X-irradiation) are given prior to exposure to a 10 T static magnetic field. It has also been

reported that treatment with trace amounts of ferrous ions in the cell culture medium and exposure to a static magnetic field increases DNA damage, which is detected using the comet assay. In addition, many studies have found a strong magnetic field that can induce orientation phenomena in cell culture.

(NE) Mizuno K, Narita E, Yamada M, Shinohara N, Miyakoshi J. ELF magnetic fields do not affect cell survival and DNA damage induced by ultraviolet B. Bioelectromagnetics. 35(2):108-115, 2014. (VT, AE, GT, IX)

We investigated whether extremely low frequency (ELF) magnetic field exposure has modification effects on cell survival after ultraviolet B (UV-B) irradiation and on repair process of DNA damage induced by UV-B irradiation in WI38VA13 subcloned 2RA and XP2OS(SV) cells. The ELF magnetic field exposure was conducted using a Helmholtz coil-based system that was designed to generate a sinusoidal magnetic field at 5 mT and 60 Hz. Cell survival was assessed by WST assay after UV-B irradiation at 20-80 J/m², ELF magnetic field exposure for 24 h, followed by incubation for 48 h. DNA damage was assessed by quantification of cyclobutane pyrimidine dimer formation and 6-4 photoproduct formation using ELISA after UV-B irradiation at 20-80 J/m² followed by ELF magnetic field exposure for 24 h. No significant changes were observed in cell survival between ELF magnetic field and sham exposures. Similarly, DNA damage induced by UV-B irradiation did not change significantly following ELF magnetic field exposure. Our results suggest that ELF magnetic field exposure at 5 mT does not have modification effect on cell survival after UV-B irradiation and on repair process of DNA damage induced by UV-B irradiation.

(E) Mohamed AF, Nasr M, Amer ME, Abuamara TMM, Abd-Elhay WM, Kaabo HF, Matar EER, El Moselhy LE, Gomah TA, El-Fatah Deban MA, Shebl RI. Anticancer and antibacterial potentials induced post short-term exposure to electromagnetic field and silver nanoparticles and related pathological and genetic alterations: in vitro study. Infect Agent Cancer 17(1):4, 2022. (VO, AE, GE)

Background: Resistance to antibiotics and anticancer therapy is a serious global health threat particularly in immunosuppressed cancer patients. Current study aimed to estimate the antibacterial and anticancer potentials of short-term exposure to extremely low frequency electromagnetic field (ELF-EMF) and silver nanoparticles (AgNPs) either in sole or combined form. **Methods:** Antibacterial activity was evaluated via determination of the bacterial viable count reduction percentage following exposure, whereas their ability to induce apoptosis in breast cancer (MCF-7) cell line was detected using annexin V-fluorescein isothiocyanate and cell cycle analysis. Also, oxidative stress potential and molecular profile were investigated.

Results: ELF-EMF and AgNPs significantly ($p < 0.01$) reduced *K. pneumoniae* viable count of compared to that of *S. aureus* in a time dependent manner till reaching 100% inhibition when ELF-EMF was applied in combination to 10 μ M/ml AgNPs for 2 h. Apoptosis induction was obvious following exposure to either ELF-EMF or AgNPs, however their apoptotic potential was intensified when applied in combination recording significantly ($p < 0.001$) induced apoptosis as indicated by elevated level of MCF-7 cells in the Pre G1 phase compared to control. S phase

arrest and accumulation of cells in G2/M phase was observed following exposure to AgNPs and EMF, respectively. Up-regulation in the expression level of p53, iNOS and NF- κ B genes as well as down-regulation of Bcl-2 and miRNA-125b genes were detected post treatment.

Conclusions: The antibacterial and anticancer potentials of these agents might be related to their ability to induce oxidative stress, suggesting their potentials as novel candidates for controlling infections and triggering cancer cells towards self-destruction.

(E) Monirul Islam M, Gianpiero Vigani G, Massimo E Maffei ME. 2020. The Geomagnetic Field (GMF) Modulates Nutrient Status and Lipid Metabolism during *Arabidopsis thaliana* Plant Development. *Plants (Basel)* 9(12):1729. (VO, AE, GE)

The Geomagnetic field (GMF) is a typical component of our planet. Plant perception of the GMF implies that any magnetic field (MF) variation would induce possible metabolic changes. In this work we assessed the role of the GMF on *Arabidopsis thaliana* Col0 mineral nutrition and lipid metabolism during plant development. We reduced the local GMF (about 40 μ T) to Near Null Magnetic Field (NNMF, about 30 nT) to evaluate the effects of GMF on *Arabidopsis* in a time-course (from rosette to seed-set) experiment by studying the lipid content (fatty acids, FA; and surface alkanes, SA) and mineral nutrients. The expression of selected genes involved in lipid metabolism was assessed by Real-Time PCR (qPCR). A progressive increase of SA with carbon numbers between 21 and 28 was found in plants exposed to NNMF from bolting to flowering developmental stages, whereas the content of some FA significantly ($p < 0.05$) increased in rosette, bolting and seed-set developmental stages. Variations in SA composition were correlated to the differential expression of several *Arabidopsis* 3-ketoacyl-CoA synthase (*KCS*) genes, including *KCS1*, *KCS5*, *KCS6*, *KCS8*, and *KCS12*, a lipid transfer protein (*LTPG1*) and a lipase (*LIP1*). Ionic analysis showed a significant variation in some micronutrients (Fe, Co, Mn and Ni) and macronutrients (Mg, K and Ca) during plant development of plants exposed to NNMF. The results of this work show that *A. thaliana* responds to variations of the GMF which are perceived as is typical of abiotic stress responses.

(E) Moraveji M, Haghighipour N, Keshvari H, Nourizadeh Abbariki T, Shokrgozar MA, Amanzadeh A. Effect of extremely low frequency electromagnetic field on MAP2 and Nestin gene expression of hair follicle dermal papilla cells. *Int J Artif Organs*. 39(6):294-299, 2016. (VT, LE, GE)

Introduction: In recent years, the extremely low frequency electromagnetic field (ELF-EMF) has attracted a great deal of scientific interest. The ELF-EMF signal is able to control ion transport across ion channels and therefore induce cell differentiation. **Aim:** The purpose of this study was to investigate the effect of ELF-EMF (50 Hz, 1 mT) on MAP2 and Nestin gene expression of dermal papilla mesenchymal cells (DPCs). **Methods:** In order to examine the effect of chemical and electromagnetic factors on gene expression, 4 experimental groups, namely chemical (cell exposure to chemical signals), EMF (exposing cells to ELF-EMF), chemical-EMF (subjecting cells to chemical signals and ELF-EMF) and control (with no treatment) groups, were prepared, treated for 5 days, and studied. To assess the effect of extended test time on the expression of neural differentiation markers (Nestin and MAP2), an

EMF group was prepared and treated for a period of 14 consecutive days. The beneficial role of EMF in inducing neural differentiation was shown by real-time PCR analysis. **Results:** The higher expression of MAP2 after 14 days compared to that after 5 days and decrease of cell proliferation on days 5 to 20 were indicative of the positive effect of extending treatment time on neural differentiation by evaluation of gene expression in EMF group.

(E) Moretti M, Villarini M, Simonucci S, Fatigoni C, Scassellati-Sforzolini G, Monarca S, Pasquini R, Angelucci M, Strappini M Effects of co-exposure to extremely low frequency (ELF) magnetic fields and benzene or benzene metabolites determined in vitro by the alkaline comet assay. Toxicol Lett. 157(2):119-128, 2005. (VT, AE, GT, IX)

In the present study, we investigated in vitro the possible genotoxic and/or co-genotoxic activity of 50 Hz (power frequency) magnetic fields (MF) by using the alkaline single cell microgel-electrophoresis (comet) assay. Sets of experiments were performed to evaluate the possible interaction between 50 Hz MF and the known leukemogen benzene. Three benzene hydroxylated metabolites were also evaluated: 1,2-benzenediol (1,2-BD, catechol), 1,4-benzenediol (1,4-BD, hydroquinone), and 1,2,4-benzenetriol (1,2,4-BT). MF (1 mT) were generated by a system consisting of a pair of parallel coils in a Helmholtz configuration. To evaluate the genotoxic potential of 50 Hz MF, Jurkat cell cultures were exposed to 1 mT MF or sham-exposed for 1h. To evaluate the co-genotoxic activity of MF, the xenobiotics (benzene, catechol, hydroquinone, and 1,2,4-benzenetriol) were added to Jurkat cells subcultures at the beginning of the exposure time. In cell cultures co-exposed to 1 mT (50 Hz) MF, benzene and catechol did not show any genotoxic activity. However, co-exposure of cell cultures to 1 mT MF and hydroquinone led to the appearance of a clear genotoxic effect. Moreover, co-exposure of cell cultures to 1 mT MF and 1,2,4-benzenetriol led to a marked increase in the genotoxicity of the ultimate metabolite of benzene. The possibility that 50 Hz (power frequency) MF might interfere with the genotoxic activity of xenobiotics has important implications, since human populations are likely to be exposed to a variety of genotoxic agents concomitantly with exposure to this type of physical agent.

(E) Mouhoub RB, Mansouri A, Aliliche K, Beghalem H, Landoulsi A, El May A. Unraveling the expression of genes involved in the biosynthesis pathway of cardiolipin and phosphatidylethanolamine in Salmonella Hadar grown under static magnetic field 200 mT. Microb Pathog 2017 Oct; 111:414-421. (VO, AE, GE)

We aimed in this work to evaluate the effect of static magnetic field 200 mT (SMF) on the expression of genes involved in the biosynthetic pathway of cardiolipin: g3pd, plsB, cdsA, pgsA, pgpA, cls and phosphatidylethanolamine: pssA and psd in *Salmonella enterica* subsp *enterica* serovar Hadar. Bacteria were exposed to a SMF during 3, 6 and 9 h. RNA extraction was followed by Reverse Transcriptase Polymerase Chain Reaction RT-PCR. The relative quantification of mRNA expression levels using 16S rRNA doesn't change during the time exposure. RT-PCR was done for two exposure experiments. The gene expression using RT-PCR present no significant difference in case of plsB, cdsA, pgpA, pgsA and psd genes during the

different exposure times. However, a significant increase was observed in the expression of g3pd and pssA genes after 6 h and for cls gene after 3 h of exposure, but any variation was notified after 9 h of exposure. So we can conclude from this study that cls, g3pd and pssA genes are required in the adaptation of Salmonella Hadar to SMF.

(E) Mustafa E, Luukkonen J, Makkonen J, Naarala J. The duration of exposure to 50 Hz magnetic fields: Influence on circadian genes and DNA damage responses in murine hematopoietic FDC-P1 cells. Mutat Res 2021 Jun 12;823:111756. doi: 10.1016/j.mrfmmm.2021.111756. Online ahead of print. (VT, AE, GE)

We investigated the effects of 50 Hz extremely low-frequency magnetic fields (MFs) on gene expression related to the circadian rhythm or DNA damage signaling and whether these fields modify DNA damage repair rate after bleomycin treatment. Murine FDC-P1 hematopoietic cells were exposed for different durations (15 min, 2 h, 12 h, and 24 h) to either 200 μ T MFs or sham-exposures. Cells were then collected for comet assay or real-time PCR to determine immediate DNA damage level and circadian rhythm gene expression, respectively. To assess DNA-damage signaling and DNA repair rate, the cells were subsequently treated with 20 μ g/mL bleomycin for 1 h and then either assayed immediately or allowed to repair their DNA for 1 or 2 h. We found that circadian rhythm-related genes were upregulated after 12 h of MF exposure and downregulated after 24 h of MF exposure, but none of the affected genes were core genes controlling the circadian rhythm. In addition, we found that the repair rate for bleomycin-induced damage was only decreased after MF exposure for 24 h. In conclusion, our findings suggest that the effects of MFs are duration-dependent; they were observed predominantly after long exposures.

(E) Mustafa E, Makinistian L, Luukkonen J, Juutilainen J, Naarala J. Do 50/60 Hz magnetic fields influence oxidative or DNA damage responses in human SH-SY5Y neuroblastoma cells? Int J Radiat Biol 2022 Mar 23;1-35. doi: 10.1080/09553002.2022.2055803. Online ahead of print. (VT, AE, GE, OX)

Purpose: We investigated possible effects of 50 Hz and 60 Hz magnetic fields (MFs) on reactive oxygen species (ROS) production, DNA damage, DNA damage repair rate, as well as gene expression related to oxidative stress and DNA damage signaling. **Materials and methods:** Human SH-SY5Y neuroblastoma cells were sham-exposed or exposed to 100 μ T_{RMS} MFs for 24 h, then assayed or further treated with 100 μ M menadione for 1 h before the assay. The levels of ROS and cytosolic superoxide anion ($O_2^{\cdot-}$) were assayed fluorometrically. DNA damage and gene expression were assayed by comet assay and RT-qPCR, respectively. To examine whether MFs affected DNA damage repair rate, cells were allowed to repair their DNA for 1 or 2 h after menadione treatment and then assayed for DNA damage. **Results:** There was suggestive evidence of a general low-magnitude increase in the expression of ROS-related genes (primarily genes with antioxidant activity) when quantified immediately after MF exposure, suggesting a response to a small increase in ROS level. The possible upregulation of ROS-related genes is supported by the finding that the level of menadione-induced ROS was consistently decreased by 50 Hz MFs (not significantly by 60 Hz MFs) in several measurements 30 - 60 min after MF exposure. MF exposures did not affect cytosolic $O_2^{\cdot-}$ levels, DNA damage, or its repair rate. Changes in the expression of DNA damage-signaling genes in the MF-exposed

cells did not exceed the expected rate of false positive findings. No firm evidence was found for differential effects from 50 Hz vs. 60 Hz MFs. **Conclusions:** While only weak effects were found on the endpoints measured, the results are consistent with MF effects on ROS signaling.

(E) Nakayama M, Nakamura A, Hondou T, Miyata H. Evaluation of cell viability, DNA single-strand breaks, and nitric oxide production in LPS-stimulated macrophage RAW264 exposed to a 50-Hz magnetic field. Int J Radiat Biol. 92(10):583-589, 2016. (VT, AE, GT, IX)

PURPOSE: Synergistic effects between cellular oxidative stress and magnetic fields may explain the adverse biological effects of 50/60 Hz magnetic fields. To determine whether this hypothesis holds in macrophage RAW264 cells, we measured DNA single-strand breaks (SSB), cell viability, and nitric oxide (NO) production in cells with or without exposure to 0.5-mT, 50-Hz magnetic fields for 24 h and with or without simultaneous stimulation via the bacterial endotoxin, lipopolysaccharide (LPS). MATERIALS AND METHODS: Macrophages stimulated with 10 ng/ml LPS for 1 h were exposed to or not exposed to a magnetic field and were then subjected to (1) the alkaline comet assay to measure SSBs, (2) trypan-blue exclusion assay for cell viability, and (3) measurements of NO for evaluation of oxidative stress. RESULTS: The 50-Hz magnetic field enhanced DNA SSB and decreased cell viability only in the LPS-stimulated macrophages in which NO production was greatly enhanced. The magnetic field alone did not alter NO production. CONCLUSION: Co-stimulation of the cell with LPS and a 50-Hz magnetic field promoted SSB and lowered cell viability, but these were not mediated by LPS-induced NO production.

(E) Nasrabadi N, Soheili ZS, Bagheri A, Ahmadi H, Amizadeh Y, Sahebjam F, Tabeie F, Rezaei Kanavi M. The effects of electromagnetic fields on cultured human retinal pigment epithelial cells. Bioelectromagnetics. 39(8):585-594, 2018. (VT, LE, GE)

A great deal of evidence has confirmed that electromagnetic fields (EMFs) can affect the central nervous system. In this study, cultured neonatal human retinal pigment epithelial (hRPE) cells were exposed to pulsed EMF of 1 mT intensity and 50 Hz frequency 8 h daily for 3 days. In addition to cell proliferation and cell death assays, immunocytochemistry for RPE65, PAX6, nestin, and cytokeratin 8/18 proteins were performed. Real-time reverse transcriptase polymerase chain reaction (RT-PCR) was performed for NES, PAX6, RPE65, and ACTA2 gene expression. Exposed hRPE cells did not demonstrate significant change in terms of cytomorphology, cell proliferation, or cell death. Protein expression of PAX6 was decreased in treated cells compared to controls and remained unchanged for RPE65, cytokeratin 8/18, and nestin. Gene expressions of NES, RPE65, and PAX6 were decreased in treated cells as compared to controls. Gene expression of ACTA2 did not significantly change. In conclusion, viability of cultivated neonatal hRPE cells did not change after short exposure to a safe dose of pulsed EMF albeit that both gene and protein expressions of retinal progenitor cell markers were reduced. Whether longer exposure durations that are being constantly produced by widely-used electronic devices may induce significant changes in these cells, needs further investigation.

(E) Nikolova T, Czyz J, Rolletschek A, Blyszczuk P, Fuchs J, Jovtchev G, Schuderer J, Kuster N, Wobus AM. Electromagnetic fields affect transcript levels of apoptosis-related genes in embryonic stem cell-derived neural progenitor cells. FASEB J 19(12):1686-1688, 2005. (VT, AE, GE)

Mouse embryonic stem (ES) cells were used as an experimental model to study the effects of electromagnetic fields (EMF). ES-derived nestin-positive neural progenitor cells were exposed to extremely low frequency EMF simulating power line magnetic fields at 50 Hz (ELF-EMF) and to radiofrequency EMF simulating the Global System for Mobile Communication (GSM) signals at 1.71 GHz (RF-EMF). Following EMF exposure, cells were analyzed for transcript levels of cell cycle regulatory, apoptosis-related, and neural-specific genes and proteins; changes in proliferation; apoptosis; and cytogenetic effects. Quantitative RT-PCR analysis revealed that ELF-EMF exposure to ES-derived neural cells significantly affected transcript levels of the apoptosis-related bcl-2, bax, and cell cycle regulatory "growth arrest DNA damage inducible" GADD45 genes, whereas mRNA levels of neural-specific genes were not affected. RF-EMF exposure of neural progenitor cells resulted in down-regulation of neural-specific Nurr1 and in up-regulation of bax and GADD45 mRNA levels. Short-term RF-EMF exposure for 6 h, but not for 48 h, resulted in a low and transient increase of DNA double-strand breaks. No effects of ELF- and RF-EMF on mitochondrial function, nuclear apoptosis, cell proliferation, and chromosomal alterations were observed. We may conclude that EMF exposure of ES-derived neural progenitor cells transiently affects the transcript level of genes related to apoptosis and cell cycle control. However, these responses are not associated with detectable changes of cell physiology, suggesting compensatory mechanisms at the translational and posttranslational level.

(NE) Okudan N, Celik I, Salbacak A, Cicekcibasi AE, Buyukmumcu M, Gökbel H. Effects of long-term 50 Hz magnetic field exposure on the micro nucleated polychromatic erythrocyte and blood lymphocyte frequency and argyrophilic nucleolar organizer regions in lymphocytes of mice. Neuro Endocrinol Lett. 31(2):208-214, 2010. (VO, LE, LI, GT)

OBJECTIVES: We aimed to investigate the effects of weak extremely low frequency electromagnetic fields (ELF-EMFs) on the nucleus size, the silver staining nucleolar organizer regions (AgNORs), the frequency of micro nucleated peripheral blood lymphocytes (MPBLs) and the micro nucleated polychromatic erythrocytes (MPCEs). **METHODS:** One hundred and twenty Swiss albino mice were equally divided into 6 groups. The study groups were exposed to 1, 2, 3, 4 and 5 microT 50 Hz-EMFs for 40 days. Micronucleus number (MN) per PBL was determined. **RESULTS:** ELF-EMF exposure caused a nonlinear decline of nucleus area. A sharp drop occurred in AgNOR area of 1 microT group, and following it gained an insignificantly higher level than that of the control group. The field did not change mean AgNOR numbers per nucleus of the groups. Relative AgNOR area had the highest level in 1 microT-exposure group, and the level was quite similar to that of the 5 microT-exposure group. The remaining groups had significantly lower values quite similar to that of the control level. The field exposure at any intensity did not affect significantly the frequency of either MPBLs or MPCEs. The number of MN per PBL in the 4 and 5 microT-exposure groups were significantly higher than those of the lower intensity exposure groups. The males in 4 microT-exposure group displayed the highest MN number per PBL, whereas values changed in a nonlinear manner.

CONCLUSIONS: The results of the present study suggest that ≤ 5 microT intensities of 50 Hz EMFs did not cause genotoxic effect on the mouse.

(E) Oladnabi M, Bagheri A, Kanavi MR, Azadmehr A, Kianmehr A. Extremely low frequency-pulsed electromagnetic fields affect proangiogenic-related gene expression in retinal pigment epithelial cells. Iran J Basic Med Sci 2019 Feb;22(2):128-133. (VT, LE, GE)

Objectives: It is known that extremely low frequency-pulsed electromagnetic fields (ELF-PEMF) influence multiple cellular and molecular processes. Retinal pigment epithelial (RPE) cells have a significant part in the emergence and pathophysiology of several ocular disorders, such as neovascularization. This study assessed the impact of ELF-PEMF on the proangiogenic features of RPE cells. **Materials and methods:** Primary cultured RPE cells were treated with ELF-PEMF (50 Hz) for three days. Using ELISA assay, we evaluated the effects of treatment on RPE cell proliferation and apoptosis. Also, RT-PCR was used to determine the gene expression of proangiogenic factors, such as matrix metalloproteinase-2 (MMP-2), MMP-9, vascular endothelial growth factors receptor 2 (VEGFR-2), hypoxia-inducible factor 1 (HIF-1 α), VEGFA, cathepsin D, connective tissue growth factor (CTGF), E2F3, tissue inhibitors of metalloproteinases 1 (TIMP-1), and TIMP-2. **Results:** No noticeable changes were observed in cell proliferation and cell death of ELF-PEMF-exposed RPE cells, while transcript levels of proangiogenic genes (HIF-1 α , VEGFA, VEGFR-2, CTGF, cathepsin D, TIMP-1, E2F3, MMP-2, and MMP-9) increased significantly. **Conclusion:** RPE cells are important for homeostasis of the retina. ELF-PEMF increased the gene expression of proangiogenic factors in RPE cells, which highlights concerns about the impact of this treatment on human health.

(E) Ozturk H, Saribal D, Gelmez YM, Deniz G, Yilmaz A, Kirectepe A, Ercan AM. Extremely low frequency electromagnetic fields exposure during the prenatal and postnatal periods alters pro-inflammatory cytokines levels by gender. Electromagn Biol Med 2022 Mar 2;1-11. doi: 10.1080/15368378.2022.2046045. Online ahead of print. (VO, LE, GE, DE)

Maternal exposure to the excessive electromagnetic fields is considered harmful to infants and associated with several health problems in life, such as neurological or immune diseases. In this present study we aimed to investigate the potential effects of extremely low-frequency electromagnetic field (ELF-EMF) exposure during the gestational and lactational period of dams on immune system parameters. The development of white blood cells (WBC), lymphocyte subpopulations (CD4⁺ T cells, CD8⁺ T cells, Natural Killer (NK) cells, and B cells) and production of T cell related cytokines were explored in the offsprings. Significant changes were found in WBC and lymphocyte counts. Although no changes in lymphocyte subunits were observed among groups, CD4⁺ cells were significantly increased in the female group exposed to ELF-EMF. Also, IL-17A and IFN- γ levels increased in plasma and spleen. The mean IL-4 level and the expression level of the IL-4 gene were not changed, in the experimental groups. But the expression of the IL-17A gene was also upregulated, which supports cytokine quantification analyses. In conclusion, ELF-EMF exposure in the prenatal and postnatal period increases the

level of IL-17A in the spleen and blood of young female rats, and it upregulates IL-17 gene expression in the spleen, resulting in CD4⁺ cell proliferation and inflammation.

(E) Panagopoulos DJ, Karabarbounis A, Lioliouis C. ELF alternating magnetic field decreases reproduction by DNA damage induction. Cell Biochem Biophys. 67(2):703-716, 2013. (VO, LE, GT, RP)

In the present experiments, the effect of 50-Hz alternating magnetic field on *Drosophila melanogaster* reproduction was studied. Newly eclosed insects were separated into identical groups of ten males and ten females and exposed to three different intensities of the ELF magnetic field (1, 11, and 21 G) continuously during the first 5 days of their adult lives. The reproductive capacity was assessed by the number of F1 pupae according to a well-defined protocol of ours. The magnetic field was found to decrease reproduction by up to 4.3%. The effect increased with increasing field intensities. The decline in reproductive capacity was found to be due to severe DNA damage (DNA fragmentation) and consequent cell death induction in the reproductive cells as determined by the TUNEL assay applied during early and mid-oogenesis (from germarium to stage 10) where physiological apoptosis does not occur. The increase in DNA damage was more significant than the corresponding decrease in reproductive capacity (up to ~7.5%). The TUNEL-positive signal denoting DNA fragmentation was observed exclusively at the two most sensitive developmental stages of oogenesis: the early and mid-oogenesis checkpoints (i.e. region 2a/2b of the germarium and stages 7-8 just before the onset of vitellogenesis)-in contrast to exposure to microwave radiation of earlier work of ours in which the DNA fragmentation was induced at all developmental stages of early and mid-oogenesis. Moreover, the TUNEL-positive signal was observed in all three types of egg chamber cells, mainly in the nurse and follicle cells and also in the oocyte, in agreement with the microwave exposure of our earlier works. According to previous reports, cell death induction in the oocyte was observed only in the case of microwave exposure and not after exposure to other stress factors as toxic chemicals or food deprivation. Now it is also observed for the first time after ELF magnetic field exposure. Finally, in contrast to microwave exposure of previous experiments of ours in which the germarium checkpoint was found to be more sensitive than stage 7-8, in the magnetic field exposure of the present experiments the mid-oogenesis checkpoint was found to be more sensitive than the germarium.

(E) Park H-J, Choi J-H, Nam M-H, Y-K. Induced Neurodifferentiation of hBM-MSCs through Activation of the ERK/CREB Pathway via Pulsed Electromagnetic Fields and Physical Stimulation Promotes Neurogenesis in Cerebral Ischemic Models. Int J Mol Sci 2022 Jan 21;23(3):1177. (VT, VO, LE, GE, WS)

Stroke is among the leading causes of death worldwide, and stroke patients are more likely to live with permanent disabilities even after treatment. Several treatments are being developed to improve the quality of life of patients; however, these treatments still have important limitations. Our study thus sought to evaluate the neural differentiation of human bone marrow mesenchymal stem cells (hBM-MSCs) at various pulsed electromagnetic field (PEMF) frequencies. Furthermore, the effects of selected frequencies in vivo were also evaluated using a mouse ischemia stroke model. Cell proliferation decreased by 20% in the PEMF group, as demonstrated

by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay, and lactate dehydrogenase (LDH) secretion increased by approximately 10% in an LDH release assay. Fluorescence-activated cell sorting (FACS) analysis demonstrated that CD73 and CD105 were downregulated in the PEMF group at 60 Hz. Moreover, microtubule-associated protein 2 (MAP-2) and neurofilament light chain (NF-L) were upregulated in cell cultures at 60 and 75 Hz. To assess the effects of PEMF in vivo, cerebral ischemia mice were exposed to a PEMF at 60 Hz. Neural-related proteins were significantly upregulated in the PEMF groups compared with the control and cell group. Upon conducting rotarod tests, the cell/PEMF group exhibited significant differences in motor coordination at 13 days post-treatment when compared with the control and stem-cell-treated group. Furthermore, the cell and cell/PEMF group exhibited a significant reduction in the expression of matrix metalloproteinase-9 (MMP-9), tumor necrosis factor-alpha (TNF- α) and interferon-gamma (IFN- γ) in the induced ischemic area compared with the control. Collectively, our findings demonstrated that PEMFs at 60 and 75 Hz could stimulate hBM-MSCs neural differentiation in vitro, in addition to promoting neurogenesis to enhance the functional recovery process by reducing the post-stroke inflammatory reaction.

(E) Patruno A, Pesce M, Grilli A, Speranza L, Franceschelli S, De Lutiis MA, Vianale G, Costantini E, Amerio P, Muraro R, Felaco M, Reale M. mTOR Activation by PI3K/Akt and ERK Signaling in Short ELF-EMF Exposed Human Keratinocytes. PLoS One 10(10):e0139644, 2015. (VT, AE, GE)

Several reports suggest that ELF-EMF exposures interact with biological processes including promotion of cell proliferation. However, the molecular mechanisms by which ELF-EMF controls cell growth are not completely understood. The present study aimed to investigate the effect of ELF-EMF on keratinocytes proliferation and molecular mechanisms involved. Effect of ELF-EMF (50 Hz, 1 mT) on HaCaT cell cycle and cells growth and viability was monitored by FACS analysis and BrdU assay. Gene expression profile by microarray and qRT-PCR validation was performed in HaCaT cells exposed or not to ELF-EMF. mTOR, Akt and MAPKs expressions were evaluated by Western blot analysis. In HaCaT cells, short ELF-EMF exposure modulates distinct patterns of gene expression involved in cell proliferation and in the cell cycle. mTOR activation resulted the main molecular target of ELF-EMF on HaCaT cells. Our data showed the increase of the canonical pathway of mTOR regulation (PI3K/Akt) and activation of ERK signaling pathways. Our results indicate that ELF-EMF selectively modulated the expression of multiple genes related to pivotal biological processes and functions that play a key role in physio-pathological mechanisms such as wound healing.

(E) Peng L, Fu C, Liang Z, Zhang Q, Xiong F, Chen L, He C, Wei Q. Pulsed Electromagnetic Fields Increase Angiogenesis and Improve Cardiac Function After Myocardial Ischemia in Mice. Circ J 2020 Jan 24;84(2):186-193.(VT, LE, GE)

Background: Previous studies have shown that pulsed electromagnetic fields (PEMF) stimulate angiogenesis and may be a potential treatment strategy to improve cardiac function after myocardial infarction (MI). This study explored the effects and its related mechanisms of PEMF in MI mice. **Methods and Results:** MI mice were used in PEMF treatment (15 Hz 1.5 mT PEMF

or 30 Hz 3.0 mT PEMF) for 45 min per day for 2 weeks. Furthermore, an in vivo Matrigel plug assay was used to observe the effect of PEMF in promoting angiogenesis. Compared with the sham PEMF group, PEMF treatment with 30 Hz 3.0 mT significantly improved heart function. PEMF treatment with 15 Hz 1.5 mT and 30 Hz 3.0 mT both increased capillary density, decreased infarction area size, increased the protein expression of vascular endothelial growth factor (VEGF), vascular endothelial growth factor receptor 2 (VEGFR2), Ser473-phosphorylated Akt (p^{Ser473}-Akt) and S1177-phosphorylated endothelial nitric oxide synthase (p^{S1177}-eNOS), and increased the mRNA level of VEGF and hypoxia inducible factor 1-alpha (HIF-1 α) in the infarct border zone. Additionally, treatment with 30 Hz 3.0 mT also increased protein and mRNA level of fibroblast growth factor 2 (FGF2), and protein level of β 1 integrin, and shows a stronger therapeutic effect.

(E) Pesqueira T, Costa-Almeida R, Gomes ME. Uncovering the effect of low-frequency static magnetic field on tendon-derived cells: from mechanosensing to tenogenesis. Sci Rep 2017 Sep 8;7(1):10948. (VT, AE, LE, GE)

Magnetotherapy has been receiving increased attention as an attractive strategy for modulating cell physiology directly at the site of injury, thereby providing the medical community with a safe and non-invasive therapy. Yet, how magnetic field influences tendon cells both at the cellular and molecular levels remains unclear. Thus, the influence of a low-frequency static magnetic field (2 Hz, 350 mT) on human tendon-derived cells was studied using different exposure times (4 and 8 h; short-term studies) and different regimens of exposure to an 8h-period of magnetic stimulation (continuous, every 24 h or every 48 h; long-term studies). Herein, 8 h stimulation in short-term studies significantly upregulated the expression of tendon-associated genes SCX, COL1A1, TNC and DCN ($p < 0.05$) and altered intracellular Ca²⁺ levels ($p < 0.05$). Additionally, every 24 h regimen of stimulation significantly upregulated COL1A1, COL3A1 and TNC at day 14 in comparison to control ($p < 0.05$), whereas continuous exposure differentially regulated the release of the immunomodulatory cytokines IL-1 β and IL-10 ($p < 0.001$) but only at day 7 in comparison to controls. Altogether, these results provide new insights on how low-frequency static magnetic field fine-tune the behaviour of tendon cells according to the magnetic settings used, which we foresee to represent an interesting candidate to guide tendon regeneration.

(E) Pilger A, Ivancsits S, Diem E, Steffens M, Kolb HA, Rüdiger HW. No effects of intermittent 50 Hz EMF on cytoplasmic free calcium and on the mitochondrial membrane potential in human diploid fibroblasts. Radiat Environ Biophys. 43(3):203-207, 2004. (VT, AE, GT)

The recently described increase in DNA strand breaks of cultured human diploid fibroblasts after intermittent exposure to extremely-low-frequency electromagnetic fields (ELF-EMF) of more than about 70 microT ELF-EMF is difficult to explain by a direct induction of covalent bond disruption. Therefore the hypothesis has been tested that ELF-EMF-induced DNA strand breaks might be mediated by cellular processes that cause alteration of the intracellular concentration of free calcium ([Ca²⁺]_i) and/or the membrane potential ($\Delta\Psi(m)$). [Ca²⁺]_i was determined by the ratiometric fura-2 technique. Changes in $\Delta\Psi(m)$ were assessed by using the potential-dependent lipophilic cationic probe JC-1. Human fibroblasts were exposed to intermittent ELF-

EMF (50 Hz, 1000 microT). Although exposure of fibroblasts to ELF-EMF resulted in a highly significant increase in DNA strand breaks as determined by the comet assay, no effect on JC-1 fluorescence emission or on $[Ca^{2+}]_i$ has been observed when comparing exposed with sham-exposed cells. Therefore, it is suggested that ELF-EMF-induced DNA strand breaks are unlikely to be caused by intracellular changes that affect $[Ca^{2+}]_i$ and/or $\Delta\psi(m)$.

(E) Potenza L, Ubaldi L, De Sanctis R, De Bellis R, Cucchiaroni L, Dachà M. Effects of a static magnetic field on cell growth and gene expression in Escherichia coli. Mutat Res. 561(1-2):53-62, 2004a. (VO, AE, GE)

Escherichia coli cultures exposed to a 300 mT static magnetic field (SMF) were studied in order to analyse possible induced changes in cellular growth and gene expression. Biomass was evaluated by visible-light spectrometry and gene expression analyses were carried out by use of RNA arbitrarily primed PCR. The bacterial strain XL-1Blue, cultivated in traditional and modified Luria-Bertani medium, was exposed to SMF generated by permanent neodymium magnetic disks. The results show alterations induced by SMF in terms of increased cell proliferation and changes in gene expression compared with control groups. Three cDNAs were found to be expressed only in the exposed cells, whereas one cDNA was more expressed in the controls. One clone, expressed only in the exposed cells, corresponds to a putative transposase. This is of particular interest in that it suggests that exposure to a magnetic field may stimulate transposition activity.

(E) Potenza L, Cucchiaroni L, Piatti E, Angelini U, Dachà M. Effects of high static magnetic field exposure on different DNAs. Bioelectromagnetics. 25(5):352-355, 2004b. (VT, AE, GT)

The effects of magnetic fields produced by permanent magnets on different DNA sources were investigated in vivo and in vitro. Escherichia coli DNA, plasmid, and amplification products of different lengths were used as the magnetic field target. The in vivo assays did not reveal any DNA alterations following exposure, demonstrating the presence of cell dependent mechanisms, such as the repair system and the buffering action of the heat shock proteins DNA K/J (Hsp 70/40). The in vitro assays displayed interactions between the magnetic field and DNA, revealing principally that magnetic field exposure induces DNA alterations in terms of point mutations. We speculate that the magnetic field can perturb DNA stability interacting with DNA directly or potentiating the activity of oxidant radicals. This genotoxic effect of the magnetic field, however, is minimized in living organisms due to the presence of protective cellular responses.

(E) Rageh MM, El-Gebaly RH, El-Bialy NS. Assessment of genotoxic and cytotoxic hazards in brain and bone marrow cells of newborn rats exposed to extremely low-frequency magnetic field. J Biomed Biotechnol. 2012:716023, 2012. (VO, LE, GT, DE, OX)

The present study aimed to evaluate the association between whole body exposure to extremely low frequency magnetic field (ELF-MF) and genotoxic, cytotoxic hazards in brain and bone marrow cells of newborn rats. Newborn rats (10 days after delivery) were exposed continuously to 50 Hz, 0.5 mT for 30 days. The control group was treated as the exposed one with the sole

difference that the rats were not exposed to magnetic field. Comet assay was used to quantify the level of DNA damage in isolated brain cells. Also bone marrow cells were flushed out to assess micronucleus induction and mitotic index. Spectrophotometric methods were used to measure the level of malondialdehyde (MDA) and the activity of glutathione (GSH) and superoxide dismutase (SOD). The results showed a significant increase in the mean tail moment indicating DNA damage in exposed group ($P < 0.01, 0.001, 0.0001$). Moreover ELF-MF exposure induced a significant ($P < 0.01, 0.001$) four folds increase in the induction of micronucleus and about three folds increase in mitotic index ($P < 0.0001$). Additionally newborn rats exposed to ELF-MF showed significant higher levels of MDA and SOD ($P < 0.05$). Meanwhile ELF-MF failed to alter the activity of GSH. In conclusion, the present study suggests an association between DNA damage and ELF-MF exposure in newborn rats.

(E) Rao S, Henderson AS. Regulation of c-fos is affected by electromagnetic fields. J Cell Biochem 63(3):358-365, 1996. (VT, AE, GE)

The goal of the present study was to determine if regulatory regions of the c-fos gene were responsive to electromagnetic field exposure. The research design used transfected cells to increase the sensitivity of assays designed to identify changes following exposure. HeLa cells were transiently transfected with plasmids containing upstream regulating regions of c-fos up to -700 base pairs, coupled with the prokaryotic reporter gene CAT. Cells were exposed to an environmentally relevant EMF of 60 Hz at 60 mGrms. CAT expression above control levels in transfected cells (region +42 to -700 bp) was observed following 5 min exposure to the electromagnetic field, with a peak at 20 min. The expression was at basal levels following 40 min exposure. Deletion analysis of upstream DNA narrowed the responsive region to 138 base pairs from -363 to -225, which contains the SRE/AP-1 sites

(NE) Rao RR, Halper J, Kisaalita WS. Effects of 60 Hz electromagnetic field exposure on APP695 transcription levels in differentiating human neuroblastoma cells. Bioelectrochemistry. 2002 Jul;57(1):9-15. (VT, AE, GE)

Epidemiological studies have suggested that workers with primary occupation that are likely to have resulted in the medium-to-high extremely low frequency (ELF) electromagnetic field (EMF) exposure are at increased risk of Alzheimer's disease (AD) pathogenesis. As a first step in investigating the possibility of an association between the ELF-EMF exposure and AD at the cellular level, we have used the differentiating IMR-32 neuroblastoma cells. In double-blind experiments, IMR-32 cells were exposed to the magnetic field intensities of 50, 100, and 200 microT at a frequency of 60 Hz for a period of 4 h at the three ages of differentiation (2, 10, and 16 days after incubation in differentiation medium). We used a custom-made Helmholtz coil setup driven by a 60-Hz sinusoidal signal from a function generator and an in-house built power amplifier. Total RNA extracted from the exposed cells was separated by the agarose gel electrophoresis and transferred to a nylon membrane for the northern hybridization. Digoxigenin-labeled APP695 RNA probes were used to detect changes in the APP695 mRNA levels in response to the ELF-EMF exposure. The results reported herein provided no support for any relationship between the APP695 gene transcription and

IMR-32 differentiation age, as well as the magnetic field exposure. This study constitutes the first step towards investigating the possibility of an association between the ELF-EMF exposure and AD manifestations at the cellular level.

(E) Reale M, De Lutiis MA, Patruno A, Speranza L, Felaco M, Grilli A, Macrì MA, Comani S, Conti P, Di Luzio S. Modulation of MCP-1 and iNOS by 50-Hz sinusoidal electromagnetic field. Nitric Oxide 15(1):50-57, 2006. (VT, AE, GE)

The purpose of this study was to investigate whether overnight exposure to 1 mT-50 Hz extremely low-frequency sinusoidal electromagnetic field (EMF) affects the expression and production of inducible nitric oxide synthase (iNOS) and monocyte chemoattractant protein-1 (MCP-1) in human monocytes. RT-PCR and Western blot analysis demonstrate that EMF exposure affects the expression of iNOS and MCP-1 in cultured human mononuclear cells at the mRNA level and protein synthesis. Interestingly, the effects of EMF exposure clearly differed with respect to the potentiation and inhibition of iNOS and MCP-1 expression. Whereas iNOS was down-regulated both at the mRNA level and at the protein level, MCP-1 was up-regulated. These results provide helpful information regarding the EMF-mediated modulation of the inflammatory response in vivo. However, additional studies are necessary to demonstrate that EMF acts as a nonpharmacological inhibitor of NO and inducer of MCP-1 in some diseases where the balance of MCP-1 and NO may be important.

(NE) Reese JA, Jostes RF, Frazier ME. Exposure of mammalian cells to 60-Hz magnetic or electric fields: analysis for DNA single-strand breaks. Bioelectromagnetics. 9(3):237-247, 1998. (VT, AE, GT)

Chinese hamster ovary (CHO) cells were exposed for 1 h to 60-Hz magnetic fields (0.1 or 2 mT), electric fields (1 or 38 V/m), or to combined magnetic and electric fields (2 mT and 38 V/m, respectively). Following exposure, the cells were lysed, and the DNA was analyzed for the presence of single-strand breaks (SSB), using the alkaline elution technique. No significant differences in numbers of DNA SSB were detected between exposed and sham-exposed cells. A positive control exposed to X-irradiation sustained SSB with a dose-related frequency. Cells exposed to nitrogen mustard (a known crosslinking agent) and X-irradiation demonstrated that the assay could detect cross-linked DNA under our conditions of electric and magnetic field exposures.

(E) Reyes-Guerrero G, Guzmán C, García DE, Camacho-Arroyo I, Vázquez-García M. Extremely low-frequency electromagnetic fields differentially regulate estrogen receptor-alpha and -beta expression in the rat olfactory bulb. Neurosci Lett. 471(2):109-113, 2010. (VO, LE, GE) (sex different)

Recently, the effects of extremely low-frequency electromagnetic fields (ELF EMF) on biological systems have been extensively investigated. In this report, the influence of ELF EMF on olfactory bulb (OB) estrogen receptor-alpha (ER alpha) mRNA and -beta (ER beta) mRNA expression was studied by RT-PCR in adult female and male rats. Results reveal for the first time that ELF EMF exerted a biphasic effect on female OB ER beta mRNA gene expression, which

increased during diestrous and decreased during estrous. We did not observe any influence of ELF EMF on female OB ER alpha mRNA expression. Our data demonstrate a fluctuating pattern of ER-alpha and -beta mRNA expression in the female OB throughout the phases of the estrous cycle in non-ELF EMF-exposed animals. Thus the highest ER alpha expression was observed in diestrous and the lowest in proestrous. The pattern of ER beta mRNA was less variable, the lowest expression was observed in diestrous. ER-alpha mRNA and -beta mRNA expression level in the male OB did not exhibit any variation either in ELF EMF-exposed or non-ELF EMF-exposed animals. In summary, ELF EMF modulate ER beta gene expression in the OB of female adult rats but not in males.

(E) Robison JG, Pendleton AR, Monson KO, Murray BK, O'Neill KL. Decreased DNA repair rates and protection from heat induced apoptosis mediated by electromagnetic field exposure. Bioelectromagnetics. 23(2):106-112, 2002. (VT, AE, GT, IX, CS)

In this study, we demonstrate that electromagnetic field (EMF) exposure results in protection from heat induced apoptosis in human cancer cell lines in a time dependent manner. Apoptosis protection was determined by growing HL-60, HL-60R, and Raji cell lines in a 0.15 mT 60 Hz sinusoidal EMF for time periods between 4 and 24 h. After induction of apoptosis, cells were analyzed by the neutral comet assay to determine the percentage of apoptotic cells. To discover the duration of this protection, cells were grown in the EMF for 24 h and then removed for 24 to 48 h before heat shock and neutral comet assays were performed. Our results demonstrate that EMF exposure offers significant protection from apoptosis (P<.0001 for HL-60 and HL-60R, P<.005 for Raji) after 12 h of exposure and that protection can last up to 48 h after removal from the EMF. In this study we further demonstrate the effect of the EMF on DNA repair rates. DNA repair data were gathered by exposing the same cell lines to the EMF for 24 h before damaging the exposed cells and non-exposed cells with H₂O₂. Cells were allowed to repair for time periods between 0 and 15 min before analysis using the alkaline comet assay. Results showed that EMF exposure significantly decreased DNA repair rates in HL-60 and HL-60R cell lines (P<.001 and P<.01 respectively), but not in the Raji cell line. Importantly, our apoptosis results show that a minimal time exposure to an EMF is needed before observed effects. This may explain previous studies showing no change in apoptosis susceptibility and repair rates when treatments and EMF exposure were administered concurrently. More research is necessary, however, before data from this in vitro study can be applied to in vivo systems.

(E) Rodríguez-De la Fuente AO, Alcocer-González JM, Heredia-Rojas JA, Rodríguez-Padilla C, Rodríguez-Flores LE, Santoyo-Stephano MA, Castañeda-Garza E, Taméz-Guerra RS. Effect of 60 Hz electromagnetic fields on the activity of hsp70 promoter: an in vivo study. Cell Biol Int Rep (2010) 2012 Mar 26;19(1):e00014. (VO, LE, GE)

Exposure to EMFs (electromagnetic fields) results in a number of important biological changes, including modification of genetic expression. We have investigated the effect of 60 Hz sinusoidal EMFs at a magnetic flux density of 80 μ T on the expression of the luciferase gene

contained in a plasmid labelled as pEMF (EMF plasmid). This gene construct contains the specific sequences for the induction of hsp70 (heat-shock protein 70) expression by EMFs, as well as the reporter for the luciferase gene. The pEMF vector was electrotransferred into quadriceps muscles of BALB/c mice that were later exposed to EMFs. Increased luciferase expression was observed in mice exposed to EMFs 2 h daily for 7 days compared with controls ($P < 0.05$). These data along with other reports in the literature suggest that EMFs can have far-reaching effects on the genome.

(NE) Ross CL, Pettenati MJ, Procita J, Cathey L, George SK, Almeida-Porada G. Evaluation of cytotoxic and genotoxic effects of extremely low-frequency electromagnetic field on mesenchymal stromal cells. *Glob Adv Health Med.* 7:2164956118777472, 2018. (VT, AE, GT)

Background: Interest in the use of extremely low-frequency (ELF) electromagnetic field (EMF) for the treatment of pain and inflammation is increasing due to the ability of this promising therapy to compete with pharmaceuticals without the adverse effects caused by drugs. However, there continues to be concerns regarding cytotoxic and genotoxic effects that may occur as a result of exposure to EMF. **Objective:** To investigate this concern, we tested the effect of our known therapeutic 5 Hz, 0.4 milliTesla (mT) EMF on a human mesenchymal stromal cell (hMSC) line to determine whether ELF-EMF exposure would cause cytotoxic or genotoxic effects. **Methods:** Treated samples along with controls were exposed to 5 Hz, 0.4 mT ELF-EMF for 20 min/day, 3×/week for 2 weeks and then assayed for cell viability, proliferation rates, and chromosome breaks. **Results:** Cytogenetic analysis of the viability and proliferation rates along with analysis of morphological genome stability showed no cytotoxicity, and no chromosome breaks per karyotype analysis-therefore no genotoxicity. **Conclusion:** Exposure to an ELF-EMF of 5 Hz, 0.4 mT for 20 min/day, 3×/week for 2 weeks does not cause cytotoxic or genotoxic effects in hMSCs.

Ruiz-Gómez MJ, Martínez-Morillo M. Electromagnetic fields and the induction of DNA strand breaks. *Electromagn Biol Med.* 28(2):201-214, 2009. (review)

The International Agency for Research on Cancer (IARC) has classified the extremely low-frequency (ELF) electromagnetic fields (EMF) as "possible carcinogenic" based on the reported effects. The purpose of this work is to review and compare the recent findings related to the induction of DNA strand breaks (DNA-SB) by magnetic field (MF) exposure. We found 29 studies (genotoxic and epigenetic) about the induction of DNA-SB by MF. 50% showed effect of MF and 50% showed no DNA-SB. Nevertheless, considering only genotoxic or only epigenetic studies, 37.5% and 69.2% found induction of DNA-SB by MF, respectively. In relation to these data it seems that MF could act as a co-inductor of DNA damage rather than as a genotoxic agent per se. Nevertheless, the published results, in some cases conflicting with negative findings, do not facilitate to obtain a common consensus about MF effects and biophysical interaction mechanisms.

(NE) Ruiz-Gómez MJ, Sendra-Portero F, Martínez-Morillo M. Effect of 2.45 mT sinusoidal 50 Hz magnetic field on *Saccharomyces cerevisiae* strains deficient in DNA strand breaks repair. *Int J Radiat Biol.* 86(7):602-611, 2010. (VT, AE, GT)

PURPOSE: To investigate whether extremely-low frequency magnetic field (MF) exposure produce alterations in the growth, cell cycle, survival and DNA damage of wild type (wt) and mutant yeast strains. **MATERIALS AND METHODS:** wt and high affinity DNA binding factor 1 (hdf1), radiation sensitive 52 (rad52), rad52 hdf1 mutant *Saccharomyces cerevisiae* strains were exposed to 2.45 mT, sinusoidal 50 Hz MF for 96 h. MF was generated by a pair of Helmholtz coils. During this time the growth was monitored by measuring the optical density at 600 nm and cell cycle evolution were analysed by microscopic morphological analysis. Then, yeast survival was assayed by the drop test and DNA was extracted and electrophoresed.

RESULTS: A significant increase in the growth was observed for rad52 strain ($P = 0.005$, Analysis of Variance [ANOVA]) and close to significance for rad52 hdf1 strain ($P = 0.069$, ANOVA). In addition, the surviving fraction values obtained for MF-exposed samples were in all cases less than for the controls, being the P value obtained for the whole set of MF-treated strains close to significance ($P = 0.066$, Student's t -test). In contrast, the cell cycle evolution and the DNA pattern obtained for wt and the mutant strains were not altered after exposure to MF.

CONCLUSIONS: The data presented in the current report show that the applied MF (2.45 mT, sinusoidal 50 Hz, 96 h) induces alterations in the growth and survival of *S. cerevisiae* strains deficient in DNA strand breaks repair. In contrast, the MF treatment does not induce alterations in the cell cycle and does not cause DNA damage.

(E) Sadri M, Abdolmaleki P, Abrun S, Beiki B, Sahraneshin Samani F
Static magnetic field effect on cell alignment, growth, and differentiation in human cord-derived mesenchymal stem cells. *Cell Mol Bioeng* 10(3):249-262, 2017. (VT, AE, GE)

This investigation is performed to evaluate the impact of static magnetic field on the Cell growth alignment, and differentiation potential in Human Mesenchymal Stem cells derived from human newborn cords. *In vitro*-cultured mesenchymal stem cells derived from human newborn cords were exposed to SMF up to 24 mT and compared with the control (unexposed) cultures.

Viability was assessed *via* Trypan Blue staining and MTT assay. Cell cycle progression was studied after flow cytometry data analysis. Sox-2, Nanog, and Oct-4 Primers used for RT-PCR experiment. Morphological studies showed that the exposed cells were significantly aligned in parallel bundles in a correlation with the magnetic field lines. Viability measurements showed a significant reduction in cell viability which was noted after exposure to static magnetic field and initiated 36 h after the end of exposure time. Flow cytometric data analysis confirmed a decrease in G1 phase cell population within the treated and cultured groups compared with the corresponding control samples. However, the induced changes were recovered in the cell cultures after the post-exposure culture recovery time which may be attributed to the cellular repair mechanisms. Furthermore, the proliferation rate and Oct-4 gene expression were reduced due to the 18 mT static magnetic field exposure. The significant proliferation rate decrease accompanied by the Sox-2, Nanog, and Oct-4 gene expression decline, suggested the differentiation inducing effects of SMF exposure. Exposure to Static Magnetic fields up to 24 mT affects mesenchymal stem cell alignment and proliferation rate as well as mRNA expression

of Sox-2, Nanog, and Oct-4 genes, therefore can be considered as a new differentiation inducer in addition to the other stimulators.

(E) Salek F, Baharara J, Shahrokhbadi KN, Amini E. The guardians of germ cells; Sertoli-derived exosomes against electromagnetic field-induced oxidative stress in mouse spermatogonial stem cells. Theriogenology 2021 Oct 1;173:112-122. (VT, CE, GE)

Nowadays, prolonged exposure to electromagnetic fields (EMF) has raised public concern about the detrimental potential of EMF on spermatogonial stem cells (SSCs) and spermatogenesis. Recent studies introduced the fundamental role of Sertoli cell paracrine signaling in the regulation of SSCs maintenance and differentiation in fertility preservation. Thus we investigated the therapeutic effect of Sertoli-derived exosomes (Sertoli-EXOs) as powerful paracrine mediators in SSCs subjected to EMF and its underlying mechanisms. SSCs and Sertoli cells were isolated from neonate mice testis, and identified by their specific markers. Then SSCs were exposed to 50 Hz EMF with intensity of 2.5 mT (1 h for 5 days) and supplemented with exosomes that were isolated from pre-pubertal Sertoli cells. Sertoli-EXOs were characterized and the uptake was observed by PKH26 labeling. The cell viability, colonization efficiency, reactive oxygen species (ROS) balance, cell cycle arrest and apoptosis induction were then analysed. SSCs were confirmed by immunocytochemistry (Oct4, Plzf) and Sertoli cells were identified through Sox9 and vimentin expression by immunocytochemistry and Real-time PCR (qRT-PCR), respectively. Our results demonstrated the detrimental effect of EMF via ROS accumulation that reduced the expression of catalase antioxidant, cell viability and colonization of SSCs. Also, AO/PI and flow cytometry analysis demonstrated the elevation of apoptosis in SSCs exposed to EMF in comparison with control. qRT-PCR data confirmed the up-regulation of apoptotic gene (Caspase-3) and down-regulation of SSCs specific gene ($GFR\alpha 1$). Consequently, the administration of Sertoli-EXOs exerted ameliorative effect on SSCs and significantly improved these changes through the regulation of oxidative stress. These findings suggest that Sertoli-EXOs have positive impact on SSCs exposed to EMF and can be useful in further investigation of Sertoli-EXOs as a novel therapeutic agent which may recover the deregulated SSCs microenvironment and spermatogenesis after exposure to EMF.

(E) Sanie-Jahromi F, Saadat I, Saadat M. Effects of extremely low frequency electromagnetic field and cisplatin on mRNA levels of some DNA repair genes. Life Sci. 166:41-45, 2016. (VT, AE, GE, IX)

AIMS: It has been shown that exposure to extremely-low frequency (<300Hz) oscillating electromagnetic field (EMF) can affect gene expression. The effects of different exposure patterns of 50-Hz EMF and co-treatment of EMF plus cisplatin (CDDP) on mRNA levels of seven genes involved in DNA repair pathways (GADD45A, XRCC1, XRCC4, Ku70, Ku80, DNA-PKcs and LIG4) were evaluated. MAIN METHODS: Two 50-Hz EMF intensities (0.25 and 0.50 mT), three exposure patterns (5min field-on/5min field-off, 15min field-on/15min field-off, 30 min field-on continuously) and two cell lines (MCF-7 and SH-SY5Y) were used. The mRNA levels were measured using quantitative real-time PCR. KEY FINDINGS: The examined genes had tendency to be down-regulated in MCF-7 cells treated with EMF. In the pattern of 15min field-on/15min field-off of the 0.50 mT EMF, no increase in mRNA levels were observed, but the mRNA levels of GADD45A, XRCC1, XRCC4, Ku80, Ku70, and LIG4

were down-regulated. A significant elevation in IC_{50} of CDDP was observed when MCF-7 and SH-SY5Y cells were co-treated with CDDP+EMF in comparison with the cells treated with CDDP alone. GADD45A mRNA levels in MCF-7 and SH-SY5Y cells co-treated with CDDP+EMF were increased and at the same time the mRNA levels of XRCC4, Ku80, Ku70 and DNA-PKcs were down-regulated. SIGNIFICANCE: Present study provides evidence that co-treatment of CDDP+EMF can enhance down-regulation of the genes involved in non-homologous end-joining pathway. It might be suggested that co-treatment of CDDP+EMF could be more promising for sensitizing cancer cells to DNA double strand breaks.

(E) Sanie-Jahromi F, Saadat M. Different profiles of the mRNA levels of DNA repair genes in MCF-7 and SH-SY5Y cells after treatment with combination of cisplatin, 50-Hz electromagnetic field and bleomycin. Biomed Pharmacother. 94:564-568, 2017. (VT, AE, GE, CS, IX)

Neurotoxicity is known to be a major dose-limiting adverse effect of cisplatin (CDDP), alone or in combination with other chemicals. DNA repair capacity serve as a neuroprotective factor against CDDP. The purpose of this study was to evaluate the effect of 50-Hz electromagnetic field (EMF) in combination with CDDP and bleomycin (Bleo) on expression of some of DNA repair genes (GADD45A, XRCC1, XRCC4, Ku70, Ku80, DNA-PKcs and LIG4) in MCF-7 (breast cancer) and SH-SY5Y (neuroblastoma) cell lines. MCF-7 and SH-SY5Y cells were pre-treated with CDDP in the presence or absence of EMF and then exposed to different concentration of Bleo. EMF (0.50mT intensity) was used in the intermittenet pattern of "15min field on/15min field off" with 30min total exposure. Cell viability assay was done and then the transcript levels of the examined genes were measured using quantitative real-time PCR in "CDDP+Bleo" and "CDDP+EMF+Bleo" treatments. Our results indicated that MCF-7 cells treated with "CDDP+EMF+Bleo" showed more susceptibility compared with "CDDP+Bleo" treated ones, while SH-SY5Y susceptibility was not changed between the two treatments. The represented data indicated that MCF-7 and SH-SY5Y cells showed non-random disagreement in DNA repair gene expression in 11 conditions (out of 14 conditions) with each other ($\chi^2=4.52$, $df=1$, $P=0.033$). This finding can be promising for sensitizing breast cancer cells while protecting against CDDP induced neuropathy in cancer patients

(E) Sanie-Jahromi F, Saadat M. Effects of electromagnetic field, cisplatin and morphine on cytotoxicity and expression levels of DNA repair genes. Mol Biol Rep. 45(5):807-814, 2018. (VT, AE, GE, IX, CS)

Morphine (Mor) is widely used as an analgesic drug in cancers and in combination with chemotherapy is known to have DNA damaging effects on non-targeted cell. This study surveyed the effect of Mor in combination with 50-Hz electromagnetic field (EMF) and co-treatment of cisplatin in combination with Mor and EMF on the expression of genes involved in DNA repair pathways. MCF-7 and SH-SY5Y cells were treated with 5.0 μ M Mor and then exposed to 50-Hz 0.50 mT EMF in the intermittent pattern of 15 min field-on/15 min field-off. Gene expression, cisplatin and bleomycin cytotoxicity were measured using real-time PCR and MTT assay. Mor treated cells showed significant down-regulation of the examined genes, while in "Mor + EMF" treatments the genes were not significantly changed. IC_{50} of cisplatin was significantly elevated in both cell lines when co-treated with "Mor + EMF" compared with Mor treated cells. Non-homologous end joining (NHEJ) related genes were significantly decreased in

co-treatment of cisplatin and "Mor + EMF" which led to bleomycin higher cytotoxicity in SH-SY5Y not in MCF-7. Our data is promising for providing a cell line-specific sensitization by combination of cisplatin and "Mor + EMF" treatment with local administration of double strand breaking agents.

(E) Sarimov R, Alipov ED, Belyaev IY. Fifty hertz magnetic fields individually affect chromatin conformation in human lymphocytes: dependence on amplitude, temperature, and initial chromatin state. Bioelectromagnetics. 32(7):570-579, 2011. (VT, AE, LI, GT)

Effects of magnetic field (MF) at 50 Hz on chromatin conformation were studied by the method of anomalous viscosity time dependence (AVTD) in human lymphocytes from two healthy donors. MF within the peak amplitude range of 5-20 μ T affected chromatin conformation. These MF effects differed significantly between studied donors, and depended on magnetic flux density and initial condensation of chromatin. While the initial state of chromatin was rather stable in one donor during one calendar year of measurements, the initial condensation varied significantly in cells from another donor. Both this variation and the MF effect depended on temperature during exposure. Despite these variations, the general rule was that MF condensed the relaxed chromatin and relaxed the condensed chromatin. Thus, in this study we show that individual effects of 50 Hz MF exposure at peak amplitudes within the range of 5-20 μ T may be observed in human lymphocytes in dependence on the initial state of chromatin and temperature.

(NE) Scarfi MR, Sannino A, Perrotta A, Sarti M, Mesirca P, Bersani F. Evaluation of genotoxic effects in human fibroblasts after intermittent exposure to 50 Hz electromagnetic fields: a confirmatory study. Radiat Res. 164(3):270-276, 2005. (NT, AE, GT)

The aim of this investigation was to confirm the main results reported in recent studies on the induction of genotoxic effects in human fibroblasts exposed to 50 Hz intermittent (5 min field on/10 min field off) sinusoidal electromagnetic fields. For this purpose, the induction of DNA single-strand breaks was evaluated by applying the alkaline single-cell gel electrophoresis (SCGE)/comet assay. To extend the study and validate the results, in the same experimental conditions, the potential genotoxicity was also tested by exposing the cells to a 50 Hz powerline signal (50 Hz frequency plus its harmonics). The cytokinesis-block micronucleus assay was applied after 24 h intermittent exposure to both sinusoidal and powerline signals to obtain information on cell cycle kinetics. The experiments were carried out on human diploid fibroblasts (ES-1). For each experimental run, exposed and sham-exposed samples were set up; positive controls were also provided by treating cells with hydrogen peroxide or mitomycin C for the comet or micronucleus assay, respectively. No statistically significant difference was detected in exposed compared to sham-exposed samples in any of the experimental conditions tested ($P > 0.05$). In contrast, the positive controls showed a statistically significant increase in DNA damage in all cases, as expected. Accordingly, our findings do not confirm the results reported previously for either comet induction or an increase in micronucleus frequency.

(E) Scassellati Sforzolini G, Moretti M, Villarini M, Fatigoni C, Pasquini R. [Evaluation of genotoxic and/or co-genotoxic effects in cells exposed in vitro to extremely-low frequency electromagnetic fields]. Ann Ig. 16(1-2):321-240, 2004. [Article in Italian] (VT, AE, GT, IX)

During the last two decades, concerns have arisen regarding a possible association between extremely-low frequency (ELF) electromagnetic fields (EMF) exposure and cancer incidence (e.g. childhood acute leukaemia, cancer of the nervous system, and lymphomas). In 1979, Wertheimer and Leeper firstly reported an excess of cancer mortality among children living in homes located near power lines and presumably exposed to elevated magnetic fields. Subsequently, a large number of epidemiological studies investigated the possible association between residential or occupational exposure to ELF-EMF and cancer. Several in vivo and in vitro models have been investigated with the effort to determine a link, if any, between such fields and mutagenesis and to determine the possible mechanism of cancer risk. However, a causal relationship between exposure to ELF-EMF and cancer has been suggested but has not been unequivocally demonstrated. In 1998, following an analysis of the results retrieved in the literature, the U.S. National Institute of Environmental Health Sciences proposed to apply a "possible human carcinogen" category (Group 2B) to ELF-EMF. More recently, in 2002, the same classification for ELF-MF was proposed by the International Agency for Research on Cancer. In this in vitro approach, to test the genotoxic and/or co-genotoxic potency of ELF-MF, we used the alkaline single-cell microgel-electrophoresis (comet) assay and the cytokinesis block micronucleus test. Co-exposure assays were performed in the presence of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), 4-nitroquinoline N-oxide (4NQO), benzene, 1,4-benzenediol (1,4-BD), or 1,2,4-benzenetriol (1,2,4-BT). An ELF-MF (50 Hz, 5 mT) was obtained by a system composed of capsulated induction coils. ELF-MF alone was unable to cause direct primary DNA damage. Whereas, an increased extent of DNA damage was observed in cells co-exposed to ELF-MF and MNNG, 1,4-BD, or 1,2,4-BT. An opposite trend was observed in cells treated with 4NQO and co-exposed to ELF-MF. Moreover, the frequency of micronucleated cells in ELF-MF-exposed cells was higher than in control cultures. Our findings suggest that the tested ELF-MF (50 Hz, 5 mT) possess genotoxic (micronucleus test) and co-genotoxic (comet assay) capabilities. The possibility that ELF-MF might interfere with the genotoxic activity of xenobiotics has important implications, since human populations are likely to be exposed to a variety of genotoxic agents concomitantly with exposure to this type of physical agent.

(E) Schmitz C, Keller E, Freuding T, Silny J, Korr H. 50-Hz magnetic field exposure influences DNA repair and mitochondrial DNA synthesis of distinct cell types in brain and kidney of adult mice. Acta Neuropathol (Berl). 107(3):257-264, 2004. (VO, LE, GT)

Despite several recent investigations, the impact of whole-body magnetic field exposure on cell-type-specific alterations due to DNA damage and DNA repair remains unclear. In this pilot study adult mice were exposed to 50-Hz magnetic field (mean value 1.5 mT) for 8 weeks or left unexposed. Five minutes after ending exposure, the mice received [(3)H]thymidine and were killed 2 h later. Autoradiographs were prepared from paraffin sections of brains and kidneys for measuring unscheduled DNA synthesis and

mitochondrial DNA synthesis, or in situ nick translation with DNA polymerase-I and [(3)H]dTTP. A significant ($P < 0.05$) increase in both unscheduled DNA synthesis and in situ nick translation was only found for epithelial cells of the choroid plexus. Thus, these two independent methods indicate that nuclear DNA damage is produced by long-lasting and strong magnetic field exposure. The fact that only plexus epithelial cells were affected might point to possible effects of magnetic fields on iron transport across the blood-cerebrospinal fluid barrier, but the mechanisms are currently not understood. Mitochondrial DNA synthesis was exclusively increased in renal epithelial cells of distal convoluted tubules and collecting ducts, i.e., cells with a very high content of mitochondria, possibly indicating increased metabolic activity of these cells.

(E) Selvamurugan N, He Z, Rifkin D, Dabovic B, Partridge NC. Pulsed Electromagnetic Field Regulates MicroRNA 21 Expression to Activate TGF- β Signaling in Human Bone Marrow Stromal Cells to Enhance Osteoblast Differentiation. Stem Cells Int 2017;2017:2450327. (VT, LE, GE)

Pulsed electromagnetic fields (PEMFs) have been documented to promote bone fracture healing in nonunions and increase lumbar spinal fusion rates. However, the molecular mechanisms by which PEMF stimulates differentiation of human bone marrow stromal cells (hBMSCs) into osteoblasts are not well understood. In this study the PEMF effects on hBMSCs were studied by microarray analysis. PEMF stimulation of hBMSCs' cell numbers mainly affected genes of cell cycle regulation, cell structure, and growth receptors or kinase pathways. In the differentiation and mineralization stages, PEMF regulated preosteoblast gene expression and notably, the transforming growth factor-beta (TGF- β) signaling pathway and microRNA 21 (miR21) were most highly regulated. PEMF stimulated activation of Smad2 and miR21-5p expression in differentiated osteoblasts, and TGF- β signaling was essential for PEMF stimulation of alkaline phosphatase mRNA expression. Smad7, an antagonist of the TGF- β signaling pathway, was found to be miR21-5p's putative target gene and PEMF caused a decrease in Smad7 expression. Expression of Runx2 was increased by PEMF treatment and the miR21-5p inhibitor prevented the PEMF stimulation of Runx2 expression in differentiating cells. Thus, PEMF could mediate its effects on bone metabolism by activation of the TGF- β signaling pathway and stimulation of expression of miR21-5p in hBMSCs.

(E) Seo NR, Lee S-H, Ju K-W, Woo JM, Kim BJ, Kim SM, Jahng JW, Lee JH. Low-frequency pulsed electromagnetic field pretreated bone marrow-derived mesenchymal stem cells promote the regeneration of crush-injured rat mental nerve. Neural Regen Res 13(1):145-153, 2018. (VT, AE, GE)

Bone marrow-derived mesenchymal stem cells (BMSCs) have been shown to promote the regeneration of injured peripheral nerves. Pulsed electromagnetic field (PEMF) reportedly promotes the proliferation and neuronal differentiation of BMSCs. Low-frequency PEMF can induce the neuronal differentiation of BMSCs in the absence of nerve growth factors. This study was designed to investigate the effects of low-frequency PEMF pretreatment on the proliferation and function of BMSCs and the effects of low-frequency PEMF pre-treated BMSCs on the

regeneration of injured peripheral nerve using in vitro and in vivo experiments. In in vitro experiments, quantitative DNA analysis was performed to determine the proliferation of BMSCs, and reverse transcription-polymerase chain reaction was performed to detect S100 (Schwann cell marker), glial fibrillary acidic protein (astrocyte marker), and brain-derived neurotrophic factor and nerve growth factor (neurotrophic factors) mRNA expression. In the in vivo experiments, rat models of crush-injured mental nerve established using clamp method were randomly injected with low-frequency PEMF pretreated BMSCs, unpretreated BMSCs or PBS at the injury site (1×10^6 cells). DiI-labeled BMSCs injected at the injury site were counted under the fluorescence microscope to determine cell survival. One or two weeks after cell injection, functional recovery of the injured nerve was assessed using the sensory test with von Frey filaments. Two weeks after cell injection, axonal regeneration was evaluated using histomorphometric analysis and retrograde labeling of trigeminal ganglion neurons. In vitro experiment results revealed that low-frequency PEMF pretreated BMSCs proliferated faster and had greater mRNA expression of growth factors than unpretreated BMSCs. In vivo experiment results revealed that compared with injection of unpretreated BMSCs, injection of low-frequency PEMF pretreated BMSCs led to higher myelinated axon count and axon density and more DiI-labeled neurons in the trigeminal ganglia, contributing to rapider functional recovery of injured mental nerve. These findings suggest that low-frequency PEMF pretreatment is a promising approach to enhance the efficacy of cell therapy for peripheral nerve injury repair

(E) Seong Y, Moon J, Kim J. Egr1 mediated the neuronal differentiation induced by extremely low-frequency electromagnetic fields. Life Sci. 102(1):16-27, 2014. (VT, LE, GE) (medical application)

AIM: There is a specific frequency of extremely low-frequency electromagnetic field (ELF-EMF) that promotes neuronal differentiation. Although several mechanisms are known to regulate ELF-EMF-induced neuronal differentiation, a key factor that mediates neurogenic potentials by the ELF-EMF is largely unknown. Also, the potential use of ELF-EMF exposure in cell transplantation assays is yet to be determined, including their possible use in ELF-EMF based therapy of neurological diseases. The aim of this study is to understand the underlying mechanisms that mediate ELF-EMF-induced neuronal differentiation and also to harness these mechanisms for cell transplantation assays. MAIN METHOD: Human bone marrow-mesenchymal stem cells (hBM-MSCs) were exposed to ELF-EMF (50 Hz frequency, 1 mT intensity) for 8 days. The hBM-MSC derived neurons were then analyzed by general molecular biology techniques including immunofluorescence and quantitative RT-PCR. To assess changes in gene expression induced by ELF-EMF exposure, we analyzed the transcriptome of neuronal cells after an 8-day ELF-EMF exposure (50 Hz, 1 mT) and compared the transcriptional profiles to control cells. KEY FINDING: We found that early growth response protein 1 (Egr1) is one of the key transcription factors in ELF-EMF-induced neuronal differentiation. In addition, we show that transplantations of ELF-EMF-induced neurons significantly alleviate symptoms in mouse models of neurodegenerative disease. SIGNIFICANCE: These findings indicate that a specific transcriptional factor, Egr1, mediates ELF-EMF-induced neuronal differentiations, and demonstrate the promise of ELF-EMF based cell replacement therapies for neurodegenerative diseases.

(E) Sharma AK, Sah S, Singla SK, Chauhan MS, Manik RS, Palta P. Exposure to Pulsed Electromagnetic Fields Improves the Developmental Competence and Quality of Somatic Cell Nuclear Transfer Buffalo (*Bubalus bubalis*) Embryos Produced Using Fibroblast Cells and Alters Their Epigenetic Status and Gene Expression. Cell Reprogram 23(5):304-315, 2021. (VO, AE, GE)

We examined the effects of treatment with pulsed electromagnetic fields (PEMFs) on cumulus cells and buffalo somatic cell nuclear transfer (SCNT) embryos. PEMF treatment (30 μ T for 3 hours) of cumulus cells increased ($p < 0.05$) the relative cell viability and cell proliferation and the expression level of *OCT4*, *NANOG*, *SOX2*, *P53*, *CCNB1*, and *GPX*, but decreased ($p < 0.05$) that of *DNMT1*, *DNMT3a*, *GSK3b*, and *BAX*, whereas the expression level of *DNMT3b*, *GLUT1*, *BCL2*, *CASPASE3*, *SOD1*, and *CATALASE* was not affected. PEMF treatment of SCNT embryos at the beginning of *in vitro* culture increased ($p < 0.05$) the blastocyst rate ($51.4\% \pm 1.36\%$ vs. $42.8\% \pm 1.29\%$) and decreased ($p < 0.01$) the apoptotic index to the level in *in vitro* fertilization blastocysts, but did not significantly alter the total cell number and the inner cell mass:trophoblast cell number ratio of blastocysts compared to the controls. PEMF treatment increased the expression level of *NANOG*, *SOX2*, *CDX2*, *GLUT1*, *P53*, and *BCL2* and decreased that of *BAX*, *CASPASE3*, *GSK3b*, and *HSP70*, but not *OCT4*, *DNMT1*, *DNMT3a*, *DNMT3b*, *HDAC1*, and *CCNB1* in blastocysts. It increased ($p < 0.001$) the global level of H3K27me3 but not H3K18ac. These results suggest that PEMF treatment of SCNT embryos improves their developmental competence, reduces the level of apoptosis, and alters the expression level of several important genes related to pluripotency, apoptosis, metabolism, and stress.

(Review) Shayeghan M, Forouzesh F, Ansari AM, Javidi MA DNMT1 and miRNAs: possible epigenetics footprints in electromagnetic fields utilization in oncology. Med Oncol 2021 Sep 8;38(10):125.

Many studies were performed to unravel the effects of different types of Electromagnetic fields (EMFs) on biological systems. Some studies were conducted to exploit EMFs for medical purposes mainly in cancer therapy. Although many studies suggest that the EMFs exposures can be effective in pre-clinical cancer issues, the treatment outcomes of these exposures on the cancer cells, especially at the molecular level, are challenging and overwhelmingly complicated yet. This article aims to review the epigenetic mechanisms that can be altered by EMFs exposures with the main emphasis on Extremely low frequency electromagnetic field (ELF-EMF). The epigenetic mechanisms are reversible and affected by environmental factors, thus, EMFs exposures can modulate these mechanisms. According to the reports, ELF-EMF exposures affect epigenetic machinery directly or through the molecular signaling pathways. ELF-EMF in association with DNA methylation, histone modification, miRNAs, and nucleosome remodeling could affect the homeostasis of cancer cells and play a role in DNA damage repairing, apoptosis induction, prevention of metastasis, differentiation, and cell cycle regulation. In general, the result of this study shows that ELF-EMF exposure probably can be effective in cancer epigenetic therapy, but more molecular and clinical investigations are needed to clarify the safe and specific dosimetric characteristics of ELF-EMF in practice.

(NE) Shen Y, Xia R, Jiang H, Chen Y, Hong L, Yu Y, Xu Z, Zeng Q. Exposure to 50Hz-sinusoidal electromagnetic field induces DNA damage-independent autophagy. Int J Biochem Cell Biol. 77(Pt A):72-79, 2016. (VT, AE, GT)

As electromagnetic field (EMF) is commonly encountered within our daily lives, the biological effects of EMF are of great concern. Autophagy is a key process for maintaining cellular homeostasis, and it can also reveal cellular responses to environmental stimuli. In this study, we aim to investigate the biological effects of a 50Hz-sinusoidal electromagnetic field on autophagy and we identified its mechanism of action in Chinese Hamster Lung (CHL) cells. CHL cells were exposed to a 50Hz sinusoidal EMF at 0.4mT for 30min or 24h. In this study, we found that a 0.4mT EMF resulted in: (i) an increase in LC3-II expression and increased autophagosome formation; (ii) no significant difference in the incidence of γ H2AX foci between the sham and exposure groups; (iii) reorganized actin filaments and increased pseudopodial extensions without promoting cell migration; and (iv) enhanced cell apoptosis when autophagy was blocked by Bafilomycin A1. These results implied that DNA damage was not directly involved in the autophagy induced by a 0.4mT 50Hz EMF. In addition, an EMF induced autophagy balanced the cellular homeostasis to protect the cells from severe adverse biological consequences.

(E) Shokrollahi S, Ghanati F, Sajedi RH, Sharifi M. Possible role of iron containing proteins in physiological responses of soybean to static magnetic field. J Plant Physiol. 226:163-171, 2018. (VO, LE, GE, OX)

Iron is a component of many proteins that have crucial roles in plant growth and development, such as ferritin and catalase. Iron also, as a ferromagnetic element, is assumed to be influenced by a static magnetic field (SMF). In the present study, we examined the relationship between ferrous content and gene expression and activity of ferritin and catalase in soybean plants under the influence of 0, 20, and 30 mT SMF for 5 day, 5 h each. Exposure to 20 mT decreased gene expression of Fe transporter, ferrous and H₂O₂ contents and gene expression, content and activity of ferritin and catalase. Opposite responses were observed under 30 mT treatments. The results suggest that SMF triggered a signaling pathway that is mediated by iron. The structure and activity of purified - from bovine liver proteins under SMF were evaluated as well. Secondary structure of proteins were not influenced by SMF (evidenced by far-UV circular dichroism), whereas their tertiary structure, size, and activity were altered (shown by fluorescence spectroscopy and dynamic light-scattering). From these results, it is likely that the number of iron atoms is involved in the nature of influence of SMF on protein structure.

(E) Singh N, Lai H. 60 Hz magnetic field exposure induces DNA crosslinks in rat brain cells. Mutat Res. 400(1-2):313-320, 1998. (VO, AE, GT)

In previous research, we found an increase in DNA strand breaks in brain cells of rats acutely exposed to a 60 Hz magnetic field (for 2 h at an intensity of 0.5 mT). DNA strand breaks were measured with a microgel electrophoresis assay using the length of DNA migration as an index. In the present experiment, we found that most of the magnetic field-induced increase in DNA migration was observed only after proteinase-K treatment,

suggesting that the field caused DNA-protein crosslinks. In addition, when brain cells from control rats were exposed to X-rays, an increase in DNA migration was observed, the extent of which was independent of proteinase-K treatment. However, the X-ray-induced increase in DNA migration was retarded in cells from animals exposed to magnetic fields even after proteinase-K treatment, suggesting that DNA-DNA crosslinks were also induced by the magnetic field. The effects of magnetic fields were also compared with those of a known DNA crosslink-inducing agent mitomycin C. The pattern of effects is similar between the two agents. These data suggest that both DNA-protein and DNA-DNA crosslinks are formed in brain cells of rats after acute exposure to a 60 Hz magnetic field.

(E) Skyberg K, Hansteen IL, Vistnes AI. Chromosomal aberrations in lymphocytes of employees in transformer and generator production exposed to electromagnetic fields and mineral oil. Bioelectromagnetics. 22(3):150-160, 2001. (HU, LE, GT, IX)

The objective was to study the risk of cytogenetic damage among high voltage laboratory workers exposed to electromagnetic fields and mineral oil. This is a cross sectional study of 24 exposed and 24 matched controls in a Norwegian transformer factory. The exposure group included employees in the high voltage laboratory and in the generator soldering department. Electric and magnetic fields and oil mist and vapor were measured. Blood samples were analyzed for chromosomal aberrations in cultured lymphocytes. In addition to conventional cultures, the lymphocytes were also treated with hydroxyurea and caffeine. This procedure inhibits DNA synthesis and repair in vitro, revealing in vivo genotoxic lesions that are repaired during conventional culturing. In conventional cultures, the exposure group and the controls showed similar values for all cytogenetic parameters. In the DNA synthesis- and repair-inhibited cultures, generator welders showed no differences compared to controls. Among high voltage laboratory testers, compared to the controls, the median number of chromatid breaks was doubled (5 vs. 2.5 per 50 cells; $P < 0.05$) the median number of chromosome breaks was 2 vs. 0.5 ($P > 0.05$) and the median number of aberrant cells was 5 vs. 3.5 ($P < 0.05$). Further analysis of the inhibited culture data from this and a previous study indicated that years of exposure and smoking increase the risk of aberrations. We conclude that there was no increase in cytogenetic damage among exposed workers compared to controls in the conventional lymphocyte assay. In inhibited cultures, however, there were indications that electromagnetic fields in combination with mineral oil exposure may produce chromosomal aberrations.

(E) Sobhanifard M, Eftekharian MM, Solgi G, Nikzad S, Salehi I, KG, Ganji M, Zamani A. Effect of Extremely Low Frequency Electromagnetic Fields on Expression of T-bet and GATA-3 Genes and Serum Interferon- γ and Interleukin-4. J Interferon Cytokine Res 39(2):125-131, 2019. (VO, LE, GE)

This study investigated the effect of various magnetic flux densities of extremely low frequency electromagnetic fields (ELF-EMF) on expression of T-box transcription factor (T-bet) and

GATA binding protein-3 (GATA-3) genes in the spleen and thymus of rats injected with human serum albumin (HSA). Moreover, serum levels of interferon (IFN)- γ and interleukin (IL)-4 were evaluated at two phases, that is, prestimulation and poststimulation with HSA. Eighty rats were separated into five groups, and four groups were exposed daily to 50 Hz EMF of 1, 100, 500, and 2000 μ T magnetic flux densities for 60 days. To activate the immune system, 100 μ g HSA was intraperitoneally injected into each rat on days 31, 44, and 58 of the regimen. Splenic and thymic T-bet and GATA-3 messenger RNA (mRNA) expression on day 61 was evaluated by reverse transcription quantitative PCR. Serum IFN- γ and IL-4 (in blood on day 31 before HSA and again on day 61) levels were evaluated by enzyme-linked immunosorbent assay. Expression of T-bet and GATA-3 mRNA was decreased in the spleen in hosts exposed to densities of 1 and 100 μ T. Serum IFN- γ and IL-4 levels were also significantly decreased in 100 μ T-exposed rats, but only at the prestimulation phase. From these findings, it appears that (30 and 60 days) ELF-EMF exposure could suppress the expression of some key genes associated with T helper (Th) cells and on some of their associated functions, that is, the ability to generate (in some cases, spontaneously) select cytokines. Whether this is attributable to effects on Th1/Th2 levels in the hosts and/or due to potential effects of the EMF on cellular functions remains to be determined.

(E) Solek P, Majchrowicz L, Bloniarz D, Krotoszynska E, Kozirowski M. Pulsed or continuous electromagnetic field induce p53/p21-mediated apoptotic signaling pathway in mouse spermatogenic cells in vitro and thus may affect male fertility. Toxicology. 382:84-92, 2017. (VT, AE, GT, OX, RP)

The impact of electromagnetic field (EMF) on the human health and surrounding environment is a common topic investigated over the years. A significant increase in the electromagnetic field concentration arouses public concern about the long-term effects of EMF on living organisms associated with many aspects. In the present study, we investigated the effects of pulsed and continuous electromagnetic field (PEMF/CEMF) on mouse spermatogenic cell lines (GC-1 spg and GC-2 spd) in terms of cellular and biochemical features in vitro. We evaluated the effect of EMF on mitochondrial metabolism, morphology, proliferation rate, viability, cell cycle progression, oxidative stress balance and regulatory proteins. Our results strongly suggest that EMF induces oxidative and nitrosative stress-mediated DNA damage, resulting in p53/p21-dependent cell cycle arrest and apoptosis. Therefore, spermatogenic cells due to the lack of antioxidant enzymes undergo oxidative and nitrosative stress-mediated cytotoxic and genotoxic events, which contribute to infertility by reduction in healthy sperm cells pool. In conclusion, electromagnetic field present in surrounding environment impairs male fertility by inducing p53/p21-mediated cell cycle arrest and apoptosis.

(NE) Song K, Im SH, Yoon YJ, Kim HM, Lee HJ, Park GS. A 60 Hz uniform electromagnetic field promotes human cell proliferation by decreasing intracellular reactive oxygen species levels. PLoS One. 13(7):e0199753, 2018. (VT, CE)

Previously, we showed that exposure of human normal and cancer cells to a 6 mT, 60 Hz gradient electromagnetic field (EMF) induced genotoxicity. Here, we investigated the cellular effects of a uniform EMF. Single or repetitive exposure to a 6 mT, 60 Hz uniform EMF neither induced DNA damage nor affected cell viability in HeLa and primary IMR-90 fibroblasts. However, continuous exposure of these cells to an EMF promoted cell proliferation. Cell

viability increased 24.4% for HeLa and 15.2% for IMR-90 cells after a total 168 h exposure by subculture. This increase in cell proliferation was directly correlated with EMF strength and exposure time. When further incubated without EMF, cell proliferation slowed down to that of unexposed cells, suggesting that the proliferative effect is reversible. The expression of cell cycle markers increased in cells continuously exposed to an EMF as expected, but the distribution of cells in each stage of the cell cycle did not change. Notably, intracellular reactive oxygen species levels decreased and phosphorylation of Akt and Erk1/2 increased in cells exposed to an EMF, suggesting that reduced levels of intracellular reactive oxygen species play a role in increased proliferation. These results demonstrate that EMF uniformity at an extremely low frequency (ELF) is an important factor in the cellular effects of ELF-EMF.

(E) Stankevičiūtė M, Jakubowska M, Pažusienė J, Makaras T, Otremba Z, Urban-Malinga B, Fey DP, Greszkiewicz M, Sauliūtė G, Baršienė J, Andrulewicz E. Genotoxic and cytotoxic effects of 50 Hz 1 mT electromagnetic field on larval rainbow trout (*Oncorhynchus mykiss*), Baltic clam (*Limecola balthica*) and common ragworm (*Hediste diversicolor*). *Aquat Toxicol.* 208:109-117, 2019. (VO, CE, GT)

The aim of this research was to assess genotoxicity and cytotoxicity responses in aquatic animals exposed to 50 Hz 1 m T electromagnetic field (EMF). Rainbow trout (*Oncorhynchus mykiss*) at early stages of development were exposed to EMF for 40 days, whereas marine benthic invertebrates - the common ragworm *Hediste diversicolor* and the Baltic clam *Limecola balthica* - for 12 days. To define genotoxicity and cytotoxicity responses in selected animals, assays of nuclear abnormalities in peripheral blood erythrocytes of *O. mykiss*, coelomocytes of *H. diversicolor* and gill cells of *L. balthica* were performed. Induction of formation of micronuclei (MN), nuclear buds (NB), nuclear buds on filament cells (NBf) and cells with blebbed nuclei (BL) were assessed as genotoxicity endpoints, and 8-shaped nuclei, fragmented (Fr), apoptotic (Ap) and binucleated (BN) cells as cytotoxicity endpoints. Exposure to EMF affected all studied species but with varying degrees. The strongest responses to EMF treatment were elicited in *L. balthica*, in which six out of the total eight analyzed geno- and cytotoxicity endpoints were significantly elevated. Significantly induced frequencies of MN were detected in *O. mykiss* and *H. diversicolor* cells, NBf and BL only in gill cells of *L. balthica*, and NB in analyzed tissues of all the test species. As cytotoxicity endpoints, a significant elevation in frequencies of cells with 8-shaped nuclei was found in *O. mykiss* and *L. balthica*, while Ap and BN was observed only in *L. balthica*. EMF exposure did not induce any significant cytotoxic activity in *H. diversicolor* coelomocytes. The present study is the first to reveal the genotoxic and cytotoxic activity of 1 m T EMF in aquatic animals, and, consequently, the first one to report the adverse effect of this factor on common marine invertebrates and early life stages of fish.

(NE) Stronati L, Testa A, Villani P, Marino C, Lovisolo GA, Conti D, Russo F, Fresegna AM, Cordelli E. Absence of genotoxicity in human blood cells exposed to 50 Hz magnetic fields as assessed by comet assay, chromosome aberration, micronucleus, and sister chromatid exchange analyses. *Bioelectromagnetics.* 25(1):41-48, 2004. (VT, AE, GT)

In the past, epidemiological studies indicated a possible correlation between the exposure to ELF fields and cancer. Public concern over possible hazards associated with exposure to extremely low frequency magnetic fields (ELFMFs) stimulated an increased scientific

research effort. More recent research and laboratory studies, however, have not been able to definitively confirm the correlation suggested by epidemiological studies. The aim of this study was to evaluate the effects of 50 Hz magnetic fields in human blood cells exposed in vitro, using several methodological approaches for the detection of genotoxicity. Whole blood samples obtained from five donors were exposed for 2 h to 50 Hz, 1 mT uniform magnetic field generated by a Helmholtz coil system. Comet assay, sister chromatid exchanges (SCE), chromosome aberrations (CA), and micronucleus (MN) tests were used to assess DNA damage, one hallmark of malignant cell transformation. The effects of a combined exposure with X-rays were also evaluated. Results obtained do not show any significant difference between ELFMFs exposed and unexposed samples. Moreover, no synergistic effect with ionizing radiation has been observed. A slight but significant decrease of cell proliferation was evident in ELFMFs treated samples and samples subjected to the combined exposure.

(NE) Sun C, Wei X, Yimaer A, Xu Z, Chen G. Ataxia telangiectasia mutated deficiency does not result in genetic susceptibility to 50 Hz magnetic fields exposure in mouse embryonic fibroblasts. Bioelectromagnetics. 39(6):476-484, 2018. (VT, AS, GT)

Extremely low frequency magnetic field (ELF-MF) has been classified as a possible carcinogen to humans by the International Agency for Research on Cancer [2002]. However, debate on the genotoxic effects of ELF-MF has continued due to lack of sufficient experimental evidence. Ataxia telangiectasia mutated (ATM) plays a central role in DNA damage repair; its deficiency can result in cellular sensitivity to DNA-damaging agents. To evaluate the genotoxicity of ELF-MF, we investigated the effects of 50 Hz MF on DNA damage in ATM-proficient ($Atm^{+/+}$) mouse embryonic fibroblasts (MEFs) and ATM-deficient ($Atm^{-/-}$) MEFs, a radiosensitive cell line. Results showed no significant difference in average number of γ H2AX foci per cell (9.37 ± 0.44 vs. 9.08 ± 0.28 , $P = 0.58$) or percentage of γ H2AX foci positive cells ($49.22 \pm 1.86\%$ vs. $49.74 \pm 1.44\%$, $P = 0.83$) between sham and exposure groups when $Atm^{+/+}$ MEFs were exposed to 50 Hz MF at 2.0 mT for 15 min. Extending exposure duration to 1 or 24 h did not significantly change γ H2AX foci formation in $Atm^{+/+}$ MEFs. Similarly, the exposure did not significantly affect γ H2AX foci formation in $Atm^{-/-}$ MEFs. Furthermore, 50 Hz MF exposure also did not significantly influence DNA fragmentation, cell viability, or cell cycle progression in either cell types. In conclusion, exposure to 50 Hz MF did not induce significant DNA damage in either $Atm^{+/+}$ or $Atm^{-/-}$ MEFs under the reported experimental conditions.

(E) Sun L, Li X, Ma H, He R, Donkor PO. Global gene expression changes reflecting pleiotropic effects of Irpex lacteus induced by low-intensity electromagnetic field. Bioelectromagnetics. 40(2):104-117, 2019. (VO, LE, GE)

A polysaccharide of *Irpex lacteus*, a white-rot fungus with lignocellulose-degrading activities, has been used as a commercial medicine for nephritis treatment. Previously, a low-intensity electromagnetic field (LI-EMF) was found to increase the biomass and polysaccharide content of *Irpex lacteus* and induce twists on the cell surface. In this study, RNA-sequencing (RNA-seq) technology was used to analyze the underlying mechanism of LI-EMF's influence on *Irpex*

lacteus. We identified 3268, 1377, and 941 differentially expressed genes (DEGs) in the LI-EMF-treated samples at recovery times of 0 h, 3 h, and 6 h, respectively, indicating a significant decline in the influence of the LI-EMF treatment on *Irpex lacteus* with the passage of recovery time. Moreover, 30 upregulated and 14 downregulated DEGs overlapped in the LI-EMF-treated samples at the recovery times of 0 h, 3 h, and 6 h, implying the important lasting effects of LI-EMF. The reliability of the RNA-seq data were validated by quantitative real-time PCR (qRT-PCR). The DEGs related to transcription factors, cell proliferation, cell wall, membrane components, amino acid biosynthesis and metabolism, and polysaccharide biosynthesis and metabolism were significantly enriched in the LI-EMF-treated samples. The experiments confirmed that the LI-EMF treatment significantly increased the content of amino acids with a considerable increase in the content of essential amino acids. Therefore, the global gene expression changes explained the pleiotropic effects of *Irpex lacteus* induced by the LI-EMF treatment. These findings provide the requisite data for the appropriate design and application of LI-EMF in the fermentation of microorganisms to increase production.

(E) Sun L-Y, Hsieh D-K, Lin P-C, Chiu H-T, Chiou TW. Pulsed electromagnetic fields accelerate proliferation and osteogenic gene expression in human bone marrow mesenchymal stem cells during osteogenic differentiation. *Bioelectromagnetics* 31(3):209-19, 2010. (VT, AE, GE)

Osteogenesis is a complex series of events involving the differentiation of mesenchymal stem cells to generate new bone. In this study, we examined the effect of pulsed electromagnetic fields (PEMFs) on cell proliferation, alkaline phosphatase (ALP) activity, mineralization of the extracellular matrix, and gene expression in bone marrow mesenchymal stem cells (BMSCs) during osteogenic differentiation. Exposure of BMSCs to PEMFs increased cell proliferation by 29.6% compared to untreated cells at day 1 of differentiation. Semi-quantitative RT-PCR indicated that PEMFs significantly altered temporal expression of osteogenesis-related genes, including a 2.7-fold increase in expression of the key osteogenesis regulatory gene *cbfa1*, compared to untreated controls. In addition, exposure to PEMFs significantly increased ALP expression during the early stages of osteogenesis and substantially enhanced mineralization near the midpoint of osteogenesis. These results suggest that PEMFs enhance early cell proliferation in BMSC-mediated osteogenesis, and accelerate the osteogenesis.

(E) Sun RG, Chen WF, Qi H, Zhang K, Bu T, Liu Y, Wang SR. Biologic effects of SMF and paclitaxel on K562 human leukemia cells. *Gen Physiol Biophys.* 31(1):1-10, 2012. (VT, AE, GT, IX)

In this study, we evaluated the ability of 8.8 mT static magnetic fields (SMF) to enhance the in vitro action of a chemotherapeutic agent, paclitaxel, against K562 human leukemia cells. We analyzed the cell proliferation, cell cycle distribution, DNA damage and alteration of cell surface and cell organelle ultrastructure after K562 cells were exposed to paclitaxel in the presence or absence of 8.8 mT SMF. The results showed that in the presence of SMF, the efficient concentration of paclitaxel on K562 cells was decreased from 50 to 10 ng/ml. Cell cycle analysis indicated that K562 cells treated with SMF plus paclitaxel were arrested at the G2 phase, which was mainly induced by paclitaxel. Through comet assay, we found that the cell cycle arrest effect

of paclitaxel with or without SMF on K562 cells was correlated with DNA damage. The results of atomic force microscopy and transmission electron microscopy observation showed that the cell ultrastructure was altered in the group treated with the combination of SMF and paclitaxel, holes and protuberances were observed, and vacuoles in cytoplasm were augmented. Our data indicated that the potency of the combination of SMF and paclitaxel was greater than that of SMF or paclitaxel alone on K562 cells, and these effects were correlated with DNA damage induced by SMF and paclitaxel. Therefore, the alteration of cell membrane permeability may be one important mechanism underlying the effects of SMF and paclitaxel on K562 cells.

(E) Sun W, Tan Q, Pan Y, Fu Y, Sun H, Chiang H. Effects of 50-Hz magnetic field exposure on hormone secretion and apoptosis-related gene expression in human first trimester villous trophoblasts in vitro. Bioelectromagnetics 2010 Oct;31(7):566-72. (VT, AE, GE)

Evidence from epidemiological and animal studies showed that exposure to extremely low frequency magnetic fields (ELF-MF) could produce deleterious effects on reproduction. In order to investigate the possible mechanism of MF exposure on reproductive effects, first trimester human chorionic villi at 8-10 weeks' gestation were obtained, and trophoblasts were isolated, cultured, and exposed to a 50-Hz MF for different durations. The human chorionic gonadotropin (hCG) and progesterone in the culture medium was measured by electrochemiluminescence immunoassay. The mRNA levels of apoptosis-related genes bcl-2, bax, caspase-3, p53, and fas in trophoblasts were analyzed using real-time RT-PCR. The results showed that exposure of trophoblasts to MF at 0.2 mT for 72 h did not affect secretion of hCG and progesterone from these cells. There was also no significant change in secretion of these hormones when trophoblasts were exposed to a 0.4 mT MF for 48 h. However, MF significantly inhibited hCG and progesterone secretion of trophoblasts after exposure for 72 h at 0.4 mT. Results of apoptosis-related gene expression analysis showed that, within 72 h of exposure at 0.4 mT, there was no significant difference between MF exposure and control on the expression pattern of each gene. Based on results of the present experiment, it is suggested that exposure to MF for a longer duration (72 h) could inhibit secretion of hCG and progesterone by human first trimester villous trophoblasts, however, the effect might not be related to trophoblast apoptosis.

(E) Suryani L, Too JH, Hassanbhai AM, Wen F, Lin DJ, Yu N, Teoh S-H Effects of Electromagnetic Field on Proliferation, Differentiation, and Mineralization of MC3T3 Cells. Tissue Eng Part C Methods 25(2):114-125, 2019. (VT, AE, GE)

We present the study about how the parameters of pulsed electromagnetic field (PEMF) stimulus affected calvarial osteoblast precursor cell in terms of growth, viability, and differentiation. This research provides insight and foundation to clinical application of noninvasive therapy using PEMF to improve bone regeneration.

(E) Suzuki Y, Ikehata M, Nakamura K, Nishioka M, Asanuma K, Koana T, Shimizu H. Induction of micronuclei in mice exposed to static magnetic fields. Mutagenesis 2001 Nov;16(6):499-501.(VO, AE, GT)

The aim of this experiment was to investigate whether static magnetic fields (SMFs) have cytogenetic effects in mouse bone marrow cells. The frequency of micronuclei was significantly increased by exposure of mice to 3.0 T for 48 and 72 h and 4.7 T for 24, 48 and 72 h. The increase in micronucleus frequency was dose dependent at all times. Micronucleus frequency at 4.7 T was higher than at 3.0 T. We consider that the increased numbers of micronuclei may be attributable to a stress reaction caused by SMFs or a direct clastogenic/spindle disturbance effect of SMFs.

(E) Svedenstal BM, Johanson KJ, Mild KH. DNA damage induced in brain cells of CBA mice exposed to magnetic fields. In Vivo. 13(6):551-552, 1999. (VO, LE, GT)

DNA migration, using single cell gel electrophoresis (comet assay), was studied on brain cells of CBA mice exposed continuously to 50 Hz, 0.5 mT magnetic fields (MF) for 2 hrs, 5 days or 14 days. No differences were observed in the groups MF-exposed for 2 hrs and 5 days compared with controls. However, in the group exposed to MF for 14 days, a significantly extended cell DNA migration was observed ($0.02 < p < 0.05$). These changes together with results from previous studies indicate that magnetic fields may have genotoxic effects in brain cells.

(NE) Szerencsi Á, Kubinyi G, Váliczkó É, Juhász P, Rudas G, Mester Á, Jánossy G, Bakos J, Thuróczy G. DNA integrity of human leukocytes after magnetic resonance imaging. Int J Radiat Biol. 89(10):870-876, 2013. (VT, AE, GT)

PURPOSE: This study focuses on the effects of high-field (3T) magnetic resonance imaging (MRI) scans on the DNA integrity of human leukocytes in vitro in order to validate the study where genotoxic effects were obtained and published by Lee et al. **MATERIALS AND METHODS:** The scanning protocol and exposure situation were the same as those used under routine clinical brain MRI scan. Peripheral blood samples from healthy non-smoking male donors were exposed to electromagnetic fields (EMF) produced by 3T magnetic resonance imaging equipment for 0, 22, 45, 67, and 89 min during the scanning procedure. Samples of positive control were exposed to ionizing radiation (4 Gy of $(60)\text{Co-}\gamma$). Single breaks of DNA in leukocytes were detected by single-cell gel electrophoresis (Comet assay). Chromosome breakage, chromosome loss and micronuclei formations were detected by a micronucleus test (MN). Three independent experiments were performed. **RESULTS:** The data of comet tail DNA%, olive tail moment and micronucleus frequency showed no DNA damages due to MRI exposure. **CONCLUSIONS:** The results of the Comet assay and the micronucleus test indicate that the applied exposure of MRI does not appear to produce breaks in the DNA and has no significant effect on DNA integrity.

(E) Tasset I, Pérez-Herrera A, Medina FJ, Arias-Carrión O, Drucker-Colín R, Túnez I. Extremely low-frequency electromagnetic fields activate the antioxidant pathway Nrf2 in a Huntington's disease-like rat model. Brain Stimul 6(1):84-86, 2013. (VO, LE, GE)

Transcranial magnetic stimulation (TMS) is a non-invasive technique used recently to treat different neuropsychiatric and neurodegenerative disorders. Despite its proven value, the mechanisms through which TMS exerts its beneficial action on neuronal function remain unclear. Recent studies have shown that its beneficial effects may be at least partly due to a neuroprotective effect on oxidative and cell damage. This study shows that TMS can modulate the Nrf2 transcription factor in a Huntington's disease-like rat model induced by 3-nitropropionic acid (3-NP). Western blot analysis demonstrated that 3-NP caused a reduction in Nrf2 in both cytoplasm and nucleus, while TMS applied to 3-NP-treated rats triggered an increase in cytoplasm and nucleus Nrf2 levels. It was therefore concluded that TMS modulates Nrf2 expression and translocation and that these mechanisms may partly explain the neuroprotective effect of TMS, as well as its antioxidant and cell protection capacity.

(E) Teodori L, Giovanetti A, Albertini MC, Rocchi M, Perniconi B, Valente MG, Coletti D. Static magnetic fields modulate X-ray-induced DNA damage in human glioblastoma primary cells. J Radiat Res. 55(2):218-227, 2014. (VT, AE, GT, IX)

Although static magnetic fields (SMFs) are used extensively in the occupational and medical fields, few comprehensive studies have investigated their possible genotoxic effect and the findings are controversial. With the advent of magnetic resonance imaging-guided radiation therapy, the potential effects of SMFs on ionizing radiation (IR) have become increasingly important. In this study we focused on the genotoxic effect of 80 mT SMFs, both alone and in combination with (i.e. preceding or following) X-ray (XR) irradiation, on primary glioblastoma cells in culture. The cells were exposed to: (i) SMFs alone; (ii) XRs alone; (iii) XR, with SMFs applied during recovery; (iv) SMFs both before and after XR irradiation. XR-induced DNA damage was analyzed by Single Cell Gel Electrophoresis assay (comet assay) using statistical tools designed to assess the tail DNA (TD) and tail length (TL) as indicators of DNA fragmentation. Mitochondrial membrane potential, known to be affected by IR, was assessed using the JC-1 mitochondrial probe. Our results showed that exposure of cells to 5 Gy of XR irradiation alone led to extensive DNA damage, which was significantly reduced by post-irradiation exposure to SMFs. The XR-induced loss of mitochondrial membrane potential was to a large extent averted by exposure to SMFs. These data suggest that SMFs modulate DNA damage and/or damage repair, possibly through a mechanism that affects mitochondria.

(NE) Testa A, Cordelli E, Stronati L, Marino C, Lovisolo GA, Fresegna AM, Conti D, Villani P. Evaluation of genotoxic effect of low level 50 Hz magnetic fields on human blood cells using different cytogenetic assays. Bioelectromagnetics. 25(8):613-619, 2004. (VT, AE, GT)

The question whether extremely low frequency magnetic fields (ELFMFs) may contribute to mutagenesis or carcinogenesis is of current interest. In order to evaluate the possible genotoxic effects of ELFMFs, human blood cells from four donors were exposed in vitro for 48 h to 50 Hz, 1 mT uniform magnetic field generated by a Helmholtz coil

system. Comet assay (SCGE), sister chromatid exchanges (SCE), chromosome aberrations (CAs), and micronucleus (MN) test were used to assess the DNA damage. ELF pretreated cells were also irradiated with 1 Gy of X-ray to investigate the possible combined effect of ELFMFs and ionizing radiation. Furthermore, nuclear division index (NDI) and proliferation index (PRI) were evaluated. Results do not evidence any DNA damage induced by ELFMF exposure or any effect on cell proliferation. Data obtained from the combined exposure to ELFMFs and ionizing radiation do not suggest any synergistic or antagonistic effect.

(E) Tai YK, Chan KKW, Fong CHH, Ramanan S, Yap JLY, Yin JN, Yip YS, Tan WR, Koh APF, Tan NS, Chan CW, Huang RYJ, Li JZ, Fröhlich J, Franco-Obregón A. Modulated TRPC1 Expression Predicts Sensitivity of Breast Cancer to Doxorubicin and Magnetic Field Therapy: Segue Towards a Precision Medicine Approach. Front Oncol 11:783803, 2022. (VT, VO, LE, IX, GE)

Chemotherapy is the mainstream treatment modality for invasive breast cancer. Unfortunately, chemotherapy-associated adverse events can result in early termination of treatment. Paradoxical effects of chemotherapy are also sometimes observed, whereby prolonged exposure to high doses of chemotherapeutic agents results in malignant states resistant to chemotherapy. In this study, potential synergism between doxorubicin (DOX) and pulsed electromagnetic field (PEMF) therapy was investigated in: 1) MCF-7 and MDA-MB-231 cells *in vitro*; 2) MCF-7 tumors implanted onto a chicken chorioallantoic membrane (CAM) and; 3) human patient-derived and MCF-7 and MDA-MB-231 breast cancer xenografts implanted into NOD-SCID gamma (NSG) mice. *In vivo*, synergism was observed in patient-derived and breast cancer cell line xenograft mouse models, wherein PEMF exposure and DOX administration individually reduced tumor size and increased apoptosis and could be augmented by combined treatments. In the CAM xenograft model, DOX and PEMF exposure also synergistically reduced tumor size as well as reduced Transient Receptor Potential Canonical 1 (TRPC1) channel expression. *In vitro*, PEMF exposure alone impaired the survival of MCF-7 and MDA-MB-231 cells, but not that of non-malignant MCF10A breast cells; the selective vulnerability of breast cancer cells to PEMF exposure was corroborated in human tumor biopsy samples. Stable overexpression of TRPC1 enhanced the vulnerability of MCF-7 cells to both DOX and PEMF exposure and promoted proliferation, whereas TRPC1 genetic silencing reduced sensitivity to both DOX and PEMF treatments and mitigated proliferation. Chronic exposure to DOX depressed TRPC1 expression, proliferation, and responses to both PEMF exposure and DOX in a manner that was reversible upon removal of DOX. TRPC1 channel overexpression and silencing positively correlated with markers of epithelial-mesenchymal transition (EMT), including *SLUG*, *SNAIL*, *VIMENTIN*, and *E-CADHERIN*, indicating increased and decreased EMT, respectively. Finally, PEMF exposure was shown to attenuate the invasiveness of MCF-7 cells in correlation with TRPC1 expression. We thus demonstrate that the expression levels of TRPC1 consistently predicted breast cancer sensitivity to DOX and PEMF interventions and positively correlated to EMT status, providing an initial rationale for the use of PEMF-based therapies as an adjuvant to DOX chemotherapy for the treatment of breast cancers characterized by elevated TRPC1 expression levels.

(E) Tian F, Nakahara T, Yoshida M, Honda N, Hirose H, Miyakoshi J. Exposure to power frequency magnetic fields suppresses X-ray-induced apoptosis transiently in Ku80-deficient xrs5 cells. Biochem Biophys Res Commun 292(2):355-361, 2002. (VT, AE, GE)

In an attempt to determine whether exposure to extremely low frequency (ELF) electromagnetic fields can affect cells, Ku80-deficient cells (xrs5) and Ku80-proficient cells (CHO-K1) were exposed to ELF electromagnetic fields. Cell survival, and the levels of the apoptosis-related genes p21, p53, phospho-p53 (Ser(15)), caspase-3 and the anti-apoptosis gene bcl-2 were determined in xrs5 and CHO-K1 cells following exposure to ELF electromagnetic fields and X-rays. It was found that exposure of xrs5 and CHO-K1 cells to 60 Hz ELF electromagnetic fields had no effect on cell survival, cell cycle distribution and protein expression. Exposure of xrs5 cells to 60 Hz ELF electromagnetic fields for 5 h after irradiation significantly inhibited G(1) cell cycle arrest induced by X-rays (1 Gy) and resulted in elevated bcl-2 expression. A significant decrease in the induction of p53, phospho-p53, caspase-3 and p21 proteins was observed in xrs5 cells when irradiation by X-rays (8 Gy) was followed by exposure to 5 mT ELF magnetic fields. Exposure of xrs5 cells to the ELF electromagnetic fields for 10 h following irradiation significantly decreased X-ray-induced apoptosis from about 1.7% to 0.7%. However, this effect was not found in CHO-K1 cells within 24 h of irradiation by X-rays alone and by X-rays combined with ELF electromagnetic fields. Exposure of xrs5 cells to 60 Hz ELF electromagnetic fields following irradiation can affect cell cycle distribution and transiently suppress apoptosis by decreasing the levels of caspase-3, p21, p53 and phospho-p53 and by increasing bcl-2 expression.

(E) Tipping DR, K E Chapman, A J Birley, M Anderson Observations on the effects of low frequency electromagnetic fields on cellular transcription in Drosophila larvae reared in field-free conditions. Bioelectromagnetics 20(2):129-131, 1999. (VO, AE, GE)

Drosophila larvae reared inside a micro-metal box with an internal field strength 0.004 microT, were treated with a magnetic field of 50 Hz, 8 microT. for 20 min. Control experienced 0.004 microT. Cellular transcript levels were assessed using slot blots and quantified using a Phosphorimager. Blots were hybridised using probes against HSP 70a, Histone 1.9, and Copia. The low frequency EMFs very significantly decreased transcript levels, indicating that experimental responses may be influenced by previous exposure or lack of previous exposure.

(NE) Tiwari R, Lakshmi NK, Bhargava SC, Ahuja YR. Epinephrine, DNA integrity and oxidative stress in workers exposed to extremely low-frequency electromagnetic fields (ELF-EMFs) at 132 kV substations. Electromagn Biol Med. 34(1):56-62. 2015. (HU, LE, GT, OX)

There is apprehension about widespread use of electrical and electromagnetic gadgets which are supposed to emit electromagnetic radiations. Reports are controversy. These electromagnetic fields (EMFs) have considerable effect on endocrine system of exposed subjects. This study was focused to assess the possible bioeffects of extremely low-frequency (ELF)-EMFs on epinephrine level, DNA damage and oxidative stress in subjects occupationally exposed to 132 kV high-voltage substations. The blood sample of 142 exposed subjects and 151 non-

exposed individuals was analyzed. Plasma epinephrine was measured by enzyme-linked immunosorbent assay, DNA damage was studied by alkaline comet assay along with oxidative stress. Epinephrine levels of sub-groups showed mean concentration of 75.22 ± 1.46 , 64.43 ± 8.26 and 48.47 ± 4.97 for high, medium and low exposed groups, respectively. DNA damage ranged between 1.69 μm and 9.91 μm . The oxidative stress levels showed significant increase. The individuals employed in the live-line procedures were found to be vulnerable for EM stress with altered epinephrine concentrations, DNA damage and increased oxidative stress.

(E) Udroui I, Cristaldi M, Ieradi LA, Bedini A, Giuliani L, Tanzarella C. Clastogenicity and aneuploidy in newborn and adult mice exposed to 50 Hz magnetic fields. Int J Radiat Biol. 82(8):561-567, 2006. (VO, LE, GT, DE)

PURPOSE: To detect possible clastogenic and aneugenic properties of a 50 Hz, 650 μT magnetic field. **MATERIALS AND METHODS:** The micronucleus test with CREST (Calcinosis, Raynaud's phenomenon, Esophageal dysmotility, Sclerodactyly, Telangiectasia) antibody staining was performed on liver and peripheral blood sampled from newborn mice exposed to an ELF (Extremely Low Frequency) magnetic field during the whole intra-uterine life (21 days), and on bone marrow and peripheral blood sampled from adult mice exposed to the same magnetic field for the same period. **RESULTS:** Data obtained in newborn mice show a significant increase in micronuclei frequencies. In absolute terms, most of the induced micronuclei were CREST-negative (i.e., formed by a chromosome fragment). However, in relative terms, ELF exposure caused a two-fold increase in CREST-negative micronuclei and a four-fold increase in CREST-positive micronuclei (i.e., formed by a whole chromosome). No significant effect was recorded on exposed adults. **CONCLUSIONS:** These findings suggest the need for investigation of aneugenic properties of ELF magnetic fields in order to establish a possible relationship to carcinogenesis.

(E) Udroui I, Antoccia A, Tanzarella C, Giuliani L, Pacchierotti F, Cordelli E, Eleuteri P, Villani P, Sgura A. Genotoxicity induced by foetal and infant exposure to magnetic fields and modulation of ionising radiation effects. PLoS One. 10(11):e0142259, 2015. (VO, LE, GT, IX, CS, DE)

BACKGROUND: Few studies have investigated the toxicity and genotoxicity of extremely low frequency magnetic fields (ELF-MF) during prenatal and neonatal development. These phases of life are characterized by cell proliferation and differentiation, which might make them sensitive to environmental stressors. Although in vitro evidences suggest that ELF-MF may modify the effects of ionizing radiation, no research has been conducted so far in vivo on the genotoxic effects of ELF-MF combined with X-rays. **AIM AND METHODS:** Aim of this study was to investigate in somatic and germ cells the effects of chronic ELF-MF exposure from mid gestation until weaning, and any possible modulation produced by ELF-MF exposure on ionizing radiation-induced damage. Mice were exposed to 50 Hz, 65 μT magnetic field, 24 hours/day, for a total of 30 days, starting from 12 days post-conception. Another group was irradiated with 1 Gy X-rays immediately before ELF-MF exposure, other groups were only X-irradiated or sham-exposed. Micronucleus test on blood erythrocytes was performed at multiple times from 1 to 140 days after birth. Additionally, 42 days after birth, genotoxic and cytotoxic effects on male

germ cells were assessed by comet assay and flow cytometric analysis. RESULTS: ELF-MF exposure had no teratogenic effect and did not affect survival, growth and development. The micronucleus test indicated that ELF-MF induced a slight genotoxic damage only after the maximum exposure time and that this effect faded away in the months following the end of exposure. ELF-MF had no effects on ionizing radiation (IR)-induced genotoxicity in erythrocytes. Differently, ELF-MF appeared to modulate the response of male germ cells to X-rays with an impact on proliferation/differentiation processes. These results point to the importance of tissue specificity and development on the impact of ELF-MF on the early stages of life and indicate the need of further research on the molecular mechanisms underlying ELF-MF biological effects.

(E) Urnukhsaikhan E, Cho H, Mishig-Ochir T, Seo YK, Park JK. Life Sci. 2016 Apr 15;151:130-138. (CS, AE, GE)

Pulsed electromagnetic fields (PEMF) are known to affect biological properties such as differentiation, regulation of transcription factor and cell proliferation. However, the cell-protective effect of PEMF exposure is largely unknown. The aim of this study is to understand the mechanisms underlying PEMF-mediated suppression of apoptosis and promotion of survival, including PEMF-induced neuronal differentiation. Treatment of induced human BM-MSCs with PEMF increased the expression of neural markers such as NF-L, NeuroD1 and Tau. Moreover, treatment of induced human BM-MSCs with PEMF greatly decreased cell death in a dose- and time-dependent manner. There is evidence that Akt and Ras are involved in neuronal survival and protection. Activation of Akt and Ras results in the regulation of survival proteins such as Bad and Bcl-xL. Thus, the Akt/Ras signaling pathway may be a desirable target for enhancing cell survival and treatment of neurological disease. Our analyses indicated that PEMF exposure dramatically increased the activity of Akt, Rsk, Creb, Erk, Bcl-xL and Bad via phosphorylation. PEMF-dependent cell protection was reversed by pretreatment with LY294002, a specific inhibitor of phosphatidylinositol 3-kinase (PI3K). Our data suggest that the PI3K/Akt/Bad signaling pathway may be a possible mechanism for the cell-protective effects of PEMF.

(E) Vergallo C, Panzarini E, Tenuzzo BA, Mariano S, Tata AM, Dini L. Moderate Static Magnetic Field (6 mT)-Induced Lipid Rafts Rearrangement Increases Silver NPs Uptake in Human Lymphocytes. Molecules. 25(6):1398, 2020. (VT, AE, GE)

One of the most relevant drawbacks in medicine is the ability of drugs and/or imaging agents to reach cells. Nanotechnology opened new horizons in drug delivery, and silver nanoparticles (AgNPs) represent a promising delivery vehicle for their adjustable size and shape, high-density surface ligand attachment, etc. AgNPs cellular uptake involves different endocytosis mechanisms, including lipid raft-mediated endocytosis. Since static magnetic fields (SMFs) exposure induces plasma membrane perturbation, including the rearrangement of lipid rafts, we investigated whether SMF could increase the amount of AgNPs able to pass the peripheral blood lymphocytes (PBLs) plasma membrane. To this purpose, the effect of 6-mT SMF exposure on the redistribution of two main lipid raft components (i.e., disialoganglioside GD3, cholesterol) and on AgNPs uptake efficiency was investigated. Results showed that 6 mT SMF: (i) induces a time-

dependent GD3 and cholesterol redistribution in plasma membrane lipid rafts and modulates gene expression of ATP-binding cassette transporter A1 (ABCA1), (ii) increases reactive oxygen species (ROS) production and lipid peroxidation, (iii) does not induce cell death and (iv) induces lipid rafts rearrangement, that, in turn, favors the uptake of AgNPs. Thus, it derives that SMF exposure could be exploited to enhance the internalization of NPs-loaded therapeutic or diagnostic molecules.

(NE) Verschaeve L, Anthonissen R, Grudniewska M, Wudarski J, Gevaert L, Maes A. Genotoxicity investigation of ELF-magnetic fields in Salmonella typhimurium with the sensitive SOS-based VITOTOX test. Bioelectromagnetics. 32(7):580-584, 2011. (VT, AE, GT, IX)

We performed a genotoxicity investigation of extremely low-frequency (ELF) magnetic fields (MFs, 50 Hz, 100 and 500 μ T, 1 and 2 h exposure) alone and in combination with known chemical mutagens using the VITOTOX test. This test is a very sensitive reporter assay of Salmonella typhimurium bacteria based on the SOS response. Our study showed that ELF-MFs do not induce SOS-based mutagenicity in S. typhimurium bacteria and do not show any synergetic effect when combined with chemical mutagens.

(NE) Verschaeve L, Wambacq S, Anthonissen R, Maes A. Co-exposure of ELF-magnetic fields and chemical mutagens: An investigation of genotoxicity with the SOS-based VITOTOX test in Salmonella typhimurium. Mutat Res. 795:31-35, 2016. (VT, AE, GT)

It is believed that extreme low frequency magnetic fields (ELF-MF) are not mutagenic, at least at exposure levels below 100 μ T. Synergistic or co-operative effects with environmental mutagens remain possible yet. We therefore investigated the effects of ELF-MF in conjunction with 4 different well known chemical mutagens having different modes of action. For this purpose the bacterial Vitotox test was used. Our study confirmed previous results which showed that a 100 μ T magnetic field (50 Hz) does not damage DNA and hence is not mutagenic in this assay and that there was also no influence on the DNA damaging capacity of the used mutagens.

(E) Vianale G, M Reale, P Amerio, M Stefanachi, S Di Luzio, R Muraro. Extremely low frequency electromagnetic field enhances human keratinocyte cell growth and decreases proinflammatory chemokine production. Br J Dermatol 158(6):1189-1196, 2008.(VT, AE, GE)

Background: Proliferation and differentiation of keratinocytes are central processes in tissue regeneration after injury. Chemokines, produced by a wide range of cell types including keratinocytes, play a regulatory role in inflammatory skin diseases. Several studies have shown that an electromagnetic field (EMF) can influence both inflammatory processes and repair mechanisms including wound healing on different tissue models. **Objectives:** To elucidate the effect of extremely low frequency EMF (ELF-EMF) on keratinocyte proliferation and production of chemokines [RANTES, monocyte chemoattractant protein (MCP)-1, macrophage

inflammatory protein (MIP)-1 alpha and interleukin (IL)-8] in order to evaluate a potential therapeutic use of magnetic fields. **Methods:** The human keratinocyte cell line HaCaT was exposed at 1 mT, 50 Hz for different lengths of time and compared with unexposed control cells. Cell growth and viability were evaluated at different exposure times by cell count and trypan blue exclusion. Chemokine production and expression were analysed by enzyme-linked immunosorbent assay (ELISA) and by real-time polymerase chain reaction. Total NF-kappaB p65 was quantified by ELISA. **Results:** Significantly increased growth rates were observed after 48 h of EMF exposure as compared with control cells, while no difference in cell viabilities were detected. Gene expression and release of RANTES, MCP-1, MIP-1 alpha and IL-8 were significantly reduced after 72 h of exposure. NF-kappaB levels became almost undetectable after only 1 h of EMF exposure, and were inversely correlated with cell density. **Conclusions:** Our results show that ELF-EMF modulates chemokine production and keratinocyte growth through inhibition of the NF-kappaB signalling pathway and thus may inhibit inflammatory processes. ELF-EMF could represent an additional therapeutic approach in the treatment of skin injury.

(E)Villarini M, Moretti M, Scassellati-Sforzolini G, Boccioli B, Pasquini R. Effects of co-exposure to extremely low frequency (50 Hz) magnetic fields and xenobiotics determined in vitro by the alkaline comet assay. *Sci Total Environ.* 361(1-3):208-219, 2006. (VT, AE, GT, IX)

In the present study, we used human peripheral blood leukocytes from 4 different donors, to investigate in vitro the possible genotoxic and/or co-genotoxic activity of extremely low frequency magnetic fields (ELF-MF) at 3 mT intensity. Two model mutagens were used to study the possible interaction between ELF-MF and xenobiotics: N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and 4-nitroquinoline N-oxide (4NQO). Primary DNA damage was evaluated by the alkaline single-cell microgel-electrophoresis ("comet") assay. Control cells (leukocytes not exposed to ELF-MF, nor treated with genotoxins) from the different blood donors showed a comparable level of basal DNA damage, whereas the contribution of individual susceptibility toward ELF-MF and the tested genotoxic compounds led to differences in the extent of DNA damage observed following exposure to the genotoxins, both in the presence and in the absence of an applied ELF-MF. A 3 mT ELF-MF alone was unable to cause direct primary DNA damage. In leukocytes exposed to ELF-MF and genotoxins, the extent of MNNG-induced DNA damage increased with exposure duration compared to sham-exposed cells. The opposite was observed in cells treated with 4NQO. In this case the extent of 4NQO-induced DNA damage was somewhat reduced in leukocytes exposed to ELF-MF compared to sham-exposed cells. Moreover, in cells exposed to ELF-MF an increased concentration of GSH was always observed, compared to sham-exposed cells. Since following GSH conjugation the genotoxic pattern of MNNG and 4NQO is quite different, an influence of ELF-MF on the activity of the enzyme involved in the synthesis of GSH leading to different activation/deactivation of the model mutagens used was hypothesized to explain the different trends observed in MNNG and 4NQO genotoxic activity in the presence of an applied ELF-MF. The possibility that ELF-MF might interfere with the genotoxic activity of xenobiotics has important implications, since

human populations are likely to be exposed to a variety of genotoxic agents concomitantly with exposure to this type of physical agent.

(E) Villarini M, Ambrosini MV, Moretti M, Dominici L, Taha E, Piobbico D, Gambelunghe C, Mariucci G. Brain hsp70 expression and DNA damage in mice exposed to extremely low frequency magnetic fields: a dose-response study. Int J Radiat Biol. 89(7):562-570, 2013. (VO, LE, GT)

Purpose: To determine whether a dose-response relationship exists among exposure to extremely low frequency magnetic fields (ELF-MF) at different densities and 70-kDa heat shock protein (hsp70) expression and DNA damage in mouse brain. Materials and Methods: Male CD1 mice were exposed to ELF-MF (50 Hz; 0.1, 0.2, 1 or 2 mT) for 7 days (15 hours/day) and sacrificed either at the end of exposure or after 24 h. Hsp70 expression was determined in cerebral cortex-striatum, hippocampus and cerebellum by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) and western blot analysis. Primary DNA damage was evaluated in the same tissues by comet assay. Sham-exposed mice were used as controls. Results: No changes in both hsp70 mRNA and corresponding protein occurred following exposure to ELF-MF, except for a weak increase in the mRNA in hippocampus of exposed mice to 0.1 mT ELF-MF. Only mice exposed to 1 or 2 mT and sacrificed immediately after exposure presented DNA strand breaks higher than controls in all the cerebral areas; such DNA breakage reverted to baseline in the mice sacrificed 24 h after exposure. Conclusions: These data show that high density ELF-MF only induce reversible brain DNA damage while they do not affect hsp70 expression.

(E) Villarini M, Dominici L, Fatigoni C, Levorato S, Vannini S, Monarca S, Moretti M. Primary DNA damage in welders occupationally exposed to extremely-low-frequency magnetic fields (ELF-MF). Ann Ig. 27(3):511-519, 2015. (HU, LE, GT) (GT less than controls)

BACKGROUND: Electric arc welding is known to involve considerable exposure to extremely-low-frequency magnetic fields (ELF-MF; 50 Hz). The aim of the present study was to evaluate individual exposure to ELF-MF during arc welding and to assess the eventually associated genotoxic hazard by evaluating primary DNA damage. METHODS: The study group comprised 21 electric arc welders (exposed) and 21 non-exposed control subjects (healthy blood donors). Occupational exposure to ELF-MF was measured using personal dosimeters worn during one complete work-shift (7 am to 5 pm). The extent of primary DNA damage was measured in peripheral blood leukocytes with the standard procedure of the alkaline comet assay. RESULTS: Tail length showed to have similar values in welders and controls. Whereas, the data showed a significant decrease for tail intensity ($p = 0.01$) and tail moment ($p = 0.02$) counts in exposed subjects compared to controls. CONCLUSIONS: The different results of our present study and published investigations from other research groups reporting positive results in the comet assay might be a result of different chromium and/or nickel (or other metals) exposure levels, which lead to DNA-protein cross-links at lower concentrations and DNA single-strand breakages at higher concentrations. Since these results are derived from a small-scale pilot study, a larger scale study should be undertaken.

(NE) Villarini M, Gambelunghe A, Giustarini D, Ambrosini MV, Fatigoni C, Rossi R, Dominici L, Levorato S, Muzi G, Piobbico D, Mariucci G. No evidence of DNA damage by co-exposure to extremely low frequency magnetic fields and aluminum on neuroblastoma cell lines. *Mutat Res.* 823:11-21, 2017.(VT, AE, GT)

Whether exposure to 50-60Hz extremely low frequency magnetic fields (ELF-MF) exerts neurotoxic effects is a debated issue. Analogously, the potential role of Aluminum (Al) in neurodegeneration is a matter of controversial debate. As all living organisms are exposed to ELF-MF and/or Al daily, we found investigating the early effects of co-exposure to ELF-MF and Al in SH-SY5Y and SK-N-BE-2 human neuroblastoma (NB) cells intriguing. SH-SY5Y and SK-N-BE-2 cells underwent exposure to 50Hz ELF-MF (0.01, 0.1 or 1mT) or AlCl₃ (4 or 40μM) or co-exposure to 50Hz ELF-MF and AlCl₃ for 1h continuously or 5h intermittently. The effects of the treatment were evaluated in terms of DNA damage, redox status changes and Hsp70 expression. The DNA damage was assessed by Comet assay; the cellular redox status was investigated by measuring the amount of reduced glutathione (GSH) and glutathione disulfide (GSSG) while the inducible Hsp70 expression was evaluated by western blot analysis and real-time RT-PCR. Neither exposure to ELF-MF or AlCl₃ alone induced DNA damage, changes in GSH/GSSG ratio or variations in Hsp70 expression with respect to the controls in both NB cell lines. Similarly, co-exposure to ELF-MF and AlCl₃ did not have any synergic toxic effects. The results of this in vitro study, which deals with the effects of co-exposure to 50Hz MF and Aluminum, seem to exclude that short-term exposure to ELF-MF in combination with Al can have harmful effects on human SH-SY5Y and SK-N-BE-2 cells.

(E) Vincenzi F, Targa M, Corciulo C, Gessi S, Merighi S, Setti S, Cadossi R, Borea PA, Varani K. The anti-tumor effect of A3 adenosine receptors is potentiated by pulsed electromagnetic fields in cultured neural cancer cells. *PLoS One* 2012;7(6):e39317. (VT, AE, GE)

A(3) adenosine receptors (ARs) play a pivotal role in the development of cancer and their activation is involved in the inhibition of tumor growth. The effects of pulsed electromagnetic fields (PEMFs) on cancer have been controversially discussed and the detailed mechanisms are not yet fully understood. In the past we have demonstrated that PEMFs increased A(2A) and A(3)AR density and functionality in human neutrophils, human and bovine synoviocytes, and bovine chondrocytes. In the same cells, PEMF exposure increased the anti-inflammatory effect mediated by A(2A) and/or A(3)ARs. The primary aim of the present study was to evaluate if PEMF exposure potentiated the anti-tumor effect of A(3)ARs in PC12 rat adrenal pheochromocytoma and U87MG human glioblastoma cell lines in comparison with rat cortical neurons. Saturation binding assays and mRNA analysis revealed that PEMF exposure up-regulated A(2A) and A(3)ARs that are well coupled to adenylate cyclase activity and cAMP production. The activation of A(2A) and A(3)ARs resulted in the decrease of nuclear factor-kappa B (NF-κB) levels in tumor cells, whilst only A(3)ARs are involved in the increase of p53 expression. A(3)AR stimulation mediated an inhibition of tumor cell proliferation evaluated by thymidine incorporation. An increase of cytotoxicity by lactate dehydrogenase (LDH) release and apoptosis by caspase-3 activation in PC12 and U87MG cells, but not in cortical neurons, was

observed following A(3)AR activation. The effect of the A(3)AR agonist in tumor cells was enhanced in the presence of PEMFs and blocked by using a well-known selective antagonist. Together these results demonstrated that PEMF exposure significantly increases the anti-tumor effect modulated by A(3)ARs.

(E) Vincenzi F, Targa M, Corciulo C, Gessi S, Merighi S, Setti S, Cadossi R, Borea PA, Varani K. The anti-tumor effect of A3 adenosine receptors is potentiated by pulsed electromagnetic fields in cultured neural cancer cells. PLoS One 2012;7(6):e39317. (VT, AE, GE)

A(3) adenosine receptors (ARs) play a pivotal role in the development of cancer and their activation is involved in the inhibition of tumor growth. The effects of pulsed electromagnetic fields (PEMFs) on cancer have been controversially discussed and the detailed mechanisms are not yet fully understood. In the past we have demonstrated that PEMFs increased A(2A) and A(3)AR density and functionality in human neutrophils, human and bovine synoviocytes, and bovine chondrocytes. In the same cells, PEMF exposure increased the anti-inflammatory effect mediated by A(2A) and/or A(3)ARs. The primary aim of the present study was to evaluate if PEMF exposure potentiated the anti-tumor effect of A(3)ARs in PC12 rat adrenal pheochromocytoma and U87MG human glioblastoma cell lines in comparison with rat cortical neurons. Saturation binding assays and mRNA analysis revealed that PEMF exposure up-regulated A(2A) and A(3)ARs that are well coupled to adenylate cyclase activity and cAMP production. The activation of A(2A) and A(3)ARs resulted in the decrease of nuclear factor-kappa B (NF-kB) levels in tumor cells, whilst only A(3)ARs are involved in the increase of p53 expression. A(3)AR stimulation mediated an inhibition of tumor cell proliferation evaluated by thymidine incorporation. An increase of cytotoxicity by lactate dehydrogenase (LDH) release and apoptosis by caspase-3 activation in PC12 and U87MG cells, but not in cortical neurons, was observed following A(3)AR activation. The effect of the A(3)AR agonist in tumor cells was enhanced in the presence of PEMFs and blocked by using a well-known selective antagonist. Together these results demonstrated that PEMF exposure significantly increases the anti-tumor effect modulated by A(3)ARs.

(NE) Vinod E, Kachroo U, Rebekah G, Thomas S, Ramasamy B. In vitro chondrogenic differentiation of human articular cartilage derived chondroprogenitors using pulsed electromagnetic field. J Clin Orthop Trauma 2020 Oct 8;14:22-28. (VT, LE, AE, GE)

Background: The ability to grow new cartilage remains the standard goal of any treatment strategy directed at cartilage repair. Chondroprogenitors have garnered interest due to their applicability in cell therapy. Pulsed electromagnetic field (PEMF) favors chondrogenesis by possible upregulation of genes belonging to TGFβ superfamily. Since TGFβ is implicated in chondrogenic signalling, the aim of the study was to evaluate the ability of PEMF to induce chondrogenesis via endogenous TGFβ production in chondroprogenitors vs differentiation using chondrogenic medium inclusive of TGFβ. Methods: Chondroprogenitors were harvested from three non-diseased human knee joints via fibronectin assay. Passage 3 pellets were subjected to four different culture conditions: a) negative control contained chondrogenic medium without

TGF β 2, b) positive control contained medium with TGF β 2, c) PEMF 1 contained medium of negative control plus single exposure to PEMF and d) PEMF 2 contained medium of negative control plus multiple exposures to PEMF. Following differentiation (day 21), pellets were assessed for gene expression of ACAN, SOX9, COL2A1, TGF β 1, TGF β 2, and TGF β 3. Alcian blue staining to detect glycosaminoglycan deposition was also performed. Medium supernatant was used to detect endogenous latent TGF- β 1 levels using ELISA. Results: All study arms exhibited comparable gene expression without any significant difference. Although positive control and PEMF study arms demonstrated notably better staining than negative control, the level of latent TGF- β 1 was seen to be significantly high in supernatant from positive control ($P < 0.05$) when compared to other groups. Conclusion: Our results indicate that PEMF induced chondrogenesis might involve other signalling molecules, which require further evaluation.

(E) Wahab MA, Podd JV, Rapley BI, Rowland RE. Elevated sister chromatid exchange frequencies in dividing human peripheral blood lymphocytes exposed to 50 Hz magnetic fields. Bioelectromagnetics. 28(4):281-288, 2007. (VT, AE, GT, WS)

The in vitro cytomolecular technique, sister chromatid exchange (SCE), was applied to test the clastogenic potentiality of extremely low frequency (ELF) electromagnetic fields (EMFs) on human peripheral blood lymphocytes (HPBLs). SCE frequencies were scored in dividing peripheral blood lymphocytes (PBLs) from six healthy male blood donors in two rounds of experiments, R1 and R2, to determine reproducibility. Lymphocyte cultures in the eight experiments conducted in each round were exposed to 50 Hz sinusoidal (continuous or pulsed) or square (continuous or pulsed) MFs at field strengths of 1 microT or 1 mT for 72 h. A significant increase in the number of SCEs/cell in the grouped experimental conditions compared to the controls was observed in both rounds. The highest SCE frequency in R1 was 10.03 for a square continuous field, and 10.39 for a square continuous field was the second highest frequency in R2. DNA crosslinking at the replication fork is proposed as a model which could explain the mechanistic link between ELF EMF exposure and increased SCE frequency.

(E) Walther M, Mayer F, Kafka W, Schütze N Effects of weak, low-frequency pulsed electromagnetic fields (BEMER type) on gene expression of human mesenchymal stem cells and chondrocytes: an in vitro study. Electromagn Biol Med 2007; 26(3):179-190. (VT, AE, GE)

In vitro effects of electromagnetic fields appear to be related to the type of electromagnetic field applied. Previously, we showed that human osteoblasts display effects of BEMER type electromagnetic field (BTEMF) on gene regulation. Here, we analyze effects of BTEMF on gene expression in human mesenchymal stem cells and chondrocytes. Primary mesenchymal stem cells from bone marrow and the chondrocyte cell line C28I2 were stimulated 5 times at 12-h intervals for 8 min each with BTEMF. RNA from treated and control cells was analyzed for gene expression using the affymetrix chip HG-U133A. A limited number of regulated gene products from both cell types mainly affect cell metabolism and cell matrix structure. There was no increased expression of cancer-related genes. RT-PCR analysis of selected transcripts partly confirmed array data. Results indicate that BTEMF in human mesenchymal stem cells and

chondrocytes provide the first indications to understanding therapeutic effects achieved with BTEMF stimulation.

(E) Wang J, Cui J, Zhu H. Suppression of type I collagen in human scleral fibroblasts treated with extremely low-frequency electromagnetic fields. Mol Vis 19:885-893, 2013. (VT, AE, GE)

Purpose: To investigate the expression differences of type I collagen (COL1A1) and its underlying mechanisms in human fetal scleral fibroblasts (HFSFs) that were treated with conditioned medium from retinal pigment epithelial (RPE) cells under extremely low-frequency electromagnetic fields (ELF-EMFs). **Methods:** The ELF-EMFs used in this study were established by slidac and artificial coils. Growth of the treated HFSFs was evaluated by a cell-counting kit-8 assay. The expression of COL1A1 and matrix metalloproteinases-2 (MMP-2) in the treated HFSFs was detected by reverse transcription PCR (RT-PCR) and western blot, and the expression of transforming growth factor- β 2 (TGF- β 2) and basic fibroblast growth factor-2 (FGF-2) in RPE cells exposed to EMFs was detected by RT-PCR. The expression of COL1A1 and MMP-2 in HFSFs was further confirmed by immunofluorescence staining. Activation of extracellular signal-regulated kinase 1/2 (ERK1/2 also called p44/p42 mitogen-activated protein kinases [MAPK]) and p38 in HFSFs was measured by western blot. **Results:** We found that exposure to ELF-EMFs resulted in a decreased proliferation rate of HFSFs and that addition of RPE supernatant medium could enhance this effect. Compared with that of the control cells, a significant decrease in collagen synthesis was detected in HFSFs under ELF-EMFs. However, the expression of MMP-2 was upregulated, which could be further enhanced via an RPE supernatant additive. The activities of ERK1/2 and p38 were significantly increased in HFSFs exposed to ELF-EMFs, and this effect could be enhanced by RPE supernatant medium additive. **Conclusions:** Our results suggested that ELF-EMFs can inhibit the expression of type I collagen in HFSFs and contribute to the remodeling of the sclera.

(E) Wang L, Li Y, Xie S, Huang J, Song K, He C. Effects of Pulsed Electromagnetic Field Therapy at Different Frequencies on Bone Mass and Microarchitecture in Osteoporotic Mice. Bioelectromagnetics 2021 Sep;42(6):441-454. (VT, LE, GE, WS)

A pulsed electromagnetic field (PEMF) can promote osteogenesis. However, studies have shown variation in the signal characteristics in terms of waveform type, intensity, frequency, and treatment duration. Among the factors that affect electromagnetic fields, frequency plays a major role. However, few studies have investigated the effects of PEMF at different frequencies in osteoporotic mice. Therefore, our objective was to determine the effect of PEMF frequency in osteoporotic mice. Forty 3-month-old female mice were randomly divided into the following five groups: sham, OVX, and OVX followed by 1.6-mT PEMF exposure groups (8 Hz, 50 Hz, and 75 Hz, 1.6 mT). The PEMF was applied for 1 h/day, 7 days/week, for 4 weeks. After 4 weeks, the micro-computed tomography showed that PEMF with (50 and 75 Hz) ameliorated the deterioration of bone microarchitecture. Improvements in the bone histological analysis were identified for PEMF with 50 and 75 Hz groups compared with the ovariectomy (OVX) controls. Osteoclast numbers were decreased in PEMF with (50 and 75 Hz). Moreover, the real-time PCR demonstrated PEMF with (50 and 75 Hz) significantly promoted the expression of the

osteoblast-related genes (ALP, OCN, Runx2), and increased the serum PINP. PEMF with (50 and 75 Hz) exerted significant inhibitory effects on the osteoclast-related mRNA expression (CTSK, NFATc1, TRAP) and bone resorption markers CTX-I and IL-1 β . Taken together, our results showed that PEMF at 50 and 75 Hz with 1.6 mT significantly ameliorate the deterioration of bone microarchitecture in OVX mice. The inhibitory effect of PEMF may be associated with IL-1 β inhibition.

(E) Wang Q, Wu W, Han X, Zheng A, Lei S, Wu J, Chen H, He C, Luo F, Liu X. Osteogenic differentiation of amniotic epithelial cells: synergism of pulsed electromagnetic field and biochemical stimuli. BMC Musculoskelet Disord 15:271, 2014. (VT, LE, GE)

Background: Pulsed electromagnetic field (PEMF) is a non-invasive physical therapy used in the treatment of fracture nonunion or delayed healing. PEMF can facilitate the osteogenic differentiation of bone marrow mesenchymal stem cells *in vitro*. Amniotic epithelial cells (AECs) have been proposed as a potential source of stem cells for cell therapy. However, whether PEMF could modulate the osteogenic differentiation of AECs is unknown. In the present study, the effects of PEMF on the osteogenic differentiation of AECs were investigated. **Methods:** AECs were isolated from amniotic membrane of human placenta by trypsin digestion and were induced by PEMF and/or osteo-induction medium. After 21 days we used real time RT-PCR and immunocytochemistry to study the expression of osteoblast markers. The signal transduction of osteogenesis was further investigated. **Results:** The PEMF stimulation, or osteo-induction medium alone could induce osteogenic differentiation of AECs, as shown by expression of osteoblast specific genes and proteins including alkaline phosphatase and osteocalcin. Furthermore, a combination of PEMF and osteo-induction medium had synergy effects on osteogenic differentiation. In our study, the gene expression of BMP-2, Runx2, β -catenin, Nrf2, Keap1 and integrin β 1 were up-regulated in the osteogenic differentiation of AECs induced by PEMF and/or osteo-induction medium. **Conclusions:** Combined application of PEMF and osteo-induction medium is synergistic for the osteogenic differentiation of AECs. It might be a novel approach in the bone regenerative medicine.

(NE) Wang Y, Liu X, Zhang Y, Wan B, Zhang J, He W, Hu D, Yang Y, Lai J, He M, Chen C. Exposure to a 50 Hz magnetic field at 100 μ T exerts no DNA damage in cardiomyocytes. Biol Open. 8(8). pii: bio041293, 2019. (VT, VO, AE, OX, GT)

The effects of exposure to magnetic fields (MFs) at electric frequencies (50-60 Hz) on carcinogenicity are still in debate. Whether exposure to MFs affects the heart is also a debated issue. This study aimed to determine whether exposure to extremely low frequency MFs (ELF-MFs) induced DNA damage in cardiomyocytes both *in vitro* and *in vivo*. Human ventricular cardiomyocytes were exposed to 50 Hz ELF-MF at 100 μ T for 1 h continuously or 75 min intermittently. The effects of the treatments were evaluated by DNA damage, redox status changes and relative signal molecular expression. Moreover, ten male Sprague-Dawley rats were exposed to a 50 Hz MF at 100 μ T for 7 days, while another 10 rats were sham exposed. The protein levels of p53 and Hsp70 in heart tissue were analyzed by western blot. The results showed that exposure to ELF-MF did not induce DNA damage, changes to cell cycle distribution or increased reactive oxygen species level. No significant differences were detected in p53 and

Hsp70 expression level between the ELF-MF and sham-exposure groups both *in vitro* and *in vivo*. All these data indicate that MFs at power-frequency may not cause DNA damage in cardiomyocytes.

(E) Wang Y, Sun Y, Zhang Z, Li Z, Zhang H, Liao Y, Tang C, Cai P. Enhancement in the ATP level and antioxidant capacity of *Caenorhabditis elegans* under continuous exposure to extremely low-frequency electromagnetic field for multiple generations. Int J Radiat Biol 2020 Oct 12;1-8. doi: 10.1080/09553002.2020.1828657. Online ahead of print.(VO, LE, GE, OX)

Purpose: Safety concerns about the effects of long-term extremely low-frequency electromagnetic field (ELF-EMF) exposure on human health have been raised. To explore the effects of continuous exposure to ELF-EMF on organisms for multiple generations, we selected *Caenorhabditis elegans* as a model organism and conducted long-term continuous exposure studies for multiple generations under 20 °C, 50 Hz, and 3 mT ELF-EMF. **Materials and methods:** Each generation of worms was treated with ELF-EMF from the egg in the same environment. After long-term exposure to ELF-EMF, the body length of the worms was detected, and 15th generation adult worms were selected as the research object. The ATP level and ATPase were detected, and the expression levels of genes encoding ATP synthase (*r53.4*, *hpo-18*, *atp-5*, *unc-32*, *atp-3*) were detected by RT-PCR. In worm's antioxidant system, the level of reactive oxygen species (ROS) was detected by dichlorofluorescein staining, and the total antioxidant capacity (T-AOC), superoxide dismutase (SOD) and catalase (CAT) activity were investigated. The expression of genes encoding superoxide dismutase (*sod-1*, *sod-2*, *sod-3*) was detected in adult (60 h) worms of the fifteenth generation (F15). **Results:** These results showed that the body length of F15 worms increased significantly, ATP content increased significantly, ATP synthase activity was significantly enhanced, and the expression levels of the *r53.4*, *hpo-18*, *atp-5*, and *atp-3* genes encoding ATPase were significantly upregulated in F15 worms. In addition, SOD activity increased significantly, and the expression levels of the *sod-1*, *sod-2*, and *sod-3* genes encoding SOD were also significantly upregulated in F15 worms. **Conclusions:** These results indicated that continuous exposure to 50 Hz, 3 mT ELF-EMF for multiple generations can increase the body length of worms, induce the synthesis of ATP and enhance the antioxidant capacity of worms.

(E) Wang Z, Sarje A, Che PL, Yarema KJ. Moderate strength (0.23-0.28 T) static magnetic fields (SMF) modulate signaling and differentiation in human embryonic cells. BMC Genomics. 10:356, 2009. (VT, AE, GE)

BACKGROUND: Compelling evidence exists that magnetic fields modulate living systems. To date, however rigorous studies have focused on identifying the molecular-level biosensor (e.g., radical ion pairs or membranes) or on the behavior of whole animals leaving a gap in understanding how molecular effects are translated into tissue-wide and organism-level responses. This study begins to bridge this gulf by investigating static magnetic fields (SMF) through global mRNA profiling in human embryonic cells coupled with software analysis to identify the affected signaling pathways. **RESULTS:** Software analysis of gene expression in cells exposed to 0.23-0.28 T SMF showed that nine signaling networks responded to SMF; of these, detailed biochemical validation was performed for the network linked to the inflammatory

cytokine IL-6. We found the short-term (<24 h) activation of IL-6 involved the coordinate up-regulation of toll-like receptor-4 (TLR4) with complementary changes to NEU3 and ST3GAL5 that reduced ganglioside GM3 in a manner that augmented the activation of TLR4 and IL-6. Loss of GM3 also provided a plausible mechanism for the attenuation of cellular responses to SMF that occurred over longer exposure periods. Finally, SMF-mediated responses were manifest at the cellular level as morphological changes and biochemical markers indicative of pre-oligodendrocyte differentiation. **CONCLUSION:** This study provides a framework describing how magnetic exposure is transduced from a plausible molecular biosensor (lipid membranes) to cell-level responses that include differentiation toward neural lineages. In addition, SMF provided a stimulus that uncovered new relationships - that exist even in the absence of magnetic fields - between gangliosides, the time-dependent regulation of IL-6 signaling by these glycosphingolipids, and the fate of embryonic cells.

(E) Wei J, Shi Q, Xiong L, Xin G, Yi T, Xiao Y, Huang W. Transcriptome profiling of cells exposed to particular and intense electromagnetic radiation emitted by the "SG-III" prototype laser facility. Sci Rep 2021 Jan 21;11(1):2017. (VT, AE, GE)

The experiment of inertial confinement fusion by the "ShengGuang (SG)-III" prototype laser facility is a transient and extreme reaction process within several nanoseconds, which could form a very complicated and intense electromagnetic field around the target chamber of the facility and may lead to harmful effect on people around. In particular, the biological effects arising from such specific environment field could hardly be ignored and have never been investigated yet, and thus, we reported on the investigation of the biological effects of radiation on HaCat cells and PC12 cells to preliminarily assess the biological safety of the target range of the "SG-III" prototype laser facility. The viability revealed that the damage of cells was dose-dependent. Then we compared the transcriptomes of exposed and unexposed PC12 cells by RNA-Seq analysis based on Illumina Novaseq 6000 platform and found that most significantly differentially expressed genes with corresponding Gene Ontology terms and pathways were strongly involved in proliferation, transformation, necrosis, inflammation response, apoptosis and DNA damage. Furthermore, we find increase in the levels of several proteins responsible for cell-cycle regulation and tumor suppression, suggesting that pathways or mechanisms regarding DNA damage repair are quickly activated. It was found that "SG-III" prototype radiation could induce DNA damage and promote apoptotic necrosis.

(NE) Williams PA, Ingebretsen RJ, Dawson RJ. 14.6 mT ELF magnetic field exposure yields no DNA breaks in model system Salmonella, but provides evidence of heat stress protection. Bioelectromagnetics. 27(6):445-450, 2006. (VT, AE, GT, IX)

In this study, we demonstrate that common extremely low frequency magnetic field (MF) exposure does not cause DNA breaks in this Salmonella test system. The data does, however, provide evidence that MF exposure induces protection from heat stress. Bacterial cultures were exposed to MF (14.6 mT 60 Hz field, cycled 5 min on, 10 min off for 4 h) and a temperature-matched control. Double- and single-stranded DNA breaks were assayed using a recombination event counter. After MF or control exposure they were grown on indicator plates from which recombination events can be quantified and the frequency of DNA strand breaks deduced. The

effect of MF was also monitored using a recombination-deficient mutant (*recA*). The results showed no significant increase in recombination events and strand breaks due to MF. Evidence of heat stress protection was determined using a cell viability assay that compared the survival rates of MF exposed and control cells after the administration of a 10 min 53 degrees C heat stress. The control cells exhibited nine times more cell mortality than the MF exposed cells. This Salmonella system provides many mutants and genetic tools for further investigation of this phenomenon.

(E) Wilson JW, Haines J, Sienkiewicz Z, Dubrova YE. The effects of extremely low frequency magnetic fields on mutation induction in mice. *Mutat Res.* 773:22-26, 2015. (VO, AE, GT)

The growing human exposure to extremely low frequency (ELF) magnetic fields has raised a considerable concern regarding their genotoxic effects. The aim of this study was to evaluate the in vivo effects of ELF magnetic fields irradiation on mutation induction in the germline and somatic tissues of male mice. Seven week old BALB/c×CBA/Ca F1 hybrid males were exposed to 10, 100 or 300µT of 50Hz magnetic fields for 2 or 15h. Using single-molecule PCR, the frequency of mutation at the mouse Expanded Simple Tandem Repeat (ESTR) locus Ms6-hm was established in sperm and blood samples of exposed and matched sham-treated males. ESTR mutation frequency was also established in sperm and blood samples taken from male mice exposed to 1Gy of acute X-rays. The frequency of ESTR mutation in DNA samples extracted from blood of mice exposed to magnetic fields did not significantly differ from that in sham-treated controls. However, there was a marginally significant increase in mutation frequency in sperm but this was not dose-dependent. In contrast, acute exposure X-rays led to significant increases in mutation frequency in sperm and blood of exposed males. The results of our study suggest that, within the range of doses analyzed here, the in vivo mutagenic effects of ELF magnetic fields are likely to be minor if not negligible.

(E)Winker R, Ivancsits S, Pilger A, Adlkofer F, Rudiger HW. Chromosomal damage in human diploid fibroblasts by intermittent exposure to extremely low-frequency electromagnetic fields. *Mutat Res.* 585(1-2):43-49, 2005. (VT, AE, GT)

Environmental exposure to extremely low-frequency electromagnetic fields (ELF-EMFs) has been implicated in the development of cancer in humans. An important basis for assessing a potential cancer risk due to ELF-EMF exposure is knowledge of biological effects on human cells at the chromosomal level. Therefore, we investigated in the present study the effect of intermittent ELF electromagnetic fields (50 Hz, sinusoidal, 5'field-on/10'field-off, 2-24 h, 1 mT) on the induction of micronuclei (MN) and chromosomal aberrations in cultured human fibroblasts. ELF-EMF radiation resulted in a time-dependent increase of micronuclei, which became significant after 10 h of intermittent exposure at a flux density of 1 mT. After approximately 15 h a constant level of micronuclei of about three times the basal level was reached. In addition, chromosomal aberrations were increased up to 10-fold above basal levels. Our data strongly indicate a clastogenic potential of intermittent low-frequency electromagnetic

fields, which may lead to considerable chromosomal damage in dividing cells.

(E) Wolf FI, Torsello A, Tedesco B, Fasanella S, Boninsegna A, D'Ascenzo M, Grassi C, Azzena GB, Cittadini A. 50-Hz extremely low frequency electromagnetic fields enhance cell proliferation and DNA damage: possible involvement of a redox mechanism. *Biochim Biophys Acta.* 1743(1-2):120-129, 2005. (VT, AE, GT, OX)

HL-60 leukemia cells, Rat-1 fibroblasts and WI-38 diploid fibroblasts were exposed for 24-72 h to 0.5-1.0-mT 50-Hz extremely low frequency electromagnetic field (ELF-EMF). This treatment induced a dose-dependent increase in the proliferation rate of all cell types, namely about 30% increase of cell proliferation after 72-h exposure to 1.0 mT. This was accompanied by increased percentage of cells in the S-phase after 12- and 48-h exposure. The ability of ELF-EMF to induce DNA damage was also investigated by measuring DNA strand breaks. A dose-dependent increase in DNA damage was observed in all cell lines, with two peaks occurring at 24 and 72 h. A similar pattern of DNA damage was observed by measuring formation of 8-OHdG adducts. The effects of ELFEMF on cell proliferation and DNA damage were prevented by pretreatment of cells with an antioxidant like alpha-tocopherol, suggesting that redox reactions were involved. Accordingly, Rat-1 fibroblasts that had been exposed to ELF-EMF for 3 or 24 h exhibited a significant increase in dichlorofluorescein-detectable reactive oxygen species, which was blunted by alpha-tocopherol pretreatment. Cells exposed to ELF-EMF and examined as early as 6 h after treatment initiation also exhibited modifications of NF kappa B related proteins (p65-p50 and I kappa B alpha), which were suggestive of increased formation of p65-p50 or p65-p65 active forms, a process usually attributed to redox reactions. These results suggest that ELF-EMF influence proliferation and DNA damage in both normal and tumor cells through the action of free radical species. This information may be of value for appraising the pathophysiologic consequences of an exposure to ELF-EMF.

(E) Wu S, Yu Q, Lai A, Tian J. Pulsed electromagnetic field induces Ca²⁺-dependent osteoblastogenesis in C3H10T1/2 mesenchymal cells through the Wnt-Ca²⁺/Wnt-β-catenin signaling pathway. *Biochem Biophys Res Commun* 503(2):715-721, 2018. (VT, LE, GE)

Pulsed electromagnetic fields (PEMFs) are effective in healing fractures and improving osteoporosis. However, their effect on mesenchymal cells remains largely unknown. In this study, the effects of PEMF on osteoblastogenesis and its underlying molecular signaling mechanisms were systematically investigated in C3H10T1/2 cells. C3H10T1/2 mesenchymal cells were exposed to 30-Hz PEMF bursts at various intensities for 3 consecutive days. The optimal PEMF exposure (30 Hz, 1 mT, 2 h/day) was applied in subsequent experiments. Our results suggest that intracellular [Ca²⁺]_i in C3H10T1/2 cells can be upregulated upon exposure to PEMF and that PEMF-induced C3H10T1/2 cell differentiation was Ca²⁺-dependent. The pro-osteogenic effect of PEMF on Ca²⁺-dependent osteoblast differentiation was then verified by alkaline phosphatase (ALP) and von Kossa staining. Furthermore, PEMF promoted the gene

expression and protein synthesis of the Wnt/ β -catenin pathway. Increased $[Ca^{2+}]_i$ in the nucleoplasm was followed by the mobilization and translocation of β -catenin into the nucleus in C3H10T1/2 cells. A model of Wnt/ β -catenin signaling and the Wnt/ Ca^{2+} signaling network is proposed. Taken together, these findings indicated for the first time that PEMF induces osteoblastogenesis through increased intracellular $[Ca^{2+}]_i$ and the Wnt- Ca^{2+} /Wnt- β -catenin signaling pathway in C3H10T1/2 mesenchymal cells.

(E) Xu C, Yin X, Lv Y, Wu C, Zhang Y, Song T. A near-null magnetic field affects cryptochrome-related hypocotyl growth and flowering in Arabidopsis. Adv Space Res 4(5):834-840, 2012. (VO, AE, GE)

The blue light receptor, cryptochrome, has been suggested to act as a magnetoreceptor based on the proposition that photochemical reactions are involved in sensing the geomagnetic field. But the effects of the geomagnetic field on cryptochrome remain unclear. Although the functions of cryptochrome have been well demonstrated for *Arabidopsis*, the effect of the geomagnetic field on the growth of *Arabidopsis* and its mechanism of action are poorly understood. We eliminated the local geomagnetic field to grow *Arabidopsis* in a near-null magnetic field and found that the inhibition of *Arabidopsis* hypocotyl growth by white light was weakened, and flowering time was delayed. The expressions of three cryptochrome-signaling-related genes, *PHYB*, *CO* and *FT* also changed; the transcript level of *PHYB* was elevated ca. 40%, and that of *CO* and *FT* was reduced ca. 40% and 50%, respectively. These data suggest that the effects of a near-null magnetic field on *Arabidopsis* are cryptochrome-related, which may be revealed by a modification of the active state of cryptochrome and the subsequent signaling cascade.

(E) Xu C, Feng S, Yu Y, Zhang Y, Wei S. Near-Null Magnetic Field Suppresses Fruit Growth in Arabidopsis. Bioelectromagnetics 42(7):593-602, 2021. (VO, LE, GE)

We previously found that a near-null magnetic field affected reproductive growth in *Arabidopsis* under white light. To test whether the effect of a near-null magnetic field on fruit growth of *Arabidopsis* is related to cryptochrome, we grew wild-type *Arabidopsis* and cryptochrome double mutant, *cry1/cry2*, in a near-null magnetic field under blue light. We found that fruit growth of wild-type *Arabidopsis* instead of the *cry1/cry2* mutant was suppressed by the near-null magnetic field. Furthermore, gibberellin (GA) levels of GA_4 , GA_9 , GA_{34} , and GA_{51} in fruits of wild-type plants in the near-null magnetic fields were significantly lower than local geomagnetic field controls. However, in *cry1/cry2* mutants, levels of the four detected GAs in fruits in the near-null magnetic fields did not differ significantly from controls. Expressions of GA20-oxidase (GA20ox) genes (*GA20ox1* and *GA20ox2*) and GA3-oxidase (GA3ox) genes (*GA3ox1* and *GA3ox3*) in fruits of wild-type plants rather than *cry1/cry2* mutants were downregulated by the near-null magnetic field. In contrast, expressions of GA2-oxidase (GA2ox) genes and GA signaling genes were not affected by the near-null magnetic field. These results indicate that

suppression of fruit growth by the near-null magnetic field is mediated by cryptochrome and that GAs are involved in the regulation of fruit growth by the near-null magnetic field.

(E) Yagci F, Kesim B. Cytotoxic and genotoxic effects on gingival fibroblasts from static magnetic fields produced by dental magnetic attachments. Gerodontology. 33(3):421-427, 2016. (VT, AE, GT)

Objective: To investigate cytotoxic and genotoxic effects of static magnetic field (SMF) produced by dental magnetic attachments on human gingival fibroblasts in vitro. **Background:** Magnetic attachments have numerous roles in dental prosthesis fixation, but few reports evaluate possible biological effects of static magnetic field (SMF) on human gingival tissues, particular genotoxic effects. **Materials and methods:** The Dyna (500-gr breakaway force) and Steco (173-gr breakaway force) dental magnetic attachments were embedded into autopolymerising acrylic resin in four different configurations each, including single and double magnets. Gingival biopsy was performed on 28 individuals during third molar extraction, and each sample was divided into two pieces for culture under SMF exposure or as a control. In total, seven test and seven control gingival fibroblast cultures were performed for each group resulting in 56 gingival fibroblast cultures. The test culture flasks were placed atop the magnet-embedded resin blocks. After cultures were terminated, mitotic index (MI) and micronucleus (MN) rates were analysed at a $p = 0.05$ significance level by Wilcoxon's test; intergroup differences were analysed with a Kruskal-Wallis test. **Results:** There was no significant difference in intragroup or intergroup MI rates. The double Dyna ($p = 0.023$) and double Steco ($p = 0.016$) groups had statistically significant intragroup differences in the MN rates. There were no statistically significant differences in MN rates in intergroup analyses. **Conclusion:** In particular, higher magnetic fields from dental magnetic attachments might be toxic genetically to human gingival fibroblasts. However, there is need for further investigations from different aspects to detect any genotoxicity.

(E) Yaguchi H, Yoshida M, Ejima Y, Miyakoshi J. Effect of high-density extremely low frequency magnetic field on sister chromatid exchanges in mouse m5S cells. Mutat Res. 440(2):189-194, 1999. (VT, AE, GT)

The induction of sister chromatid exchanges (SCEs) was evaluated in the cultured mouse m5S cells after exposure to extremely low frequency magnetic field (ELFMF; 5, 50 and 400 mT). Exposure to 5 mT and 50 mT ELFMF led to a very small increase in the frequency of SCEs, but no significant difference was observed between exposed and unexposed control cells. The cells exposed to 400 mT ELFMF exhibited a significant elevation of the SCE frequencies. There was no significant difference between data from treatments with mitomycin-C (MMC) alone and from combined treatments of MMC plus ELFMF (400 mT) at any MMC concentrations from 4 to 40 nM. These results suggest that exposure to highest-density ELFMF of 400 mT may induce DNA damage, resulting in an elevation of the SCE frequencies. We suppose that there may be a threshold for the elevation of the SCE frequencies, that is at least over the magnetic density of 50 mT.

(E) Yaguchi H, Yoshida M, Ding GR, Shingu K, Miyakoshi J. Increased chromatid-type chromosomal aberrations in mouse m5S cells exposed to power-line frequency magnetic fields. Int J Radiat Biol. 76(12):1677-1684, 2000.(VT, AE, GT)

Purpose: To investigate the induction of chromosomal aberrations in mouse m5S cells after exposure to power-line frequency magnetic fields (extremely low frequency magnetic fields; ELFMF) at high-flux densities. **Material and method:** m5S cells were either untreated or pretreated during the G1 phase with mitomycin C (MMC, 1 microM) for 1 h or 3 Gy X-rays, and then exposed to ELFMF at three different flux densities (5 and 50 mT at 60 Hz, 400 mT at 50 Hz) for 40 h. Unexposed control cells were incubated for the same period in a conventional CO2 incubator. Chromosomal aberrations were analysed in the first post-treatment metaphases. Cell kinetics were assessed by DNA flow cytometry and the mitotic index. **Results and conclusions:** ELFMF enhanced the formation of spontaneous and MMC- or X-ray-induced chromosomal aberrations, in a flux-density-dependent manner. Statistically significant increases in the frequency of chromosomal aberrations were observed in cells exposed to 400 mT ELFMF with respect to unexposed controls. The aberrations induced by ELFMF were mostly chromatid-type, not chromosome-type. The cells exposed to 400 mT ELFMF exhibited a three-fold higher level of chromatid-type aberrations than did the unexposed cells. Flow cytometric and mitotic index analyses revealed that the S or G2 arrest following MMC or X-irradiation was more profound in ELFMF-exposed cells than in unexposed cells. Our results suggest that ELFMF can interfere with post-replication repair, resulting in increased levels of chromatid-type chromosomal aberrations induced spontaneously and by DNA damaging agents.

(E) Yao L, Li Y, Knapp J, Smith P. (2015). Exploration of molecular pathways mediating electric field-directed Schwann cell migration by RNA-seq. J Cell Physiol 230(7):1515-1524. (VT, AE, GE)

In peripheral nervous systems, Schwann cells wrap around axons of motor and sensory neurons to form the myelin sheath. Following spinal cord injury, Schwann cells regenerate and migrate to the lesion and are involved in the spinal cord regeneration process. Transplantation of Schwann cells into injured neural tissue results in enhanced spinal axonal regeneration. Effective directional migration of Schwann cells is critical in the neural regeneration process. In this study, we report that Schwann cells migrate anodally in an applied electric field (EF). The directedness and displacement of anodal migration increased significantly when the strength of the EF increased from 50 mV/mm to 200 mV/mm. The EF did not significantly affect the cell migration speed. To explore the genes and signaling pathways that regulate cell migration in EFs, we performed a comparative analysis of differential gene expression between cells stimulated with an EF (100 mV/mm) and those without using next-generation RNA sequencing, verified by RT-qPCR. Based on the cut-off criteria ($FC > 1.2$, $q < 0.05$), we identified 1,045 up-regulated and 1,636 down-regulated genes in control cells versus EF-stimulated cells. A Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis found that compared to the control group, 21 pathways are down-regulated, while 10 pathways are up-regulated. Differentially expressed genes participate in multiple cellular signaling pathways involved in the regulation of cell migration, including pathways of regulation of actin cytoskeleton, focal adhesion, and PI3K-Akt.

(E) Yao F, Li Z, Cheng L, Zhang L, Zha X, Jing J Low frequency pulsed electromagnetic field promotes differentiation of oligodendrocyte precursor cells through upregulation of miR-219-5p in vitro. Life Sci 223:185-193, 2019. (VT, LE, GE)

Aim: Spinal cord injury (SCI) is a common demyelinating disorder of the central nervous system. The differentiation of oligodendrocyte precursor cells (OPCs) into mature oligodendrocytes (OLs), which induce myelination, plays a critical role in the functional recovery following SCI. In this study, the effect of low frequency pulsed electromagnetic field (PEMF) on the differentiation of OPCs and the potential underlying mechanisms were investigated. **Main methods:** OPCs were randomly divided into the PEMF and non-PEMF (NPEMF) groups. Immunofluorescence and western blot assays were performed to assess the expression levels of OLs stage-specific markers after 3, 7, 14, and 21 days of PEMF or NPEMF exposure. qRT-PCR was used to further assess the expression levels of miR-219-5p, miR-338, miR-138, and miR-9, which are associated with OPCs differentiation, and the expression levels of genes associated with miR-219-5p. Finally, following PEMF or NPEMF exposure, qRT-PCR and western blot assays were performed to explore the relationship between miR-219-5p and Lingo1 and between miR-219-5p and PEMF in promoting OPCs differentiation. **Key findings:** PEMF promoted the differentiation of OPCs. PEMF upregulated the expression level of miR-219-5p and downregulated the expression level of Lingo1 during the differentiation of OPCs. Under PEMF exposure, miR-219-5p targeted Lingo1 and reversed the inhibitory effect of miR-219-5p inhibitor on OPCs differentiation. In addition, PEMF synergized with miR-219-5p to promote OPCs differentiation. **Significance:** Our results, for the first time, indicated that PEMF promoted OPCs differentiation by regulating miR-219-5p activity in vitro.

(E) Yin C, Luo X, Duan Y, Duan W, Zhang H, He Y, Sun G, Sun X. Neuroprotective effects of lotus seedpod procyanidins on extremely low frequency electromagnetic field-induced neurotoxicity in primary cultured hippocampal neurons. Biomed Pharmacother. 82:628-639, 2016. (VT, LE, GT, OX)

The present study investigated the protective effects of lotus seedpod procyanidins (LSPCs) on extremely low frequency electromagnetic field (ELF-EMF)-induced neurotoxicity in primary cultured rat hippocampal neurons and the underlying molecular mechanism. The results of MTT, morphological observation, superoxide dismutase (SOD) and malondialdehyde (MDA) assays showed that compared with control, incubating neurons under ELF-EMF exposure significantly decreased cell viability and increased the number of apoptotic cells, whereas LSPCs evidently protected the hippocampal neurons against ELF-EMF-induced cell damage. Moreover, a certain concentration of LSPCs inhibited the elevation of intracellular reactive oxygen species (ROS) and Ca²⁺ level, as well as prevented the disruption of mitochondrial membrane potential induced by ELF-EMF exposure. In addition, supplementation with LSPCs could alleviate DNA damage, block cell cycle arrest at S phase, and inhibit apoptosis and necrosis of hippocampal neurons under ELF-EMF exposure. Further study demonstrated that LSPCs up-regulated the activations of Bcl-2, Bcl-xl proteins and suppressed the expressions of Bad, Bax proteins caused by ELF-EMF exposure. In conclusion, these findings revealed that LSPCs protected against ELF-EMF-induced neurotoxicity through inhibiting oxidative stress and mitochondrial apoptotic pathway.

(E)Yokus B, Cakir DU, Akdag MZ, Sert C, Mete N. Oxidative DNA damage in rats exposed to extremely low frequency electro magnetic fields. Free Radic Res. 39(3):317-323, 2005. (VT, AE, GT)

Extremely low frequency (ELF) electromagnetic field (EMF) is thought to prolong the life of free radicals and can act as a promoter or co-promoter of cancer. 8-hydroxy-2'-deoxyguanosine (8OHdG) is one of the predominant forms of radical-induced lesions to DNA and is a potential tool to assess the cancer risk. We examined the effects of extremely low frequency electro magnetic field (ELF-EMF) (50 Hz, 0.97 mT) on 8OHdG levels in DNA and thiobarbituric acid reactive substances (TBARS) in plasma. To examine the possible time-dependent changes resulting from magnetic field, 8OHdG and TBARS were quantitated at 50 and 100 days. Our results showed that the exposure to ELF-EMF induced oxidative DNA damage and lipid peroxidation (LPO). The 8OHdG levels of exposed group (4.39±0.88 and 5.29±1.16 8OHdG/dG.10⁵), respectively) were significantly higher than sham group at 50 and 100 days (3.02±0.63 and 3.46±0.38 8OHdG/dG.10⁵) (p<0.001, p<0.001). The higher TBARS levels were also detected in the exposure group both on 50 and 100 days (p<0.001, p<0.001). In addition, the extent of DNA damage and LPO would depend on the exposure time (p<0.05 and p<0.05). Our data may have important implications for the long-term exposure to ELFEMF which may cause oxidative DNA damage.

(E) Yukus B, Akdag MZ, Dasdag S, Cakir DU, Kizil M. Extremely low frequency magnetic fields cause oxidative DNA damage in rats. Int J Radiat Biol. 84(10):789-795, 2008. (VO, LE, GT, OX)

PURPOSE: To detect the genotoxic effects of extremely low frequency (ELF) -magnetic fields (MF) on oxidative DNA base modifications [8-hydroxyguanine (8-OH-Gua), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) and 4,6-diamino-5-formamidopyrimidine (FapyAde)] in rat leucocytes, measured following exposure to ELF-MF. **MATERIALS AND METHODS:** After exposure to ELF-MF (50 Hz, 100 and 500 microT, for 2 hours/day during 10 months), DNA was extracted, and measurement of DNA lesions was achieved by gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS). **RESULTS:** Levels of FapyAde, FapyGua and 8OHdG in DNA were increased by both 100 microT and 500 microT ELF-MF as compared to a cage-control and a sham group; however, statistical significance was observed only in the group exposed to 100 microT. **CONCLUSION:** This is the first study to report that ELF-MF exposure generates oxidatively induced DNA base modifications which are mutagenic in mammalian cells, such as FapyGua, FapyAde and 8-OH-Gua, in vivo. This may explain previous studies showing DNA damage and genomic instability. These findings support the hypothesis that chronic exposure to 50-Hz MF may be potentially genotoxic. However, the intensity of ELF-MF has an important influence on the extent of DNA damage.

(E) Yoon HE, Lee JS, Myung SH, Lee YS. Increased γ -H2AX by exposure to a 60-Hz magnetic fields combined with ionizing radiation, but not hydrogen peroxide, in non-tumorigenic human cell lines. Int J Radiat Biol. 90(4):291-298, 2014. (VT, AE, GT, IX)

Purpose: Genotoxic effects have been considered the gold standard to determine if an environmental factor is a carcinogen, but the currently available data for extremely low frequency time-varying magnetic fields (ELF-MFs) remain controversial. As an environmental stimulus, the effect of ELF-MF on cellular DNA may be subtle. Therefore, a more sensitive method and systematic research strategy are warranted to evaluate genotoxicity. Materials and methods: We investigated the effect of ELF-MFs in combination with ionizing radiation (IR) or H₂O₂ on the DNA damage response of expression of phosphorylated H2AX (γ -H2AX) and production of γ -H2AX foci in non-tumorigenic human cell systems consisting of human lung fibroblast WI38 cells and human lung epithelial L132 cells. Results: Exposure to a 60-Hz, 2 mT ELF-MFs for 6 h produced increased γ -H2AX expression, as well as γ -H2AX foci production, a common DNA double-strand break (DSB) marker. However, exposure to a 1 mT ELF-MFs did not have the same effect. Moreover, 2 mT ELF-MFs exposure potentiated the expression of γ -H2AX and γ -H2AX foci production when combined with IR, but not when combined with H₂O₂. Conclusions: ELF-MFs could affect the DNA damage response and, in combination with different stimuli, provide different effects on γ -H2AX.

(E) Yuan LQ, Wang C, Lu DF, Zhao XD, Tan LH, Chen X. Induction of apoptosis and ferroptosis by a tumor suppressing magnetic field through ROS-mediated DNA damage. Aging (Albany NY). 2020 Feb 18;12. doi: 10.18632/aging.102836. [Epub ahead of print] (VT, LE, GT, OX)

Magnetic field (MF) is being used in antitumor treatment; however, the underlying biological mechanisms remain unclear. In this study, the potency and mechanism of a previously published tumor suppressing MF exposure protocol were further investigated. This protocol, characterized as a 50 Hz electromagnetic field modulated by static MF with time-average intensity of 5.1 mT, when applied for 2 h daily for over 3 consecutive days, selectively inhibited the growth of a broad spectrum of tumor cell lines including lung cancer, gastric cancer, pancreatic cancer and neuroblastoma. The level of intracellular reactive oxygen species (ROS) increased shortly after field exposure and persisted. Subsequently, pronounced DNA damage and activation of DNA repair pathways were identified both in vitro and in vivo. Furthermore, use of free radical scavenger alleviated DNA damage and partially reduced cell death. Finally, this field was found to inhibit cell proliferation, and simultaneously induced two types of programmed cell death, apoptosis and ferroptosis. In conclusion, this tumor suppressing MF could determine cell fate through ROS-induced DNA damage, inducing oxidative stress and activation of the DNA damage repair pathways, eventually lead to apoptosis and ferroptosis, as well as inhibition of tumor growth.

Zaporozhan V, Ponomarenko A Zaporozhan V, Ponomarenko A Mechanisms of geomagnetic field influence on gene expression using influenza as a model system: basics of physical epidemiology. Int J Environ Res Public Health. 7(3):938-965, 2010. (Review)

Recent studies demonstrate distinct changes in gene expression in cells exposed to a weak magnetic field (MF). Mechanisms of this phenomenon are not understood yet. We propose that

proteins of the Cryptochrome family (CRY) are "epigenetic sensors" of the MF fluctuations, i.e., magnetic field-sensitive part of the epigenetic controlling mechanism. It was shown that CRY represses activity of the major circadian transcriptional complex CLOCK/BMAL1. At the same time, function of CRY, is apparently highly responsive to weak MF because of radical pairs that periodically arise in the functionally active site of CRY and mediate the radical pair mechanism of magnetoreception. It is known that the circadian complex influences function of every organ and tissue, including modulation of both NF-kappaB- and glucocorticoids- dependent signaling pathways. Thus, MFs and solar cycles-dependent geomagnetic field fluctuations are capable of altering expression of genes related to function of NF-kappaB, hormones and other biological regulators. Notably, NF-kappaB, along with its significant role in immune response, also participates in differential regulation of influenza virus RNA synthesis. Presented data suggests that in the case of global application (example-geomagnetic field), MF-mediated regulation may have epidemiological and other consequences.

(E) Zavareh FA, Abdi S, Entezari M. Up-regulation of miR-144 and miR-375 in the human gastric cancer cell line following the exposure to extremely low-frequency electromagnetic fields. Int J Radiat Biol 2021 Jun 14;1-26. doi: 10.1080/09553002.2021.1941376. Online ahead of print. (VT, AE, GE)

Purpose: Recently, therapeutic effects of extremely low-frequency electromagnetic field (ELF-EMF), as complementary and alternative medicine, used in the oncology field to control disease symptoms and life quality improvement. Micro RNAs (miRs) expression changes in response to ELF-EMFs were detected in some research projects. MiRs are responsible for the post-transcriptional regulation of gene expression in the cell. This study aimed to evaluate the expression changes of miR-144 and miR-375 in the human gastric adenocarcinoma cell line (AGS) under the exposure of ELF-EMF. **Materials and methods:** After 24 h pre-incubation, AGS cells were exposed to 50 Hz ELF-EMF with a magnetic flux density of 0.2 and 2 mT for 18 h, continuously and discontinuously (1.5h on/1.5h off). A separate sham exposure group was used for each exposure condition. Cell viability was evaluated by MTT assay. Changes of miR-144 expression levels in AGS cells immediately after exposure and 18 and 36 h after the exposure cut off was calculated by QRT-PCR. **Results:** The cell viability of AGS cells was decreased under the exposure of 0.2 and 2 mT EMFs when compared to the control. Up-regulation of miR-144 and miR-375 were observed in AGS cells under the exposure of continuous and discontinuous magnetic flux densities of 0.2 and 2 mT. The results indicated that the miR levels were significantly decreased 18 and 36 h after finishing the exposure, but not reached the normal range. **Conclusions:** The results of this investigation indicated that weak and moderate intermittent 50 Hz ELF-EMFs can induce changes in miRNA expression. Given the role of miR-144 in cell proliferation and tumor suppressor role of miR-375 in cancer cells, overexpression of these two miRs under the exposure of ELF-EMF could be effective in growth inhibition and controlling gastric cancer cells. Changes in gene expression are largely reversible after the magnetic field is cut off.

(E) Zendehdel R, Yu IJ, Hajipour-Verdom B, Panjali Z. DNA effects of low level occupational exposure to extremely low frequency electromagnetic fields (50/60 Hz). Toxicol Ind Health. 35(6):424-430, 2019. (HU, LE, GT)

AIMS: Exposure to extremely low frequency magnetic fields (ELF-MF) occurs from natural and artificial sources. Although ELF-MF has been classified as a suspected humans carcinogen agent by the International Agency for Research on Cancer, little is known of the effects of ELF-MF at lower exposure levels of the recommended range. In the present study, DNA damage in the peripheral blood cells of power line workers was investigated. MATERIALS AND METHODS: Occupational exposure to ELF-MF in a power plant was measured using the National Institute for Occupational Safety and Health (NIOSH) manual. Single-strand breaks (SSBs) in DNA were evaluated in 29 male utility workers as the exposed population and 28 male support personnel as the control subjects using the comet assay. Effects of ELF-MF on subjects were evaluated using DNA percent in tails, tail length, olive length, and tail moment. RESULTS: Occupational exposure levels to ELF-MF in the utility workers were less than the threshold limit values (TLV) recommended by the American Conference of Government Industrial Hygienist (ACGIH). The median value of the magnetic field at the working sites was 0.85 μ T. Induction of DNA damage was observed for the exposed workers compared with the controls. Olive length, tail moment, and tail DNA percent increased significantly ($p < 0.05$) in the utility workers. CONCLUSIONS: Exposure to ELF-MF at levels less than the ACGIH exposure limit can produce DNA strand breaks.

(E) Zhang H, Cheng Y, Luo X, Duan Y. Protective effect of procyanidins extracted from the lotus seedpod on immune function injury induced by extremely low frequency electromagnetic field. Biomed Pharmacother. 82:364-372, 2016. (VO, CE, GT, GE, OX)

This study aimed to evaluate the protective effect of Lotus seedpod procyanidins (LSPCs) from extremely low frequency electromagnetic field (ELF-EMF) exposure (50Hz, 8mT, 28 days) and their protective mechanism against radiation damage. The results showed that LSPCs increased the organ index of mice and made the damaged blood-producing function and cytokine (INF- γ , TNF- α , IL-2, IL-6 and IL-10 in spleen) levels by ELF-EMF-irradiation recovered to normal appearance. And experimental results proved that dosing LSPCs inhibit more stagnation of splenocytes in G0/G1 phase caused by ELF-EMF, thus the spleen cells from G0/G1 phase to S phase shift, restore normal cell metabolism, promote the splenocytes proliferation, reduced the apoptosis of spleen cells, effectively protect the damage induced by the ELF-EMF radiation. In addition, LSPCs prevented the decline of DNA content caused by ELF-EMF. Western blot determined the levels of apoptosis genes including Bcl-2, Bax, Bcl-cl, Caspase-3 and Caspase-9. The results revealed that a significant suppression in Bcl-2 expression and increase in Bax, Caspase-3 and Caspase-9 expression in splenic cells in ELF-EMF group. However, LSPCs restored these changes. Taking these results together, it may be summarized that LSPCs could protect hematopoietic tissues and the immune system from ELF-EMF. And it may be hypothesized that ELF-EMF-induced apoptosis in splenocytes might occur via triggers the trans-activation of Bax and activates caspases-3 and -9, which then cleaves the death substrates, leading to apoptosis in splenocytes of mice treated with ELF-EMF.

***(E) Zhang M, Wang J, Sun Q, Zhang H, Chen P, Li Q, Wang Y, Qiao G. Immune response of mollusk Onchidium struma to extremely low-frequency electromagnetic fields (ELF-EMF,**

50 Hz) exposure based on immune-related enzyme activity and De novo transcriptome analysis. *Fish Shellfish Immunol* 2020, 98:574-584. (VO, AC, GE)

Along with rapid offshore and onshore wind power development in modern society, extremely low frequency electromagnetic fields (ELF-EMF) is produced extensively in the habits of aquatic organisms. However, the biological effects of ELF-EMF on aquatic organisms are almost sparse. In this study, *Onchidium struma* without shell was chosen to aim whether ELF-EMF can elicit immune response of mollusk based on immune-related enzyme activities and gene expression through high-throughput transcriptome sequencing. Three experimental groups, i.e. ELF-EMF unexposed control group (C), ELF-EMF (50 Hz, 100 μ T) exposed E1 group, and ELF-EMF (50 Hz, 500 μ T) exposed E2 group, were set, and coelomocytes were collected to analyze. The results showed that total coelomocyte and spherulocyte density in E1 group increased significantly compared to groups C and E2 ($P < 0.05$). There were no significant differences on amoebocyte and chromatocyte density among groups C, E1 and E2. ELF-EMF exposure could significantly increase immune-related enzyme activities in coelomic fluid of *O. struma*, including acidic phosphatase, alkaline phosphatase, antioxidative capacity, catalase, superoxide dismutase, and polyphenol oxidase ($P < 0.05$). A total of 54.32 Mb and 55.27 Mb raw reads with average length of 1520 bp were obtained from coelomocytes of *O. struma* in unexposed and exposed groups, respectively. There were 341 differentially expressed genes (DGEs) between unexposed and exposed groups, including 209 up-regulated and 132 down-regulated unigenes. All the DGEs were allocated to 14 Kyoto Encyclopedia of Genes and Genomes pathways, and five pathways were associated with immune response, including TLR/TNF/NOD-like receptor/MAPK/Fc epsilon RI signaling pathways. Altogether, short-term (to one week) exposure of *O. struma* to lower luxy density ELF-EMF (<500 μ T) could elicit the immune response, and antioxidant system is recommended as indicators of immunological effects. Hopefully, this study will further provide insights into exploring biomarker for evaluation of the effect of ELF-EMF exposure on aquatic organisms regarding to field density, frequency and exposure duration, and provide good guidance for exploitation and utilization of renewable energy.

(E) Zhang Y, Zhang D, Zhu B, Zhang H, Sun Y, Sun C. Effects of dietary green tea polyphenol supplementation on the health of workers exposed to high-voltage power lines. *Environ Toxicol Pharmacol.* 46:183-187, 2016. (HU, CE, GT, OX)

Although it has been several decades since the focus on the effect of extremely low frequency electromagnetic fields (ELF-EMF) of high-voltage power lines on human health, no consistent conclusion has been drawn. The present study aimed to investigate the change in oxidative stress after exposure to ELF-EMFs, and potential protective effects of green tea polyphenol supplementation (GTPS) on ELF-EMFs induced oxidative stress. A total of 867 subjects, including workers with or without exposure to ELF-EMFs of 110-420kV power lines, participated and were randomized into GTPS and placebo treatment groups. Oxidative stress and oxidative damage to DNA were assessed by urinary tests of 8-isoprostane and 8-OHdG.

Significant increased urinary 8-isoprostane and 8-OHdG were observed in workers with ELF-EMFs exposure, which were diminished after 12 months of GTPS. No protective effects of GTPS on oxidative stress and oxidative damage to DNA were observed after three months of GTPS withdraw. We found a negative impact of high-voltage power lines on the health of workers. Long-term GTPS could be an efficient protection against the health issues induced by high-voltage power lines.

(E) Zhang Z, Zhang J, Yang C-J, Lian H-Y, Yu H, Huang X-M, Cai P. Coupling Mechanism of Electromagnetic Field and Thermal Stress on *Drosophila melanogaster*. PLoS One 11(9):e0162675, 2016.(VO, AE, GE, OX),

Temperature is an important factor in research on the biological effects of extremely low-frequency electromagnetic field (ELF-EMF), but interactions between ELF-EMF and temperature remain unknown. The effects of ELF-EMF (50 Hz, 3 mT) on the lifespan, locomotion, heat shock response (HSR), and oxidative stress (OS) of Canton-Special (CS) and mutant w1118 flies were investigated at 25°C and 35°C (thermal stress). Results showed that thermal stress accelerated the death rates of CS and w1118 flies, shortened their lifespan, and influenced their locomotion rhythm and activity. The upregulated expression levels of heat shock protein (HSP) 22, HSP26, and HSP70 indicated that HSR was enhanced. Thermal stress-induced OS response increased malondialdehyde content, enhanced superoxide dismutase activity, and decreased reactive oxygen species level. The effects of thermal stress on the death rates, lifespan, locomotion, and HSP gene expression of flies, especially w1118 line, were also enhanced by ELF-EMF. In conclusion, thermal stress weakened the physiological function and promoted the HSR and OS of flies. ELF-EMF aggravated damages and enhanced thermal stress-induced HSP and OS response. Therefore, thermal stress and ELF-EMF elicited a synergistic effect.

(E) Zheng L, Zhang L, Chen L, Jiang J, Zhou X, Wang M, Fan Y Static magnetic field regulates proliferation, migration, differentiation, and YAP/TAZ activation of human dental pulp stem cells. J Tissue Eng Regen Med 12(10):2029-2040, 2018. (VT, AE, GE)

The dental pulp stem cells (DPSCs) are a population of mesenchymal stem cells, which have multilineage potential and high proliferation. DPSCs are regarded as a promising tool for tissue regeneration of dentine, dental pulp, bone, cartilage, and muscle. Recently, magnetic materials have become commonly applied in dental clinics. Static magnetic field has been reported to regulate the proliferation, migration, or differentiation of stem cells. However, whether static magnetic fields affect DPSCs is still unknown. In our study, we investigated the effect of static magnetic field on the proliferation, migration, and differentiation of DPSCs. The results indicated that static magnetic field rearranged the cytoskeleton of DPSCs. A static magnetic field of 1 mT increased DPSC proliferation, as well as the gene expression of several growth factors such as FGF-2, TGF- β , and VEGF. Moreover, the static magnetic field promoted the migration of DPSCs by regulating MMP-1 and MMP-2 gene expression. Static magnetic field of 1 mT also induced osteo/odontogenesis and mineralization in DPSCs. Otherwise, the static magnetic field recruited YAP/TAZ to the nucleus, inhibited the phosphorylation of YAP/TAZ, and upregulated the two YAP/TAZ-regulated genes, CTGF and ANKRD1. Cytoskeleton inhibitor, cytochalasin D, obviously inhibited the nuclear localization of YAP/TAZ. When YAP/TAZ were knocked-down, the static magnetic field-induced mineralization of DPSCs was diminished. Our findings

provide an insight into the effect of static magnetic field on DPSCs and provide the foundation for the future tissue regeneration.

(E) Zheng Y, Hao Y, Xia B, Mei L, Li S, Gao X, Ma T, Wei B, Tan Z, Lan P, Luo Z, Jing D, Huang J. Circadian Rhythm Modulates the Therapeutic Activity of Pulsed Electromagnetic Fields on Intervertebral Disc Degeneration in Rats. Oxid Med Cell Longev 2022:9067611, 2022. (VT, VO, LE, GE)

Circadian rhythm (CR) imparts significant benefits in treating multiple diseases, such as heart diseases and arthritis. But the CR effect on intervertebral disc degeneration (IVDD) therapy remains unclear. Recent studies revealed that pulsed electromagnetic fields (PEMF) are capable of alleviating IVDD. In this study, we evaluated the CR-mediated regulation of PEMF therapeutic effect on IVDD induced by rat tail disc needle puncture. Our results demonstrated that the daytime PEMF stimulation (DPEMF) is more effective than the nighttime PEMF (NPEMF) in delaying IVDD. Moreover, the rats treated with DPEMF maintained better disc stability and histology after 8 weeks, relative to NPEMF. CR and PEMF cotherapies were also examined in cellular models, whereby serum shock was used to induce different levels of clock gene expression in the nucleus pulposus (NP), thus imitating CR *in vitro*. PEMF at ZT8 (higher level of clock gene expression) correlated with a higher extracellular matrix (ECM) component expression, compared to ZT20 (lower level of clock gene expression). Taken together, these data suggest a strong role of CR in regulating the beneficial effect of PEMF on IVDD. Our findings provide a potential clinical significance of CR in optimizing PEMF positive effects on IVDD.

(NE) Zhu K, Lv Y, Cheng Q, Hua J, Zeng Q. Extremely low frequency magnetic fields do not induce DNA damage in human lens epithelial cells in vitro. Anat Rec (Hoboken). 299(5):688-697, 2016. (VT, AE, GT)

Non-ionizing radiations, e.g., radiofrequency electromagnetic fields, could induce DNA damage and oxidative stress in human lens epithelial cells (LECs) which can be early events in cataractogenesis. Extremely low frequency magnetic fields (ELF MF) as another common form of man-made electromagnetic fields has been considered as suspected human carcinogen by International Agency for Research on Cancer (IARC) and become a focus that people pay more and more attentions to. This study aimed to determine whether ELF MF can induce DNA damage in cultured human LECs at a relatively low intensity. Human LECs were exposed or sham-exposed to a 50 Hz ELF MF which produced by a well-designed exposure system at the intensity of 0.4 mT. DNA damage in human LECs was examined by the phosphorylated form of histone variant H2AX (γ H2AX) foci formation assay and further explored with western blot, flow cytometry, and alkaline comet assay. Immunofluorescence analysis showed that 0.4 mT ELF MF did not significantly increase γ H2AX foci formation in human LECs after 2, 6, 12, 24, or 48 hr exposure. No significant differences had been detected in γ H2AX expression level between the ELF MF- and sham-exposure groups, while no obvious chromosomal DNA fragmentation was detected by alkaline comet assay after ELF MF exposure. The results indicate an absence of genotoxicity in ELF MF-exposed human epithelial cells and do not support the hypothesis that environmental ELF MF might be causally led to genomic instability via chromosomal damage

response processes. Neither short nor long term continuous exposure to 50 Hz ELF MF at 0.4 mT could induce DNA damage in human lens epithelial cells in vitro.

(E) Zmyslony M, Palus J, Jajte J, Dziubaltowska E, Rajkowska E. DNA damage in rat lymphocytes treated in vitro with iron cations and exposed to 7 mT magnetic fields (static or 50 Hz). Mutat Res. 453(1):89-96, 2000. (VT, AE, GT, IX)

The present study was undertaken to verify a hypothesis that exposure of the cells to static or 50 Hz magnetic fields (MF) and simultaneous treatment with a known oxidant, ferrous chloride, may affect the oxidative deterioration of DNA molecules. The comet assay was chosen for the assessment of DNA damage. The experiments were performed on isolated rat lymphocytes incubated for 3h in Helmholtz coils at 7 mT static or 50 Hz MF. During MF exposure, part of the cell samples were incubated with 0.01 microM H_2O_2 and another one with 10 microg/ml $FeCl_2$, the rest serving as controls. Lymphocyte exposure to MF at 7 mT did not increase the number of cells with DNA damage in the comet assay. Incubation of lymphocytes with 10 microg/ml $FeCl_2$ did not produce a detectable damage of DNA either. However, when the $FeCl_2$ -incubated lymphocytes were simultaneously exposed to 7 mT MF, the number of damaged cells was significantly increased and reached about 20% for static MF and 15% for power frequency MF. In the control samples about 97% of the cells did not have any DNA damage. It is not possible at present to offer a reasonable explanation for the findings of this investigation - the high increase in the number of lymphocytes showing symptoms of DNA damage in the comet assay, following simultaneous exposure to the combination of two non-cytotoxic factors - 10 microg/ml $FeCl_2$ and 7 mT MF. In view of the obtained results we can only hypothesise that under the influence of simultaneous exposure to $FeCl_2$ and static or 50 Hz MF, the number of reactive oxygen species generated by iron cations may increase substantially. Further studies will be necessary to confirm this hypothesis and define the biological significance of the observed effect.

(E) Zmyslony M, Palus J, Dziubaltowska E, Politanski P, Mamrot P, Rajkowska E, Kamedula M. Effects of in vitro exposure to power frequency magnetic fields on UV-induced DNA damage of rat lymphocytes. Bioelectromagnetics. 25(7):560-562, 2004. (VT, AE, GT, IX)

The mechanisms of biological effects of 50/60 Hz (power frequency) magnetic fields (MF) are still poorly understood. There are a number of studies indicating that MF affect biochemical processes in which free radicals are involved, such as the biological objects' response to ultraviolet radiation (UVA). Therefore, the present study was aimed to assess the effect of 50 Hz MFs on the oxidative deterioration of DNA in rat lymphocytes irradiated in vitro by UVA. UVA radiation (150 J/m²) was applied for 5 min for all groups and 50 Hz MF (40 microT rms) exposure was applied for some of the groups for 5 or 60 min. The level of DNA damage was assessed using the alkaline comet assay, the fluorescence microscope, and image analysis. It has been found that the 1 h exposure to

MF caused an evident increase in all parameters consistent with damaged DNA. This suggest that MF affects the radical pairs generated during the oxidative or enzymatic processes of DNA repair.

April 24, 2022

Research on Genetic Effects of Radiofrequency Radiation (RFR)

RFR Genetic Effects Studies (current to April 24, 2022)

Of 423 total studies: E – 291 (68%) NE = 132 (32%)

(E = reported biological effect NE = reported no biological effect)

(VT-in vitro study; VO- in vivo study; HU- human study; LE- long term/repeated exposure; AE- acute exposure; LI- low intensity; GT- genotoxic effect, e.g., DNA damage, micronucleus formation, chromosome alterations; GE- gene expression; OX- oxidative effects, i.e., involvement of free radicals and oxidative enzymes; IX- interaction with other factors to cause genetic effects; DE- effects on developing animals; RP- reproduction, e.g., sperm damage; WS- waveform specific effect, e.g., modulation and frequency; CS- cell type specific effect; EP-epigenetic effect).

(NE) Agarwal A, Desai NR, Makker K, Varghese A, Mouradi R, Sabanegh E, Sharma R. Effects of radiofrequency electromagnetic waves (RF-EMW) from cellular phones on human ejaculated semen: an in vitro pilot study. Fertil Steril 92: 1318-1325, 2009. (VT, HU, AE, GT, RP, OX)

OBJECTIVE: To evaluate effects of cellular phone radiofrequency electromagnetic waves (RF-EMW) during talk mode on unprocessed (neat) ejaculated human semen. **DESIGN:** Prospective pilot study. **SETTING:** Center for reproductive medicine laboratory in tertiary hospital setting. **SAMPLES:** Neat semen samples from normal healthy donors (n = 23) and infertile patients (n = 9). **INTERVENTION(S):** After liquefaction, neat semen samples were divided into two aliquots. One aliquot (experimental) from each patient was exposed to cellular phone radiation (in talk mode) for 1 h, and the second aliquot (unexposed) served as the control sample under identical conditions. **MAIN OUTCOME MEASURE(S):** Evaluation of sperm parameters (motility, viability), reactive oxygen species (ROS), total antioxidant capacity (TAC) of semen, ROS-TAC score, and sperm DNA damage. **RESULT(S):** Samples exposed to RF-EMW showed a significant decrease in sperm motility and viability, increase in ROS level, and decrease in ROS-TAC score. Levels of TAC and DNA damage showed no significant differences from the unexposed group. **CONCLUSION(S):** Radiofrequency electromagnetic waves emitted from cell phones may lead to oxidative stress in human semen. We speculate that keeping the cell phone in a trouser pocket in talk mode may negatively affect spermatozoa and impair male fertility.

(E) Aitken RJ, Bennetts LE, Sawyer D, Wiklendt AM, King BV. Impact of radio frequency electromagnetic radiation on DNA integrity in the male germline. Inter J Androl 28:171-179, 2005. (VO, LE, LI, GT, RP)

Concern has arisen over human exposures to radio frequency electromagnetic radiation (RFEMR), including a recent report indicating that regular mobile phone use can negatively impact upon human semen quality. These effects would be particularly serious if the biological effects of RFEMR included the induction of DNA damage in male germ cells. In this study, mice were exposed to 900 MHz RFEMR at a specific absorption rate of approximately 90 mW/kg inside a waveguide for 7 days at 12 h per day. Following exposure, DNA damage to caudal epididymal spermatozoa was assessed by quantitative PCR (QPCR) as well as alkaline and pulsed-field gel electrophoresis. The treated mice were overtly normal and all assessment criteria, including sperm number, morphology and vitality were not significantly affected. Gel electrophoresis revealed no gross evidence of increased single- or double-DNA strand breakage in spermatozoa taken from treated animals. However, a detailed analysis of DNA integrity using QPCR revealed statistically significant damage to both the mitochondrial genome ($p < 0.05$) and the nuclear β -globin locus ($p < 0.01$). This study suggests that while RFEMR does not have a dramatic impact on male germ cell development, a significant genotoxic effect on epididymal spermatozoa is evident and deserves further investigation.

(E) Akdag MZ, Dasdag S, Canturk F, Karabulut D, Caner Y, Adalier N. Does prolonged radiofrequency radiation emitted from Wi-Fi devices induce DNA damage in various tissues of rats? J Chem Neuroanat. 75(Pt B):116-122, 2016. (VO, LE, GT, RP)

Wireless internet (Wi-Fi) providers have become essential in our daily lives, as wireless technology is evolving at a dizzying pace. Although there are different frequency generators, one of the most commonly used Wi-Fi devices are 2.4GHz frequency generators. These devices are heavily used in all areas of life but the effect of radiofrequency (RF) radiation emission on users is generally ignored. Yet, an increasing share of the public expresses concern on this issue. Therefore, this study intends to respond to the growing public concern. The purpose of this study is to reveal whether long term exposure of 2.4GHz frequency RF radiation will cause DNA damage of different tissues such as brain, kidney, liver, and skin tissue and testicular tissues of rats. The study was conducted on 16 adult male Wistar-Albino rats. The rats in the experimental group ($n=8$) were exposed to 2.4GHz frequency radiation for over a year. The rats in the sham control group ($n=8$) were subjected to the same experimental conditions except the Wi-Fi generator was turned off. After the exposure period was complete the possible DNA damage on the rat's brain, liver, kidney, skin, and testicular tissues was detected through the single cell gel electrophoresis assay (comet) method. The amount of DNA damage was measured as percentage tail DNA value. Based on the DNA damage results determined by the single cell gel electrophoresis (Comet) method, it was found that the % tail DNA values of the brain, kidney, liver, and skin tissues of the rats in the experimental group increased more than those in the control group. The increase of the DNA damage in all tissues was not significant ($p>0.05$).

However the increase of the DNA damage in rat testes tissue was significant ($p < 0.01$). In conclusion, long-term exposure to 2.4GHz RF radiation (Wi-Fi) does not cause DNA damage of the organs investigated in this study except testes. The results of this study indicated that testes are more sensitive organ to RF radiation.

(E) Akdag M, Dasdag S, Canturk F, Akdag MZ. Exposure to non-ionizing electromagnetic fields emitted from mobile phones induced DNA damage in human ear canal hair follicle cells. Electromagn Biol Med. 37(2):66-75, 2018. (HU, LE, GT)

The aim of this study was to investigate effect of radiofrequency radiation (RFR) emitted from mobile phones on DNA damage in follicle cells of hair in the ear canal. The study was carried out on 56 men (age range: 30-60 years old) in four treatment groups with $n = 14$ in each group. The groups were defined as follows: people who did not use a mobile phone (Control), people use mobile phones for 0-30 min/day (second group), people use mobile phones for 30-60 min/day (third group) and people use mobile phones for more than 60 min/day (fourth group). Ear canal hair follicle cells taken from the subjects were analyzed by the Comet Assay to determine DNA damages. The Comet Assay parameters measured were head length, tail length, comet length, percentage of head DNA, tail DNA percentage, tail moment, and Olive tail moment. Results of the study showed that DNA damage indicators were higher in the RFR exposure groups than in the control subjects. In addition, DNA damage increased with the daily duration of exposure. In conclusion, RFR emitted from mobile phones has a potential to produce DNA damage in follicle cells of hair in the ear canal. Therefore, mobile phone users have to pay more attention when using wireless phones.

(E) Akhavan-Sigari R, Baf MM, Ariabod V, Rohde V, Rahighi S. Connection between cell phone use, p53 gene expression in different zones of glioblastoma multiforme and survival prognoses. Rare Tumors. 6(3):5350, 2014. (HU, LE, GE)

The aim of this paper is to investigate p53 gene expression in the central and peripheral zones of glioblastoma multiforme using a real-time reverse transcription polymerase chain reaction (RT-PCR) technique in patients who use cell phones ≥ 3 hours a day and determine its relationship to clinicopathological findings and overall survival. Sixty-three patients (38 males and 25 females), diagnosed with glioblastoma multiforme (GBM), underwent tumor resection between 2008 and 2011. Patient ages ranged from 25 to 88 years, with a mean age of 55. The levels of expression of p53 in the central and peripheral zone of the GBM were quantified by RT-PCR. Data on p53 gene expression from the central and peripheral zone, the related malignancy and the clinicopathological findings (age, gender, tumor location and size), as well as overall survival, were analyzed. Forty-one out of 63 patients (65%) with the highest level of cell phone use (≥ 3 hours/day) had higher mutant type p53 expression in the peripheral zone of the glioblastoma; the difference was statistically significant ($P = 0.034$). Results from the present study on the use of mobile phones for ≥ 3 hours a day show a consistent pattern of increased risk for the mutant type of p53 gene expression in the peripheral zone of the glioblastoma, and that this increase was significantly correlated with shorter overall survival time. The risk was not higher for ipsilateral

exposure. We found that the mutant type of p53 gene expression in the peripheral zone of the glioblastoma was increased in 65% of patients using cell phones ≥ 3 hours a day.

(E) Albaqami M, Hammad M, Pooam M, Procopio M, Sameti M, Ritz T, Ahmad M, Martino CF. 2020. Arabidopsis cryptochrome is responsive to Radiofrequency (RF) electromagnetic fields. *Sci Rep* 10(1):11260. **(VO, AE, GE)**

How living systems respond to weak electromagnetic fields represents one of the major unsolved challenges in sensory biology. Recent evidence has implicated cryptochrome, an evolutionarily conserved flavoprotein receptor, in magnetic field responses of organisms ranging from plants to migratory birds. However, whether cryptochromes fulfill the criteria to function as biological magnetosensors remains to be established. Currently, theoretical predictions on the underlying mechanism of chemical magnetoreception have been supported by experimental observations that exposure to radiofrequency (RF) in the MHz range disrupt bird orientation and mammalian cellular respiration. Here we show that, in keeping with certain quantum physical hypotheses, a weak 7 MHz radiofrequency magnetic field significantly reduces the biological responsiveness to blue light of the cryptochrome receptor cry1 in Arabidopsis seedlings. Using an in vivo phosphorylation assay that specifically detects activated cryptochrome, we demonstrate that RF exposure reduces conformational changes associated with biological activity. RF exposure furthermore alters cryptochrome-dependent plant growth responses and gene expression to a degree consistent with theoretical predictions. To our knowledge this represents the first demonstration of a biological receptor responding to RF exposure, providing important new implications for magnetosensing as well as possible future applications in biotechnology and medicine.

(E) Alkis ME, Bilgin HM, Akpolat V, Dasdag S, Yegin K, Yavas MC, Akdag MZ. **Effect of 900-1800-, and 2100-MHz radiofrequency radiation on DNA and oxidative stress in brain.** *Electromagn Biol Med.* 38(1):32-47, 2019a. **(VO, LE, LI, GT, OX)**

Ubiquitous and ever increasing use of mobile phones led to the growing concern about the effects of radiofrequency radiation (RFR) emitted by cell phones on biological systems. The aim of this study is to explore whether long-term RFR exposure at different frequencies affects DNA damage and oxidant-antioxidant parameters in the blood and brain tissue of rats. 28 male Sprague Dawley rats were randomly divided into four equal groups (n = 7). They were identified as Group 1: sham-control, Group 2: 900 MHz, Group 3: 1800 MHz, and Group 4: 2100 MHz. Experimental groups of rats were exposed to RFR 2 h/day for 6 months. The sham-control group of rats was subjected to the same experimental condition but generator was turned off. Specific absorption rates (SARs) at brain with 1 g average were calculated as 0.0845 W/kg, 0.04563 W/kg, and 0.03957, at 900 MHz, 1800 MHz, and 2100 MHz, respectively. Additionally, malondialdehyde (MDA), 8-hydroxydeoxyguanosine (8-OHdG), total antioxidant status (TAS), and total oxidant status (TOS) analyses were conducted in the brain tissue samples. Results of the study showed that DNA damage and oxidative stress indicators were found higher in the RFR exposure groups than in the sham-control group. In conclusion, 900-, 1800-, and 2100-MHz RFR emitted from mobile phones may cause oxidative damage, induce increase in lipid

peroxidation, and increase oxidative DNA damage formation in the frontal lobe of the rat brain tissues. Furthermore, 2100-MHz RFR may cause formation of DNA single-strand breaks.

(E) Alkis MS, Akdag MZ, Dasdag S, Yegin K, Akpolat V. Single-strand DNA breaks and oxidative changes in rat testes exposed to radiofrequency radiation emitted from cellular phones, *Biotechnology & Biotechnological Equipment*, 33:1, 1733-1740, 2019b. (VO, LE, GT, OX)

The testes are a sensitive organ to electromagnetic pollution and people are concerned about the harmful effects of the radiofrequency radiation (RFR) emitted from cellular phones. Therefore, the purpose of this study was to investigate the effects of long-term exposure to different RFR frequencies on single-strand DNA breaks and oxidative changes in rat testicular tissue. Twenty-eight male Sprague–Dawley rats were divided randomly into four groups. Three groups were exposed to radiation emitted from 900, 1800 and 2100 MHz RF generators, 2 h/day for 6 months. The sham-control group was kept under the same experimental conditions but the RFR generator was turned off. Immediately after the last exposure, testes were removed and DNA damage, 8-hydroxydeoxyguanosine (8-OHdG), malondialdehyde (MDA), total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) were analyzed. The results of this study indicated that RFR increased TOS, OSI, MDA and 8-OHdG ($p < 0.05$). TAS levels in the exposed group were lower than in the sham group ($p < 0.05$). In terms of DNA damage, the tail intensities in the comet assay were higher in the exposure groups ($p < 0.05$). This study demonstrated that long-term exposure to RFR emitted by cellular phones may cause oxidative stress and oxidative DNA damage in rat testicular tissue and may generate DNA single-strand breaks at high frequencies (1800 and 2100 MHz). Our results showed that some RFR emitted from cellular phones has potential to lead to cell damage in the testes.

(E) Alkis ME, Akdag MZ, Dasdag S. Effects of Low-Intensity Microwave Radiation on Oxidant-Antioxidant Parameters and DNA Damage in the Liver of Rats. *Bioelectromagnetics* 42(1):76-85, 2021.(VO,LE, OX, GT)

The continuously increasing usage of cell phones has raised concerns about the adverse effects of microwave radiation (MWR) emitted by cell phones on health. Several in vitro and in vivo studies have claimed that MWR may cause various kinds of damage in tissues. The aim of this study is to examine the possible effects of exposure to low-intensity MWR on DNA and oxidative damage in the livers of rats. Eighteen Sprague-Dawley male rats were divided into three equal groups randomly ($n = 6$). Group 1 (Sham-control): rats were kept under conditions the same as those of other groups, except for MWR exposure. Group 2: rats exposed to 1800 MHz (SAR: 0.62 W/kg) at 0.127 ± 0.04 mW/cm² power density, and Group 3: rats exposed to 2,100 MHz (SAR: 0.2 W/kg) at 0.038 ± 0.03 mW/cm² power density. Microwave application groups were exposed to MWR 2 h/day for 7 months. At the end of the exposure period, the rats were sacrificed and DNA damage, malondialdehyde (MDA), 8-hydroxydeoxyguanosine (8-OHdG), and total oxidant-antioxidant parameter analyses were conducted in their liver tissue samples. It was found that 1800 and 2100 MHz low-intensity MWR caused a significant increase in MDA, 8-OHdG, total oxidant status, oxidative stress index, and comet assay tail intensity ($P <$

0.05), while total antioxidant status levels ($P < 0.05$) decreased. The results of our study showed that whole-body exposure to 1800 and 2100 MHz low-intensity MWR emitted by cell phones can induce oxidative stress by altering oxidant-antioxidant parameters and lead to DNA strand breaks and oxidative DNA damage in the liver of rats.

(NE) Al-Serori H, Kundi M, Ferk F, Mišik M, Nersesyan A, Murbach M, Lah TT, Knasmüller S. Evaluation of the potential of mobile phone specific electromagnetic fields (UMTS) to produce micronuclei in human glioblastoma cell lines. Toxicol In Vitro. 40:264-271, 2017. (VT, E, GT)

Some epidemiological studies indicate that mobile phones cause glioblastomas in humans. Since it is known that genomic instability plays a key role in the etiology of cancer, we investigated the effects of the universal mobile telecommunications system radiofrequency (UMTS-RF) signal, which is used in "smart" phones, on micronucleus (MN) formation and other anomalies such as nuclear buds (NBUDs) and nucleoplasmic bridges (NPBs). MN are formed by structural and numerical aberrations, NBs reflect gene amplification and NPBs are formed from dicentric chromosomes. The experiments were conducted with human glioblastoma cell lines, which differ in regard to their p53 status, namely U87 (wild-type) and U251 (mutated). The cells were cultivated for 16h in presence and absence of fetal calf serum and exposed to different SAR doses (0.25, 0.50 and 1.00W/kg), which reflect the exposure of humans, in presence and absence of mitomycin C as former studies indicate that RF may cause synergistic effects in combination with this drug. We found no evidence for induction of MN and other anomalies. However, with the highest dose, induction of apoptosis was observed in U251 cells on the basis of the morphological features of the cells. Our findings indicate that the UMTS-RF signal does not cause chromosomal damage in glioblastoma cells; the mechanisms which lead to induction of programmed cell death will be investigated in further studies.

(E) Al-Serori H, Ferk F, Kundi M, Bileck A, Gerner C, Mišik M, Nersesyan A, Waldherr M, Murbach M, Lah TT, Herold-Mende C, Collins AR, Knasmüller S. Mobile phone specific electromagnetic fields induce transient DNA damage and nucleotide excision repair in serum-deprived human glioblastoma cells. PLoS One. 13(4):e0193677, 2018. (VT, AE, GT)

Some epidemiological studies indicate that the use of mobile phones causes cancer in humans (in particular glioblastomas). It is known that DNA damage plays a key role in malignant transformation; therefore, we investigated the impact of the UMTS signal which is widely used in mobile telecommunications, on DNA stability in ten different human cell lines (six brain derived cell lines, lymphocytes, fibroblasts, liver and buccal tissue derived cells) under conditions relevant for users (SAR 0.25 to 1.00 W/kg). We found no evidence for induction of damage in single cell gel electrophoresis assays when the cells were cultivated with serum. However, clear positive effects were seen in a p53 proficient glioblastoma line (U87) when the

cells were grown under serum free conditions, while no effects were found in p53 deficient glioblastoma cells (U251). Further experiments showed that the damage disappears rapidly in U87 and that exposure induced nucleotide excision repair (NER) and does not cause double strand breaks (DSBs). The observation of NER induction is supported by results of a proteome analysis indicating that several proteins involved in NER are up-regulated after exposure to UMTS; additionally, we found limited evidence for the activation of the γ -interferon pathway. The present findings show that the signal causes transient genetic instability in glioma derived cells and activates cellular defense systems.

(NE) Antonopoulos A, Eisenbrandt H, Obe G, Effects of high-frequency electromagnetic fields on human lymphocytes in vitro. Mutat Res 395(2-3): 209-214, 1997. (VT, AE, GT)

Human peripheral lymphocytes were incubated in the presence of high-frequency electromagnetic fields of 380, 900 and 1800 MHz. The measured endpoints were cell cycle progression and the frequencies of sister-chromatid exchanges. No differences between treated and control cultures could be found.

(E) Aravinda VSS, Kandregula CR, Muppa R, Krishna MM, Nikitha BS, Yenni M. A cross-sectional and histological analysis to understand the cytological effects of cell phone radiation on buccal mucosa of children. J Indian Soc Pedod Prev Dent 2022;40(1):74-80. (HU, LE, GT)

Context: The ongoing pandemic has affected all the spheres of life and one of the severely affected avenues is the education of a child. The online education has seen an upward curve since the start of COVID-19 pandemic. Schools globally have adopted online class tutorials as the main method to impart education and directly increasing the screen time for a child.

Aim: The aim of the present study was to evaluate the cytological effects of prolonged mobile phone usage on the buccal mucosa of children.

Settings and design: Stratified sampling was used for the selection of subjects for the study. After a questionnaire regarding the usage of a mobile phone was distributed among the parents of children. Among them, 90 children were selected on the basis of pattern and frequency of mobile phone usage in the child. **Materials and methodology:** The children were divided into three groups based on the per day hours of viewing of mobile phone, i.e., Group 1: Usage of 1-2 h a day, Group 2: Usage of 3-6 h a day, and Group 3: Usage of >6 h a day. The time frame taken into consideration was 1 year after the pandemic started. This was specifically to understand the impact of the online education. Swab was obtained by using the conventional ice-cream stick method from the buccal mucosa.

Statistical analysis: The samples were subjected to histological and microscopical analysis to observe for cytological changes. One-way ANOVA was used to determine the statistical significance if any. **Results:** The results obtained clearly showed that Group 3 (>6 h usage per day) showed the highest number of cellular and chromosomal aberrations which was significant.

Conclusion: The results indicated that impact due to the prolonged screen time on the buccal mucosa is significant. A direct proportionality was seen between the apoptotic changes and chromosomal aberrations and the number of daily hour usage.

(E) Atasoy HI, Gunal MY, Atasoy P, Elgun S, Bugdayci G. Immunohistopathologic demonstration of deleterious effects on growing rat testes of radiofrequency waves emitted from conventional Wi-Fi devices. J Pediatr Urol. 9:223-229, 2013. (VO; LE, GT, OX, RP)

OBJECTIVE: To investigate effects on rat testes of radiofrequency radiation emitted from indoor Wi-Fi Internet access devices using 802.11.g wireless standards. METHODS: Ten Wistar albino male rats were divided into experimental and control groups, with five rats per group. Standard wireless gateways communicating at 2.437 GHz were used as radiofrequency wave sources. The experimental group was exposed to radiofrequency energy for 24 h a day for 20 weeks. The rats were sacrificed at the end of the study. Intracardiac blood was sampled for serum 8-hydroxy-2'-deoxyguanosine levels. Testes were removed and examined histologically and immunohistochemically. Testis tissues were analyzed for malondialdehyde levels and prooxidant-antioxidant enzyme activities. RESULTS: We observed significant increases in serum 8-hydroxy-2'-deoxyguanosine levels and 8-hydroxyguanosine staining in the testes of the experimental group indicating DNA damage due to exposure ($p < 0.05$). We also found decreased levels of catalase and glutathione peroxidase activity in the experimental group, which may have been due to radiofrequency effects on enzyme activity ($p < 0.05$). CONCLUSIONS: These findings raise questions about the safety of radiofrequency exposure from Wi-Fi Internet access devices for growing organisms of reproductive age, with a potential effect on both fertility and the integrity of germ cells.

(E) Atlı Şekeroğlu Z, Akar A, Sekeroğlu V. Evaluation of the cytogenotoxic damage in immature and mature rats exposed to 900 MHz radio frequency electromagnetic fields. Int J Radiat Biol. 89(11):985-992, 2013. (VO, LE, GT, DE)

Purpose: One of the most important issues regarding radio frequency electromagnetic fields (RF-EMF) is their effect on genetic material. Therefore, we investigated the cytogenotoxic effects of 900 MHz radio frequency electromagnetic fields (RF-EMF) and the effect of a recovery period after exposure to RF-EMF on bone marrow cells of immature and mature rats. Materials and methods: The immature and mature rats in treatment groups were exposed to RF-EMF for 2 h/day for 45 days. Average electrical field values for immature and mature rats were 28.1 ± 4.8 V/m and 20.0 ± 3.2 V/m, respectively. Whole-body specific absorption rate (SAR) values for immature and mature rats were in the range of 0.38-0.78 W/kg, and 0.31-0.52 W/kg during the 45 days, respectively. Two recovery groups were kept for 15 days after RF-EMF exposure. Results: Significant differences were observed in chromosome aberrations (CA), micronucleus (MN) frequency, mitotic index (MI) and ratio of polychromatic erythrocytes (PCE) in all treatment and recovery groups. The cytogenotoxic damage in immature rats was statistically higher than the mature rats. The recovery period did not reduce the damage to the same extent as the corresponding control groups. Conclusions: The exposure of RF-EMF leads to cytotoxic and genotoxic damage in immature and mature rats. More sensitive studies are required to elucidate the possible carcinogenic risk of EMF exposure in humans, especially children.

(E) Avendaño C, Mata A, Sanchez Sarmiento CA, Doncel GF. Use of laptop computers connected to internet through Wi-Fi decreases human sperm motility and increases sperm DNA fragmentation. Fertil Steril 97:39-45, 2012. (HU, AE, GT, RP)

OBJECTIVE: To evaluate the effects of laptop computers connected to local area networks wirelessly (Wi-Fi) on human spermatozoa. **DESIGN:** Prospective in vitro study. **SETTING:** Center for reproductive medicine. **PATIENT(S):** Semen samples from 29 healthy donors. **INTERVENTION(S):** Motile sperm were selected by swim up. Each sperm suspension was divided into two aliquots. One sperm aliquot (experimental) from each patient was exposed to an internet-connected laptop by Wi-Fi for 4 hours, whereas the second aliquot (unexposed) was used as control, incubated under identical conditions without being exposed to the laptop. **MAIN OUTCOME MEASURE(S):** Evaluation of sperm motility, viability, and DNA fragmentation. **RESULT(S):** Donor sperm samples, mostly normozoospermic, exposed ex vivo during 4 hours to a wireless internet-connected laptop showed a significant decrease in progressive sperm motility and an increase in sperm DNA fragmentation. Levels of dead sperm showed no significant differences between the two groups. **CONCLUSION(S):** To our knowledge, this is the first study to evaluate the direct impact of laptop use on human spermatozoa. Ex vivo exposure of human spermatozoa to a wireless internet-connected laptop decreased motility and induced DNA fragmentation by a nonthermal effect. We speculate that keeping a laptop connected wirelessly to the internet on the lap near the testes may result in decreased male fertility. Further in vitro and in vivo studies are needed to prove this contention.

(E) Balakrishnan K, Murali V, Rathika C, Manikandan T, Malini RP, Kumar RA, Krishnan M. Hsp70 is an independent stress marker among frequent users of mobile phones. J Environ Pathol Toxicol Oncol. 33(4):339-347, 2014. (HU, LE, GE)

The aim of this study was to measure the serum concentrations of heat shock protein (HSP) 70 and C-reactive protein (CRP) and the expression levels of the hsp70 gene among frequent users of mobile phones (FUMPs). We enrolled 120 employees of information technology (IT)/IT enabled service companies (FUMPs; IT professionals) and 102 infrequent users of mobile phones (IFUMPs; people from non-IT professions) as controls. The serum concentrations of HSP70 and CRP were measured by enzyme-linked immunosorbant assay and hsp70 gene expression by reverse transcription polymerase chain reaction. Significantly higher concentrations of serum HSP70 ($P < 0.00012$) and CRP ($P < 0.04$) were observed among FUMPs than IFUMPs. A higher level of hsp70 gene expression (fold induction) was observed among FUMPs than IFUMPs ($P < 7.06 \times 10^{-13}$). In contrast to the duration of exposure-dependent increase of serum concentration of CRP, the serum HSP70 concentration was found to be independent of the duration of exposure to mobile phones. Thus, the study convincingly demonstrated the role of serum HSP and CRP as systemic inflammatory biomarkers for mobile phone-induced radiation.

(E) Balode, Z, Assessment of radio-frequency electromagnetic radiation by the micronucleus test in bovine peripheral erythrocytes. Sci Total Environ 180(1):81-85, 1996. (VO, LE, GT)

Previous bioindicative studies in the Skrunnda Radio Location Station area have focused on the somatic influence of electromagnetic radiation on plants, but it is also important to study genetic effects. We have chosen cows as test animals for cytogenetical evaluation because they live in the same general exposure area as humans, are confined to specific locations and are

chronically exposed to radiation. Blood samples were obtained from female Latvian Brown cows from a farm close to and in front of the Skrunda Radar and from cows in a control area. A simplified alternative to the Schiff method of DNA staining for identification of micronuclei in peripheral erythrocytes was applied. Microscopically, micronuclei in peripheral blood erythrocytes were round in shape and exhibited a strong red colour. They are easily detectable as the only coloured bodies in the uncoloured erythrocytes. From each individual animal 2000 erythrocytes were examined at a magnification of x 1000 for the presence of micronuclei. The counting of micronuclei in peripheral erythrocytes gave low average incidences, 0.6 per 1000 in the exposed group and 0.1 per 1000 in the control, but statistically significant ($P < 0.01$) differences were found in the frequency distribution between the control and exposed groups.

(E) Baohong Wang, Jiliang H, Lifen J, Deqiang L, Wei Z, Jianlin L, Hongping D. Studying the synergistic damage effects induced by 1.8 GHz radiofrequency field radiation (RFR) with four chemical mutagens on human lymphocyte DNA using comet assay in vitro. *Mutat Res* 578:149-57, 2005. (VT, AE, GT, IX)

The aim of this investigation was to study the synergistic DNA damage effects in human lymphocytes induced by 1.8GHz radiofrequency field radiation (RFR, SAR of 3W/kg) with four chemical mutagens, i.e. mitomycin C (MMC, DNA crosslinker), bleomycin (BLM, radiomimetic agent), methyl methanesulfonate (MMS, alkylating agent), and 4-nitroquinoline-1-oxide (4NQO, UV-mimetic agent). The DNA damage of lymphocytes exposed to RFR and/or with chemical mutagens was detected at two incubation time (0 or 21h) after treatment with comet assay in vitro. Three combinative exposure ways were used. Cells were exposed to RFR and chemical mutagens for 2 and 3h, respectively. Tail length (TL) and tail moment (TM) were utilized as DNA damage indexes. The results showed no difference of DNA damage indexes between RFR group and control group at 0 and 21h incubation after exposure ($P > 0.05$). There were significant difference of DNA damage indexes between MMC group and RFR+MMC co-exposure group at 0 and 21h incubation after treatment ($P < 0.01$). Also the significant difference of DNA damage indexes between 4NQO group and RFR+4NQO co-exposure group at 0 and 21h incubation after treatment was observed ($P < 0.05$ or $P < 0.01$). The DNA damage in RFR+BLM co-exposure groups and RFR+MMS co-exposure groups was not significantly increased, as compared with corresponding BLM and MMS groups ($P > 0.05$). The experimental results indicated 1.8GHz RFR (SAR, 3W/kg) for 2h did not induce the human lymphocyte DNA damage effects in vitro, but could enhance the human lymphocyte DNA damage effects induced by MMC and 4NQO. The synergistic DNA damage effects of 1.8GHz RFR with BLM or MMS were not obvious.

(E) Baohong W, Lifen J, Lanjuan L, Jianlin L, Deqiang L, Wei Z, Jiliang H. Evaluating the combinative effects on human lymphocyte DNA damage induced by ultraviolet ray C plus 1.8GHz microwaves using comet assay in vitro. *Toxicology*. 232(3):311-316, 2007. (VT, AE, GT, IX)

The objective of this study was to observe whether 1.8GHz microwaves (MW) (SAR, 3 W/kg) exposure can influence human lymphocyte DNA damage induced by ultraviolet ray C (UVC). The lymphocytes, which were from three young healthy donors, were exposed to 254 nm UVC at the doses of 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0 J m⁻², respectively. The lymphocytes were

irradiated by 1.8GHz MW (SAR, 3 W/kg) for 0, 1.5 and 4 h. The combinative exposure of UVC plus MW was conducted. The treated cells were incubated for 0, 1.5 and 4 h. Finally, comet assay was used to measure DNA damage of above treated lymphocytes. The results indicated that the difference of DNA damage induced between MW group and control group was not significant ($P>0.05$). The MTLs induced by UVC were 1.71 ± 0.09 , 2.02 ± 0.08 , 2.27 ± 0.17 , 2.27 ± 0.06 , 2.25 ± 0.12 , 2.24 ± 0.11 microm, respectively, which were significantly higher than that (0.96 ± 0.05 microm) of control ($P<0.01$). MTLs of some sub-groups in combinative exposure groups at 1.5-h incubation were significantly lower than those of corresponding UVC sub-groups ($P<0.01$ or $P<0.05$). However, MTLs of some sub-groups in combinative exposure groups at 4-h incubation were significantly higher than those of corresponding UVC sub-groups ($P<0.01$ or $P<0.05$). In this experiment it was found that 1.8GHz (SAR, 3 W/kg) MW exposure for 1.5 and 4 h did not enhance significantly human lymphocyte DNA damage, but could reduce and increase DNA damage of human lymphocytes induced by UVC at 1.5-h and 4-h incubation, respectively.

(E) Banerjee S, Singh NN, Sreedhar G, Mukherjee S. Analysis of the genotoxic effects of mobile phone radiation using buccal micronucleus assay: a comparative evaluation. J Clin Diagn Res 10(3):ZC82-85, 2016. (HU, LE, GT)

Introduction: Micronucleus (MN) is considered to be a reliable marker for genotoxic damage and it determines the presence and the extent of the chromosomal damage. The MN is formed due to DNA damage or chromosomal disarrangements. The MN has a close association with cancer incidences. In the new era, mobile phones are constantly gaining popularity specifically in the young generation, but this device uses radiofrequency radiation that may have a possible carcinogenic effect. The available reports related to the carcinogenic effect of mobile radiation on oral mucosa are contradictory. **Aim:** To explore the effects of mobile phone radiation on the MN frequency in oral mucosal cells. **Materials and methods:** The subjects were divided into two major groups: low mobile phone users and high mobile phone users. Subjects who used their mobile phone since less than five years and less than three hours a week comprised of the first group and those who used their mobile since more than five years and more than 10 hours a week comprised of the second group. Net surfing and text messaging was not considered in this study. Exfoliated buccal mucosal cells were collected from both the groups and the cells were stained with DNA-specific stain acridine orange. Thousand exfoliated buccal mucosal cells were screened and the cells which were positive for micronuclei were counted. The micronucleus frequency was represented as mean \pm SD, and unpaired Student t-test was used for intergroup comparisons. **Results:** The number of micronucleated cells/ 1000 exfoliated buccal mucosal cells was found to be significantly increased in high mobile phone users group than the low mobile phone users group. The use of mobile phone with the associated complaint of warmth around the ear showed a maximum increase in the number of micronucleated cells /1000 exfoliated buccal mucosal cells. **Conclusion:** Mobile phone radiation even in the permissible range when used for longer duration causes significant genotoxicity. The genotoxicity can be avoided to some extent by the regular use of headphones.

(E) Beaubois E, Girard S, Lallechere S, Davies E, Paladian F, Bonnet P, Ledoigt G, Vian A. Intercellular communication in plants: evidence for two rapidly transmitted

systemic signals generated in response to electromagnetic field stimulation in tomato. Plant Cell Environ 30(7):834-844. 2007. (VO, AE, GE, LI)

Exposing all of a wild-type tomato plant to electromagnetic radiation evoked rapid and substantial accumulation of basic leucine-zipper transcription factor (bZIP) mRNA in the terminal leaf (#4) with kinetics very similar to that seen in response to wounding, while in the abscisic acid (ABA) mutant (Sitiens), the response was more rapid, but transient. Submitting just the oldest leaf (#1) of a wild-type plant to irradiation evoked bZIP mRNA accumulation both locally in the exposed leaf and systemically in the unexposed (distant) leaf #4, although systemic accumulation was delayed somewhat. Accumulation of Pin2 mRNA was less than bZIP in both the exposed and distant leaves in wild type, but there was no delay in the systemic response. In Sitiens, bZIP mRNA accumulation was far less than in wild type in both local and distant leaves, while Pin2 mRNA accumulation was stronger in the exposed leaf, but totally prevented in the systemic leaf. In the jasmonic acid (JA) mutant (JL-5) and in wild-type plants treated with the ABA biosynthesis inhibitor, naproxen, responses were similar to those in the ABA mutant, while treatment of the exposed leaf with calcium antagonists totally abolished both local and systemic increases in bZIP transcript accumulation.

(E) Bektas H, Dasdag S, Bektas MS. Comparison of effects of 2.4 GHz Wi-Fi and mobile phone exposure on human placenta and cord blood. Biotechn Biotechnological Equipment, 34:1, 154-162, 2020.(HU, CE, OX, GT)

The aim of this study was to investigate the effects of radiofrequency radiation emitted from Wi-Fi systems and mobile phones on cord blood and placenta. The study included 149 pregnant women who were divided in subgroups: unexposed (control), mobile phone exposed, Wi-Fi exposed and mobile phone plus Wi-Fi exposed groups. Immediately after birth, placenta and cord blood samples were collected and protein carbonyl (PCO), malondialdehyde (MDA), total oxidant status (TOS), total antioxidant status, 8-hydroxy-20-deoxyguanosine (8-OHdG) levels and DNA single strand breaks were analysed. The results of the study showed an increase in 8-OHdG, MDA, PCO and TOS in cord blood and placenta in the group exposed to mobile phones during gestation. However, the group exposed to Wi-Fi did not show alterations in the studied oxidative stress parameters. On the other hand, tail intensity and tail moment of DNA in the mobile phone exposure groups were higher than those in the control and Wi-Fi exposure groups. In conclusion, the results of this study indicated that mobile phone exposure during pregnancy could have an important potential to cause oxidative stress and DNA damage in cord blood and placenta. The results of this study also indicated that combined effects of Wi-Fi plus mobile phone exposure have a higher potential to cause synergistic harmful effects.

(E) Belyaev IYa, Alipov YD, Shcheglov VS, Lystsov VN, Resonance effect of microwaves on the genome conformational state of E. coli cells. Z Naturforsch [C] 47(7-8):621-627,1992. (VT, AE, LI, GT)

The effect of low intensity microwaves on the conformational state of the genome of X-irradiated E. coli cells was studied by the method of viscosity anomalous time dependencies. It has been established that within the ranges of 51.62-51.84 GHz and 41.25-41.50 GHz the

frequency dependence of the observed effect has a resonance nature with a resonance half-width of the order of 100 MHz. The power dependence of the microwave effect within the range of 0.1-200 microW/cm² has shown that a power density of 1 microW/cm² is sufficient to suppress radiation-induced repair of the genome conformational state. The effect of microwave suppression of repair is well reproduced and does not depend on the sequence of cell exposure to X-rays and microwave radiation in the millimeter band. The results obtained indicate the role of the cell genome in the resonant interaction of cells with low intensity millimeter waves

(E) Belyaev IY, Hillert L, Protopopova M, Tamm C, Malmgren LO, Persson BR, Selivanova G, Harms-Ringdahl M. 915 MHz microwaves and 50 Hz magnetic field affect chromatin conformation and 53BP1 foci in human lymphocytes from hypersensitive and healthy persons. Bioelectromagnetics 26:173-184, 2005. (VT, AE, LI, GT)

We used exposure to microwaves from a global system for mobile communication (GSM) mobile phone (915 MHz, specific absorption rate (SAR) 37 mW/kg) and power frequency magnetic field (50 Hz, 15 μ T peak value) to investigate the response of lymphocytes from healthy subjects and from persons reporting hypersensitivity to electromagnetic field (EMF). The hypersensitive and healthy donors were matched by gender and age and the data were analyzed blind to treatment condition. The changes in chromatin conformation were measured with the method of anomalous viscosity time dependencies (AVTD). 53BP1 protein, which has been shown to colocalize in foci with DNA double strand breaks (DSBs), was analyzed by immunostaining in situ. Exposure at room temperature to either 915 MHz or 50 Hz resulted in significant condensation of chromatin, shown as AVTD changes, which was similar to the effect of heat shock at 41 degrees C. No significant differences in responses between normal and hypersensitive subjects were detected. Neither 915 MHz nor 50 Hz exposure induced 53BP1 foci. On the contrary, a distinct decrease in background level of 53BP1 signaling was observed upon these exposures as well as after heat shock treatments. This decrease correlated with the AVTD data and may indicate decrease in accessibility of 53BP1 to antibodies because of stress-induced chromatin condensation. Apoptosis was determined by morphological changes and by apoptotic fragmentation of DNA as analyzed by pulsed-field gel electrophoresis (PFGE). No apoptosis was induced by exposure to 50 Hz and 915 MHz microwaves. In conclusion, 50 Hz magnetic field and 915 MHz microwaves under specified conditions of exposure induced comparable responses in lymphocytes from healthy and hypersensitive donors that were similar but not identical to stress response induced by heat shock.

(E) Belyaev IY, Koch CB, Terenius O, Roxstromto 915 MHz GSM microwaves induces changes in gene expression but not double stranded DNA breaks or effects on chromatin conformation. Bioelectromagnetics 27:295-306, 2006. (VO, AE, GE)

We investigated whether exposure of rat brain to microwaves (MWs) of global system for mobile communication (GSM) induces DNA breaks, changes in chromatin conformation and in gene expression. An exposure installation was used based on a test mobile phone employing a GSM signal at 915 MHz, all standard modulations included, output power level in pulses 2 W, specific absorption rate (SAR) 0.4 mW/g. Rats were exposed or sham exposed to MWs during 2 h. After exposure, cell suspensions were prepared from brain samples, as well as from spleen and thymus. For analysis of gene expression patterns, total RNA was extracted from cerebellum.

Changes in chromatin conformation, which are indicative of stress response and genotoxic effects, were measured by the method of anomalous viscosity time dependencies (AVTD). DNA double strand breaks (DSBs) were analyzed by pulsed-field gel electrophoresis (PFGE). Effects of MW exposure were observed on neither conformation of chromatin nor DNA DSBs. Gene expression profiles were obtained by Affymetrix U34 GeneChips representing 8800 rat genes and analyzed with the Affymetrix Microarray Suite (MAS) 5.0 software. In cerebellum from all exposed animals, 11 genes were upregulated in a range of 1.34-2.74 fold and one gene was downregulated 0.48-fold ($P < .0025$). The induced genes encode proteins with diverse functions including neurotransmitter regulation, blood-brain barrier (BBB), and melatonin production. The data shows that GSM MWs at 915 MHz did not induce PFGE-detectable DNA double stranded breaks or changes in chromatin conformation, but affected expression of genes in rat brain cells

(E) Belyaev IY, Markovà E, Hillert L, Malmgren LO, Persson BR. Microwaves from UMTS/GSM mobile phones induce long-lasting inhibition of 53BP1/gamma-H2AX DNA repair foci in human lymphocytes. Bioelectromagnetics 30:129-141, 2009. (VT, AE, LI, GT, WS)

We have recently described frequency-dependent effects of mobile phone microwaves (MWs) of global system for mobile communication (GSM) on human lymphocytes from persons reporting hypersensitivity to electromagnetic fields and healthy persons. Contrary to GSM, universal global telecommunications system (UMTS) mobile phones emit wide-band MW signals. Hypothetically, UMTS MWs may result in higher biological effects compared to GSM signal because of eventual "effective" frequencies within the wideband. Here, we report for the first time that UMTS MWs affect chromatin and inhibit formation of DNA double-strand breaks co-localizing 53BP1/gamma-H2AX DNA repair foci in human lymphocytes from hypersensitive and healthy persons and confirm that effects of GSM MWs depend on carrier frequency. Remarkably, the effects of MWs on 53BP1/gamma-H2AX foci persisted up to 72 h following exposure of cells, even longer than the stress response following heat shock. The data are in line with the hypothesis that the type of signal, UMTS MWs, may have higher biological efficiency and possibly larger health risk effects compared to GSM radiation emissions. No significant differences in effects between groups of healthy and hypersensitive subjects were observed, except for the effects of UMTS MWs and GSM-915 MHz MWs on the formation of the DNA repair foci, which were different for hypersensitive ($P < 0.02[53BP1]/0.01[\text{gamma-H2AX}]$) but not for control subjects ($P > 0.05$). The non-parametric statistics used here did not indicate specificity of the differences revealed between the effects of GSM and UMTS MWs on cells from hypersensitive subjects and more data are needed to study the nature of these differences.

Bhargav H, Srinivasan TM, Varambally S, Gangadhar BN, Koka P. Effect of mobile phone-induced electromagnetic field on brain hemodynamics and human stem cell functioning: possible mechanistic link to cancer risk and early diagnostic value of electronphotonic imaging. J Stem Cells. 10(4):287-294, 2015. (review)

The mobile phones (MP) are low power radio devices which work on electromagnetic fields (EMFs), in the frequency range of 900-1800 MHz. Exposure to MP-EMFs may affect brain physiology and lead to various health hazards including brain tumors. Earlier studies with positron emission tomography (PET) have found alterations in cerebral blood flow (CBF) after

acute exposure to MPEMFs. It is widely accepted that DNA double-strand breaks (DSBs) and their misrepair in stem cells are critical events in the multistage origination of various leukemia and tumors, including brain tumors such as gliomas. Both significant imbalance in DSB repair and severe stress response have been triggered by MPEMFs and EMFs from cell towers. It has been shown that stem cells are most sensitive to microwave exposure and react to more frequencies than do differentiated cells. This may be important for cancer risk assessment and indicates that stem cells are the most relevant cellular model for validating safe mobile communication signals. Recently developed technology for recording the human bioelectromagnetic (BEM) field using Electron photonic Imaging (EPI) or Gas Discharge Visualisation (GDV) technique provides useful information about the human BEM. Studies have recorded acute effects of Mobile Phone Electromagnetic Fields (MPEMFs) using EPI and found quantifiable effects on human BEM field. Present manuscript reviews evidences of altered brain physiology and stem cell functioning due to mobile phone/cell tower radiations, its association with increased cancer risk and explores early diagnostic value of EPI imaging in detecting EMF induced changes on human BEM.

(NE) Bisht KS, Moros EG, Straube WL, Baty JD, Roti Roti JL, The Effect of 835.62 MHz FDMA or 847.74 MHz CDMA modulated radiofrequency radiation on the induction of micronuclei in C3H 10T $\frac{1}{2}$ cells. Radiat. Res. 157: 506–515, 2002.(VT, AE, GT)

To determine if radiofrequency (RF) radiation induces the formation of micronuclei, C3H 10T $\frac{1}{2}$ cells were exposed to 835.62 MHz frequency division multiple access (FDMA) or 847.74 MHz code division multiple access (CDMA) modulated RF radiation. After the exposure to RF radiation, the micronucleus assay was performed by the cytokinesis block method using cytochalasin B treatment. The micronuclei appearing after mitosis were scored in binucleated cells using acridine orange staining. The frequency of micronuclei was scored both as the percentage of binucleated cells with micronuclei and as the number of micronuclei per 100 binucleated cells. Treatment of cells with cytochalasin B at a concentration of 2 μ g/ml for 22 h was found to yield the maximum number of binucleated cells in C3H 10T $\frac{1}{2}$ cells. The method used for the micronucleus assay in the present study detected a highly significant dose response for both indices of micronucleus production in the dose range of 0.1–1.2 Gy and it was sensitive enough to detect a significant ($P > 0.05$) increase in micronuclei after doses of 0.3 Gy in exponentially growing cells and after 0.9 Gy in plateau-phase cells. Exponentially growing cells or plateau-phase cells were exposed to CDMA (3.2 or 4.8 W/kg) or FDMA (3.2 or 5.1 W/kg) RF radiation for 3, 8, 16 or 24 h. In three repeat experiments, no exposure condition was found by analysis of variance to result in a significant increase relative to sham-exposed cells either in the percentage of binucleated cells with micronuclei or in the number of micronuclei per 100 binucleated cells. In this study, data from cells exposed to different RF signals at two SARs were compared to a common sham-exposed sample. We used the Dunnett's test, which is specifically designed for this purpose, and found no significant exposure-related differences for either plateau-phase cells or exponentially growing cells. Thus the results of this study are not consistent with the possibility that these RF radiations induce micronuclei.

(E) Bourdineaud JP, Šrut M, Štambuk A, Tkalec M, Brèthes D, Malarić K, Klobučar GIV. Electromagnetic fields at a mobile phone frequency (900 MHz) trigger the onset of general stress response along with DNA modifications in Eisenia fetida earthworms. Arh Hig Rada Toksikol. 68(2):142-152, 2017. (VO, AE, LI, GT, OX)

Eisenia fetida earthworms were exposed to electromagnetic field (EMF) at a mobile phone frequency (900 MHz) and at field levels ranging from 10 to 120 V m⁻¹ for a period of two hours (corresponding to specific absorption rates ranging from 0.13 to 9.33 mW kg⁻¹). Potential effects of longer exposure (four hours), field modulation, and a recovery period of 24 h after two hours of exposure were addressed at the field level of 23 V m⁻¹. All exposure treatments induced significant DNA modifications as assessed by a quantitative random amplified polymorphic DNA-PCR. Even after 24 h of recovery following a two hour-exposure, the number of probe hybridisation sites displayed a significant two-fold decrease as compared to untreated control earthworms, implying a loss of hybridisation sites and a persistent genotoxic effect of EMF. Expression of genes involved in the response to general stress (HSP70 encoding the 70 kDa heat shock protein, and MEKK1 involved in signal transduction), oxidative stress (CAT, encoding catalase), and chemical and immune defence (LYS, encoding lysenin, and MYD, encoding a myeloid differentiation factor) were up-regulated after exposure to 10 and modulated 23 V m⁻¹ field levels. Western blots showing an increased quantity of HSP70 and MTCO1 proteins confirmed this stress response.

(NE) Bourthoumieu S, Joubert V, Marin B, Collin A, Leveque P, Terro F, Yardin C. Cytogenetic studies in human cells exposed in vitro to GSM-900 MHz radiofrequency radiation using R-banded karyotyping. Radiat Res 174:712-718, 2010. (VT, AE, GT)

It is important to determine the possible effects of exposure to radiofrequency (RF) radiation on the genetic material of cells since damage to the DNA of somatic cells may be linked to cancer development or cell death and damage to germ cells may lead to genetic damage in next and subsequent generations. The objective of this study was to investigate whether exposure to radiofrequency radiation similar to that emitted by mobile phones of second-generation standard Global System for Mobile Communication (GSM) induces genotoxic effects in cultured human cells. The cytogenetic effects of GSM-900 MHz (GSM-900) RF radiation were investigated using R-banded karyotyping after in vitro exposure of human cells (amniotic cells) for 24 h. The average specific absorption rate (SAR) was 0.25 W/kg. The exposures were carried out in wire-patch cells (WPCs) under strictly controlled conditions of temperature. The genotoxic effect was assessed immediately or 24 h after exposure using four different samples. One hundred metaphase cells were analyzed per assay. Positive controls were provided by using bleomycin. We found no direct cytogenetic effects of GSM-900 either 0 h or 24 h after exposure. To the best of our knowledge, our work is the first to study genotoxicity using complete R-banded karyotyping, which allows visualizing all the chromosomal rearrangements, either numerical or structural.

(NE) Bourthoumieu S, Terro F, Leveque P, Collin A, Joubert V, Yardin C. Aneuploidy studies in human cells exposed in vitro to GSM-900 MHz radiofrequency radiation using FISH. Int J Radiat Biol 87:400-408, 2011. (VT, AE, GT)

PURPOSE: Since previous research found an increase in the rate of aneuploidies in human lymphocytes exposed to radiofrequencies, it seems important to perform further studies. The objective of this study was then to investigate whether the exposure to RF (radiofrequency) radiation similar to that emitted by mobile phones of a second generation standard, i.e., Global System for Mobile communication (GSM) may induce aneuploidy in cultured human cells. **MATERIALS AND METHODS:** The potential induction of genomic instability by GSM-900 MHz radiofrequency (GSM-900) was investigated after in vitro exposure of human amniotic cells for 24 h to average-specific absorption rates (SAR) of 0.25, 1, 2 and 4 W/kg in the temperature range of 36.3-39.7°C. The exposures were carried out in a wire-patch cell (WPC). The rate of aneuploidy of chromosomes 11 and 17 was determined by interphase FISH (Fluorescence In Situ Hybridisation) immediately after independent exposure of three different donors for 24 h. At least 100 interphase cells were analysed per assay. **RESULTS:** No significant change in the rate of aneuploidy of chromosomes 11 and 17 was found following exposure to GSM-900 for 24 h at average SAR up to 4 W/kg. **CONCLUSION:** Our study did not show any in vitro aneuploidogenic effect of GSM using FISH and is not in agreement with the results of previous research.

(NE) Bourthoumieu S, Magnaudeix A, Terro F, Leveque P, Collin A, Yardin C. Study of p53 expression and post-transcriptional modifications after GSM-900 radiofrequency exposure of human amniotic cells. Bioelectromagnetics. 3(1):52-60, 2013. (VT, AE, GT)

The potential effects of radiofrequency (RF) exposure on the genetic material of cells are very important to determine since genome instability of somatic cells may be linked to cancer development. In response to genetic damage, the p53 protein is activated and can induce cell cycle arrest allowing more time for DNA repair or elimination of damaged cells through apoptosis. The objective of this study was to investigate whether the exposure to RF electromagnetic fields, similar to those emitted by mobile phones of the second generation standard, Global System for Mobile Communications (GSM), may induce expression of the p53 protein and its activation by post-translational modifications in cultured human cells. The potential induction of p53 expression and activation by GSM-900 was investigated after in vitro exposure of human amniotic cells for 24 h to average specific absorption rates (SARs) of 0.25, 1, 2, and 4 W/kg in the temperature range of 36.3-39.7 °C. The exposures were carried out using a wire-patch cell (WPC) under strictly controlled conditions of temperature. Expression and activation of p53 by phosphorylation at serine 15 and 37 were studied using Western blot assay immediately after three independent exposures of cell cultures provided from three different donors. Bleomycin-exposed cells were used as a positive control. According to our results, no significant changes in the expression and activation of the p53 protein by phosphorylation at serine 15 and 37 were found following exposure to GSM-900 for 24 h at average SARs up to 4 W/kg in human embryonic cells.

(E) Brech A, Kubinyi G, Németh Z, Bakos J, Fiocchi S, Thuróczy G. Genotoxic effects of intermediate frequency magnetic fields on blood leukocytes in vitro. Mutat Res Genet Toxicol Environ Mutagen 845:403060, 2019.(VT, AE, GT)

The widespread presence of electromagnetic sources in daily life has initiated several studies on the effects of radiofrequency and power frequency fields. Only few investigations on the genotoxic effects of exposure to intermediate frequency magnetic fields (IF-MF) have been done so far. Therefore, the aim of this study was to evaluate possible genotoxic effects of exposure to 123.90 kHz and 250.80 kHz IF-MF on canine and human blood. Blood was exposed to IF-MF at 630 A/m (0.79 mT) and 80 A/m (0.10 m T) with exposure durations of 1-5 h (hourly), 20 and 24 h. Cylindrically divided Petri dish system was developed for in vitro exposures where different induced current could be achieved in the samples at the same magnetic flux density level. For the assessment of genotoxicity the alkaline comet assay was applied. We detected a statistically significant increase in DNA damage only following 20 h exposure to IF-MF.

(E) Burlaka A, Tsybulin O, Sidorik E, Lukin S, Polishuk V, Tshmistrenko S, Yakymenko I. Overproduction of free radical species in embryonal cells exposed to low intensity radiofrequency radiation. Exp Oncol. 35(3):219-225, 2013. (VO, LE, GT, LI, DE, OX)

Aim: Long-term exposure of humans to low intensity radiofrequency electromagnetic radiation (RF-EMR) leads to a statistically significant increase in tumor incidence. Mechanisms of such the effects are unclear, but features of oxidative stress in living cells under RF-EMR exposure were previously reported. Our study aims to assess a production of initial free radical species, which lead to oxidative stress in the cell. Materials and Methods: Embryos of Japanese quails were exposed in ovo to extremely low intensity RF-EMR of GSM 900 MHz (0.25 μ W/cm²) during 158-360 h discontinuously (48 c - ON, 12 c - OFF) before and in the initial stages of development. The levels of superoxide (O₂^{·-}), nitrogen oxide (NO[·]), thiobarbituric acid reactive substances (TBARS), 8-oxo-2'-deoxyguanosine (8-oxo-dG) and antioxidant enzymes' activities were assessed in cells/tissues of 38-h, 5- and 10-day RF-EMR exposed and unexposed embryos. Results: The exposure resulted in a significant persistent overproduction of superoxide and nitrogen oxide in embryo cells during all period of analyses. As a result, significantly increased levels of TBARS and 8-oxo-dG followed by significantly decreased levels of superoxide dismutase and catalase activities were developed in the exposed embryo cells. Conclusion: Exposure of developing quail embryos to extremely low intensity RF-EMR of GSM 900 MHz during at least one hundred and fifty-eight hours leads to a significant overproduction of free radicals/reactive oxygen species and oxidative damage of DNA in embryo cells. These oxidative changes may lead to pathologies up to oncogenic transformation of cells.

(E) Busljeta I, Trosic I, Milkovic-Kraus S. Erythropoietic changes in rats after 2.45 GJz nonthermal irradiation. Int J Hyg Environ Health. 207(6):549-554, 2004.(VO, LE, GT)

The purpose of this study was to observe the erythropoietic changes in rats subchronically exposed to radiofrequency microwave (RF/MW) irradiation at nonthermal level. Adult male Wistar rats (N=40) were exposed to 2.45 GHz continuous RF/MW fields for 2 hours daily, 7 days a week, at 5-10 mW/cm². Exposed animals were divided into four subgroups (n=10 animals in each subgroup) in order to be irradiated for 2, 8, 15 and 30 days. Animals were sacrificed on the final irradiation day of each treated subgroup. Unexposed rats were used as control (N=24). Six animals were included into the each control subgroup. Bone marrow smears were examined to

determine absolute counts of anuclear cells and erythropoietic precursor cells. The absolute erythrocyte count, haemoglobin and haematocrit values were observed in the peripheral blood by an automatic cell counter. The bone marrow cytogenetic analysis was accomplished by micronucleus (MN) tests. In the exposed animals erythrocyte count, haemoglobin and haematocrit were increased in peripheral blood on irradiation days 8 and 15. Concurrently, anuclear cells and erythropoietic precursor cells were significantly decreased ($p < 0.05$) in the bone marrow on day 15, but micronucleated cells' frequency was increased. In the applied experimental condition, RF/MW radiation might cause disturbance in red cell maturation and proliferation, and induce micronucleus formation in erythropoietic cells.

(E) Buttiglione M, Roca L, Montemurno E, Vitiello F, Capozzi V, Cibelli G. Radiofrequency radiation (900 MHz) induces Egr-1 gene expression and affects cell-cycle control in human neuroblastoma cells. J Cell Physiol. 213(3):759-767, 2007. (VT, AE, GE)

Many environmental signals, including ionizing radiation and UV rays, induce activation of Egr-1 gene, thus affecting cell growth and apoptosis. The paucity and the controversial knowledge about the effect of electromagnetic fields (EMF) exposure of nerve cells prompted us to investigate the bioeffects of radiofrequency (RF) radiation on SH-SY5Y neuroblastoma cells. The effect of a modulated RF field of 900 MHz, generated by a wire patch cell (WPC) antenna exposure system on Egr-1 gene expression, was studied as a function of time. Short-term exposures induced a transient increase in Egr-1 mRNA level paralleled with activation of the MAPK subtypes ERK1/2 and SAPK/JNK. The effects of RF radiations on cell growth rate and apoptosis were also studied. Exposure to RF radiation had an anti-proliferative activity in SH-SY5Y cells with a significant effect observed at 24 h. RF radiation impaired cell cycle progression, reaching a significant G2-M arrest. In addition, the appearance of the sub-G1 peak, a hallmark of apoptosis, was highlighted after a 24-h exposure, together with a significant decrease in mRNA levels of Bcl-2 and survivin genes, both interfering with signaling between G2-M arrest and apoptosis. Our results provide evidence that exposure to a 900 MHz-modulated RF radiation affect both Egr-1 gene expression and cell regulatory functions, involving apoptosis inhibitors like Bcl-2 and survivin, thus providing important insights into a potentially broad mechanism for controlling in vitro cell viability.

(E) Cam ST, Seyhan N. Single-strand DNA breaks in human hair root cells exposed to mobile phone radiation. Int J Radiat Biol 88(5):420-424, 2012. (HU, AE, GT)

Purpose: To analyze the short term effects of radiofrequency radiation (RFR) exposure on genomic deoxyribonucleic acid (DNA) of human hair root cells. Subjects and methods: Hair samples were collected from 8 healthy human subjects immediately before and after using a 900-MHz GSM (Global System for Mobile Communications) mobile phone for 15 and 30 minutes. Single-strand DNA breaks of hair root cells from the samples were determined using the 'comet assay'. Results: The data showed that talking on a mobile phone for 15 or 30 minutes significantly increased ($p < .05$) single-strand DNA breaks in cells of hair roots close to the phone. Comparing the 15-min and 30-min data using the paired t-test also showed that significantly more damages resulted after 30 minutes than after 15 minutes of phone use. Conclusions: A short-term exposure (15 and 30 minutes) to RFR (900-MHz) from a mobile

phone caused a significant increase in DNA single-strand breaks in human hair root cells located around the ear which is used for the phone calls.

(E) Campisi A, Gulino M, Acquaviva R, Bellia P, Raciti G, Grasso R, Musumeci F, Vanella A, Triglia A. Reactive oxygen species levels and DNA fragmentation on astrocytes in primary culture after acute exposure to low intensity microwave electromagnetic field. *Neurosci Lett* 473:52-55. 2010. (VT, AE, GT, LI, OX, WS)

The exposure of primary rat neocortical astroglial cell cultures to acute electromagnetic fields (EMF) in the microwave range was studied. Differentiated astroglial cell cultures at 14 days in vitro were exposed for 5, 10, or 20 min to either 900 MHz continuous waves or 900 MHz waves modulated in amplitude at 50 Hz using a sinusoidal waveform and 100% modulation index. The strength of the electric field (rms value) at the sample position was 10V/m. No change in cellular viability evaluated by MTT test and lactate dehydrogenase release was observed. A significant increase in ROS levels and DNA fragmentation was found only after exposure of the astrocytes to modulated EMF for 20 min. No evident effects were detected when shorter time intervals or continuous waves were used. The irradiation conditions allowed the exclusion of any possible thermal effect. Our data demonstrate, for the first time, that even acute exposure to low intensity EMF induces ROS production and DNA fragmentation in astrocytes in primary cultures, which also represent the principal target of modulated EMF. Our findings also suggest the hypothesis that the effects could be due to hyperstimulation of the glutamate receptors, which play a crucial role in acute and chronic brain damage. Furthermore, the results show the importance of the amplitude modulation in the interaction between EMF and neocortical astrocytes.

(E) Caraglia M, Marra M, Mancinelli F, D'Ambrosio G, Massa R, Giordano A, Budillon A, Abbruzzese A, Bismuto E. Electromagnetic fields at mobile phone frequency induce apoptosis and inactivation of the multi-chaperone complex in human epidermoid cancer cells. *J Cell Physiol* 204(2):539-548, 2005.(VT, AE, GE)

The exposure to non-thermal microwave electromagnetic field (MW-EMF) at 1.95 MHz, a frequency used in mobile communication, affects the refolding kinetics of eukaryotic proteins (Mancinelli et al., 2004). On these basis we have evaluated the in vivo effect of MW-EMF in human epidermoid cancer KB cells. We have found that MW-EMF induces time-dependent apoptosis (45% after 3 h) that is paralleled by an about 2.5-fold decrease of the expression of ras and Raf-1 and of the activity of ras and Erk-1/2. Although also the expression of Akt was reduced its activity was unchanged likely as a consequence of the increased expression of its upstream activator PI3K. In the same experimental conditions an about 2.5-fold increase of the ubiquitination of ras and Raf-1 was also found and the addition for 12 h of proteasome inhibitor lactacystin at 10 microM caused an accumulation of the ubiquitinated isoforms of ras and Raf-1 and counteracted the effects of MW-EMF on ras and Raf-1 expression suggesting an increased proteasome-dependent degradation induced by MW-EMF. The exposure of KB cells to MW-EMF induced a differential activation of stress-dependent pathway with an increase of JNK-1 activity and HSP70 and 27 expression and with a reduction of p38 kinase activity and HSP90 expression. The overexpression of HSP90 induced by transfection of KB cells with a plasmid encoding for the factor completely antagonized the apoptosis and the inactivation of the ras --> Erk-dependent survival signal induced by MW-EMF. Conversely, the inhibition of Erk activity

induced by 12 h exposure to 10 mM Mek-1 inhibitor U0126 antagonized the effects induced by HSP90 transfection on apoptosis caused by MW-EMF. In conclusion, these results demonstrate for the first time that MW-EMF induces apoptosis through the inactivation of the ras --> Erk survival signaling due to enhanced degradation of ras and Raf-1 determined by decreased expression of HSP90 and the consequent increase of proteasome dependent degradation.

(E) Carballo-Quintás M, Martínez-Silva I, Cadarso-Suárez C, Alvarez-Figueiras M, Ares-Pena FJ, López-Martín E. A study of neurotoxic biomarkers, c-fos and GFAP after acute exposure to GSM radiation at 900 MHz in the picrotoxin model of rat brains. Neurotoxicology. 32(4):478-494, 2011. (VO, AE, GE,IX)

The acute effects of microwave exposure from the Global System for Mobile Communication (GSM) were studied in rats, using 900MHz radiation at an intensity similar to mobile phone emissions. Acute subconvulsive doses of picrotoxin were then administered to the rats and an experimental model of seizure-proneness was created from the data. Seventy-two adult male Sprague-Dawley rats underwent immunochemical testing of relevant anatomical areas to measure induction of the c-fos neuronal marker after 90min and 24h, and of the glial fibrillary acidic protein (GFAP) 72h after acute exposure to a 900MHz electromagnetic field (EMF). The experimental set-up facilitated measurement of absorbed power, from which the average specific absorption rate was calculated using the finite-difference time-domain (FDTD) 2h after exposure to EMF radiation at 1.45W/kg in picrotoxin-treated rats and 1.38W/kg in untreated rats. Ninety minutes after radiation high levels of c-fos expression were recorded in the neocortex and paleocortex along with low hippocampus activation in picrotoxin treated animals. Most brain areas, except the limbic cortical region, showed important increases in neuronal activation 24h after picrotoxin and radiation. Three days after picrotoxin treatment, radiation effects were still apparent in the neocortex, dentate gyrus and CA3, but a significant decrease in activity was noted in the piriform and entorhinal cortex. During this time, glial reactivity increased with every seizure in irradiated, picrotoxin-treated brain regions. Our results reveal that c-fos and glial markers were triggered by the combined stress of non-thermal irradiation and the toxic effect of picrotoxin on cerebral tissues.

(E) Cervellati F, Valacchi G, Lunghi L, Fabbri E, Valbonesi P, Marci R, Biondi C, Vesce F. 17- β -estradiol counteracts the effects of high frequency electromagnetic fields on trophoblastic connexins and integrins. Oxid Med Cell Longev. 2013:280850, 2013. (VT, AE, GE, IX)

We investigated the effect of high-frequency electromagnetic fields (HF-EMFs) and 17- β -estradiol on connexins (Cxs), integrins (Ints), and estrogen receptor (ER) expression, as well as on ultrastructure of trophoblast-derived HTR-8/SVneo cells. HF-EMF, 17- β -estradiol, and their combination induced an increase of Cx40 and Cx43 mRNA expression. HF-EMF decreased Int alpha1 and β 1 mRNA levels but enhanced Int alpha5 mRNA expression. All the Ints mRNA expressions were increased by 17- β -estradiol and exposure to both stimuli. ER- β mRNA was reduced by HF-EMF but augmented by 17- β -estradiol alone or with HF-EMF. ER- β immunofluorescence showed a cytoplasmic localization in sham and HF-EMF exposed cells which became nuclear after treatment with hormone or both stimuli. Electron microscopy

evidenced a loss of cellular contact in exposed cells which appeared counteracted by 17- β -estradiol. We demonstrate that 17- β -estradiol modulates Cxs and Ints as well as ER- β expression induced by HF-EMF, suggesting an influence of both stimuli on trophoblast differentiation and migration.

(E) Chandel S, Kaur S, Issa M, Singh HP, Batish DR, Kohli RK. Exposure to mobile phone radiations at 2350 MHz incites cyto- and genotoxic effects in root meristems of *Allium cepa*. J Environ Health Sci Eng. 17(1):97-104, 2019a. (VO, AE, GT)

BACKGROUND: The exponential increase of electromagnetic field radiations (EMF-r) in the natural environment has raked up the controversies regarding their biological effects. Concern regarding the putative capacity of EMF-r to affect living beings has been growing due to the ongoing elevation in the use of high frequency EMF-r in communication systems, e.g. Mobile phones. **METHODS:** In the present study, we tried to examine the cyto- and genotoxic potential of mobile phone EMF-r at 2350 MHz using onions (*Allium cepa* L.). Fresh adventitious onion roots were exposed to continuous EMF-r at 2350 MHz for different time periods (1 h, 2 h and 4 h). The evaluation of cytotoxicity was done in terms of mitotic index (MI), phase index and chromosomal aberrations. Genotoxicity was investigated employing comet assay in terms of changes in % HDNA (head DNA) and % TDNA (tail DNA), TM (tail moment) and OTM (olive tail moment). Data were analyzed using one-way ANOVA and mean values were separated using post hoc Tukey's test. **RESULTS:** The results manifested a significant increase of MI and chromosomal aberrations (%) upon 4 h, and ≥ 2 h of exposure, respectively, as compared to the control. No specific changes in phase index in response to EMF-r exposure were observed. The % HDNA and % TDNA values exhibited significant changes in contrast to that of control upon 2 h and 4 h of exposure, respectively. However, TM and OTM did not change significantly. **CONCLUSIONS:** Our results infer that continuous exposures of radiofrequency EMF-r (2350 MHz) for long durations have a potential of inciting cyto- and genotoxic effects in onion root meristems.

(E) Chandel S, Kaur S, Issa M, Singh HP, Batish DR, Kohli RK. Appraisal of immediate and late effects of mobile phone radiations at 2100 MHz on mitotic activity and DNA integrity in root meristems of *Allium cepa*. Protoplasma. 256(5):1399-1407, 2019b. (VO, AE, GT)

The present study evaluated the potential of 2100 MHz radiofrequency radiations to act as cytotoxic and genotoxic agent. Fresh onion (*Allium cepa* L.) roots were exposed to electromagnetic field radiations (EMF-r) for different durations (1 h and 4 h) and evaluated for mitotic index (MI), phase index, chromosomal aberrations, and DNA damage. DNA damage was investigated with the help of the comet assay by assessing various parameters like % head DNA (HDNA), % tail DNA (TDNA), tail moment (TM), and olive tail moment (OTM). Effects of EMF-r exposure were also compared with that of methyl methanesulfonate (MMS; 90 μ M), which acted as a positive control. The post-exposure effects of EMF-r after providing the test plants with an acclimatization period of 24 h were also evaluated. Compared to the control, a significant increase in the MI and aberration percentage was recorded upon 4 h of exposure. However, no specific trend of phase index in response to exposure was detected. EMF-r

exposure incited DNA damage with a significant decrease in HDNA accompanied by an increase in TDNA upon exposure of 4 h. However, TM and OTM did not change significantly upon exposure as compared to that of control. Analysis of the post-exposure effects of EMF-r did not show any significant change/recovery. Our data, thus, suggest the potential cytotoxic and genotoxic nature of 2100 MHz EMF-r. Our study bears great significance in view of the swiftly emergent EMF-r in the surrounding environment and their potential for inciting aberrations at the chromosomal level, thus posing a genetic hazard.

(NE) Chang SK, Choi JS, Gil HW, Yang JO, Lee EY, Jeon YS, Lee ZW, Lee M, Hong MY, Ho Son T, Hong SY. Genotoxicity evaluation of electromagnetic fields generated by 835-MHz mobile phone frequency band. Eur J Cancer Prev 14:175-179, 2005. (VT, AE, GT) (Some interaction effects with chemicals are reported in this paper.)

It is still unclear whether the exposure to electromagnetic fields (EMFs) generated by mobile phone radiation is directly linked to cancer. We examined the biological effects of an EMF at 835 MHz, the most widely used communication frequency band in Korean CDMA mobile phone networks, on bacterial reverse mutation (Ames assay) and DNA stability (in vitro DNA degradation). In the Ames assay, tester strains alone or combined with positive mutagen were applied in an artificial mobile phone frequency EMF generator with continuous waveform at a specific absorption rate (SAR) of 4 W/kg for 48 h. In the presence of the 835-MHz EMF radiation, incubation with positive mutagen 4-nitroquinoline-1-oxide and cumene hydroxide further increased the mutation rate in Escherichia coli WP2 and TA102, respectively, while the contrary results in Salmonella typhimurium TA98 and TA1535 treated with 4-nitroquinoline-1-oxide and sodium azide, respectively, were shown as antimutagenic. However, these mutagenic or co-mutagenic effects of 835-MHz radiation were not significantly repeated in other relevant strains with same mutation type. In the DNA degradation test, the exposure to 835-MHz EMF did not change the rate of degradation observed using plasmid pBluescriptSK(+) as an indicator. Thus, we suggest that 835-MHz EMF under the conditions of our study neither affected the reverse mutation frequency nor accelerated DNA degradation in vitro.

(E) Chaturvedi CM, Singh VP, Singh P, Basu P, Singaravel M, Shukla RK, Dhawan A, Pati AK, Gangwar RK, and Singh SP. 2.45 GHz (CW) microwave irradiation alters circadian organization, spatial memory, DNA structure in the brain cells and blood cell counts of male mice, Mus musculus. Progress In Electromagnetics Research B, Vol. 29, 23-42, 2011. (VO, LE, GT)

Present study examines biological effects of 2.45 GHz microwave radiation in Parkes strain mice. Forty-day-old mice were exposed to CW (continuous wave) microwave radiation (2 h/day for 30 days). Locomotor activity was recorded on running wheel for 12 days prior to microwave exposure (pre-exposure), 7 days during the first week of exposure (short-term exposure) and another 7-day spell during the last week of the 30-day exposure period (long-term exposure). Morris water maze test was performed from 17th to 22nd day of exposure. At the termination of the exposure, blood was processed for hematological parameters, brain for comet assay, epididymis for sperm count and motility and serum for SGOT (serum glutamate oxaloacetate transaminase) and SGPT (serum glutamate pyruvate transaminase). The results show that long-term radiation-exposed group exhibited a positive ψ (phase angle difference) for the onset of activity with reference to lights-off timing and most of the activity occurred within the light

fraction of the LD (light: dark) cycle. Microwave radiation caused an increase in erythrocyte and leukocyte counts, a significant DNA strand break in brain cells and the loss of spatial memory in mice. This report for the first time provides experimental evidence that continuous exposure to low intensity microwave radiation may have an adverse effect on the brain function by altering circadian system and rate of DNA damage.

(NE) Chauhan V, Mariampillai A, Bellier PV, Qutob SS, Gajda GB, Lemay E, Thansandote A, McNamee JP. Gene expression analysis of a human lymphoblastoma cell line exposed in vitro to an intermittent 1.9 GHz pulse-modulated radiofrequency field. Radiat Res. 165(4):424-429, 2006a. (VT, AE, GE)

This study was designed to determine whether radiofrequency (RF) fields of the type used for wireless communications could elicit a cellular stress response. As general indicators of a cellular stress response, we monitored changes in proto-oncogene and heat-shock protein expression. Exponentially growing human lymphoblastoma cells (TK6) were exposed to 1.9 GHz pulse-modulated RF fields at average specific absorption rates (SARs) of 1 and 10 W/kg. Perturbations in the expression levels of the proto-oncogenes FOS, JUN and MYC after exposure to sham and RF fields were assessed by real-time RT-PCR. In addition, the transcript levels of the cellular stress proteins HSP27 and inducible HSP70 were also monitored. We demonstrated that transcript levels of these genes in RF-field-exposed cells showed no significant difference in relation to the sham treatment group. However, concurrent positive (heat-shock) control samples displayed a significant elevation in the expression of HSP27, HSP70, FOS and JUN. Conversely, the levels of MYC mRNA were found to decline in the positive (heat-shock) control. In conclusion, our study found no evidence that the 1.9 GHz RF-field exposure caused a general stress response in TK6 cells under our experimental conditions.

(NE) Chauhan V, Mariampillai A, Gajda GB, Thansandote A, McNamee JP. Analysis of proto-oncogene and heat-shock protein gene expression in human derived cell-lines exposed in vitro to an intermittent 1.9 GHz pulse-modulated radiofrequency field. Int J Radiat Biol. 82(5):347-354, 2006b. (VT, AE, GE)

Purpose: Several studies have reported that radiofrequency (RF) fields, as emitted by mobile phones, may cause changes in gene expression in cultured human cell-lines. The current study was undertaken to evaluate this possibility in two human-derived immune cell-lines. Materials and methods: HL-60 and Mono-Mac-6 (MM6) cells were individually exposed to intermittent (5 min on, 10 min off) 1.9 GHz pulse-modulated RF fields at a average specific absorption rate (SAR) of 1 and 10 W/kg at 37 +/- 0.5 degrees C for 6 h. Concurrent negative and positive (heat-shock for 1 h at 43 degrees C) controls were conducted with each experiment. Immediately following RF field exposure (T = 6 h) and 18 h post-exposure (T = 24 h), cell pellets were collected from each of the culture dishes and analyzed for transcript levels of proto-oncogenes (c-jun, c-myc and c-fos) and the stress-related genes (heat shock proteins (HSP) HSP27 and HSP70B) by quantitative reverse transcriptase polymerase chain reaction (RT-PCR). Results: No significant effects were observed in mRNA expression of HSP27, HSP70, c-jun, c-myc or c-fos

between the sham and RF-exposed groups, in either of the two cell-lines. However, the positive (heat-shock) control group displayed a significant elevation in the expression of HSP27, HSP70, c-fos and c-jun in both cell-lines at T = 6 and 24 h, relative to the sham and negative control groups. Conclusion: This study found no evidence that exposure of cells to non-thermalizing levels of 1.9 GHz pulse-modulated RF fields can cause any detectable change in stress-related gene expression.

(NE) Chauhan V, Qutob SS, Lui S, Mariampillai A, Bellier PV, Yauk CL, Douglas GR, Williams A, McNamee JP. Analysis of gene expression in two human-derived cell lines exposed in vitro to a 1.9 GHz pulse-modulated radiofrequency field. Proteomics. 7(21):3896-3905, 2007. (VT, AE, GE)

There is considerable controversy surrounding the biological effects of radiofrequency (RF) fields, as emitted by mobile phones. Previous work from our laboratory has shown no effect related to the exposure of 1.9 GHz pulse-modulated RF fields on the expression of 22,000 genes in a human glioblastoma-derived cell-line (U87MG) at 6 h following a 4 h RF field exposure period. As a follow-up to this study, we have now examined the effect of RF field exposure on the possible expression of late onset genes in U87MG cells after a 24 h RF exposure period. In addition, a human monocyte-derived cell-line (Mono-Mac-6, MM6) was exposed to intermittent (5 min ON, 10 min OFF) RF fields for 6 h and then gene expression was assessed immediately after exposure and at 18 h postexposure. Both cell lines were exposed to 1.9 GHz pulse-modulated RF fields for 6 or 24 h at specific absorption rates (SARs) of 0.1-10.0 W/kg. In support of our previous results, we found no evidence that nonthermal RF field exposure could alter gene expression in either cultured U87MG or MM6 cells, relative to nonirradiated control groups. However, exposure of both cell-lines to heat-shock conditions (43 degrees C for 1 h) caused an alteration in the expression of a number of well-characterized heat-shock proteins.

(E) Chavdoula ED, Panagopoulos DJ, Margaritis LH. Comparison of biological effects between continuous and intermittent exposure to GSM-900-MHz mobile phone radiation: detection of apoptotic cell-death features. Mutat Res 700:51-61, 2010. (VO, LE, GT, RP)

In the present study we used a 6-min daily exposure of dipteran flies, *Drosophila melanogaster*, to GSM-900 MHz (Global System for Mobile Telecommunications) mobile phone electromagnetic radiation (EMR), to compare the effects between the continuous and four different intermittent exposures of 6min total duration, and also to test whether intermittent exposure provides any cumulative effects on the insect's reproductive capacity as well as on the induction of apoptotic cell death. According to our previous experiments, a 6-min continuous exposure per day for five days to GSM-900 MHz and DCS-1800 MHz (Digital Cellular System) mobile phone radiation, brought about a large decrease in the insect's reproductive capacity, as defined by the number of F pupae. This decrease was found to be non thermal and correlated with an increased percentage of induced fragmented DNA in the egg chambers' cells at early- and mid-oogenesis. In the present experiments we show that intermittent exposure also decreases the reproductive capacity and alters the actin cytoskeleton network of the egg chambers, another known aspect of cell death that was not investigated in previous experiments, and that the effect is also due to DNA fragmentation. Intermittent exposures with 10-min intervals between

exposure sessions proved to be almost equally effective as continuous exposure of the same total duration, whereas longer intervals between the exposures seemed to allow the organism the time required to recover and partly overcome the above-mentioned effects of the GSM exposure.

(NE) Chemeris NK, Gapeyev AB, Sirota NP, Gudkova OY, Kornienko NV, Tankanag AV, Konovalov IV, Buzoverya ME, Suvorov VG, Logunov VA. DNA damage in frog erythrocytes after in vitro exposure to a high peak-power pulsed electromagnetic field. Mutat Res. 558(1-2): 27-34, 2004. (VT, AE, GT)

Till the present time, the genotoxic effects of high peak-power pulsed electromagnetic fields (HPPP EMF) on cultured cells have not been studied. We investigated possible genotoxic effects of HPPP EMF (8.8 GHz, 180 ns pulse width, peak power 65 kW, repetition rate 50 Hz) on erythrocytes of the frog *Xenopus laevis*. We used the alkaline comet assay, which is a highly sensitive method to assess DNA single-strand breaks and alkali-labile lesions. Blood samples were exposed to HPPP EMF for 40 min in rectangular wave guide. The specific absorption rate (SAR) calculated from temperature kinetics was about 1.6 kW/kg (peak SAR was about 300 MW/kg). The temperature rise in the blood samples at steady state was 3.5 +/- 0.1 degrees C. The data show that the increase in DNA damage after exposure of erythrocytes to HPPP EMF was induced by the rise in temperature in the exposed cell suspension. This was confirmed in experiments in which cells were incubated for 40 min under the corresponding temperature conditions. The results allow us to conclude that HPPP EMF-exposure at the given modality did not cause any a-thermal genotoxic effect on frog erythrocytes in vitro.

(NE) Chemeris NK, Gapeyev AB, Sirota NP, Gudkova OYu, Tankanag AV, Konovalov IV, Buzoverya ME, Suvorov VG, Logunov VA. Lack of direct DNA damage in human blood leukocytes and lymphocytes after in vitro exposure to high power microwave pulses. Bioelectromagnetics 2006 Apr;27(3):197-203. (VT, AE, GT)

Currently, the potential genotoxicity of high power microwave pulses (HPMP) is not clear. Using the alkaline single cell gel electrophoresis assay, also known as the alkaline comet assay, we studied the effects of HPMP (8.8 GHz, 180 ns pulse width, peak power 65 kW, pulse repetition frequency 50 Hz) on DNA of human whole-blood leukocytes and isolated lymphocytes. The cell suspensions were exposed to HPMP for 40 min in a rectangular waveguide. The average SAR calculated from the temperature kinetics was about 1.6 kW/kg (peak SAR was about 300 MW/kg). The steady-state temperature rise in the 50 microl samples exposed to HPMP was 3.5 +/- 0.1 degrees C. In independent experiments, we did not find any statistically significant DNA damage manifested immediately after in vitro HPMP exposure of human blood leukocytes or lymphocytes or after HPMP exposure of leukocytes subsequently incubated at 37 degrees C for 30 min. Our results indicate that HPMP under the given exposure conditions did not induce DNA strand breaks, alkali-labile sites, and incomplete excision repair sites, which could be detected by the alkaline comet assay.

(E) Chen G, Lu D, Chiang H, Leszczynski D, Xu Z. Using model organism *Saccharomyces cerevisiae* to evaluate the effects of ELF-MF and RF-EMF exposure on global gene expression. *Bioelectromagnetics*. 33(7):550-560, 2012 . (VO, AE, GE)

The potential health hazard of exposure to electromagnetic fields (EMF) continues to cause public concern. However, the possibility of biological and health effects of exposure to EMF remains controversial and their biophysical mechanisms are unknown. In the present study, we used *Saccharomyces cerevisiae* to identify genes responding to extremely low frequency magnetic fields (ELF-MF) and to radiofrequency EMF (RF-EMF) exposures. The yeast cells were exposed for 6 h to either 0.4 mT 50 Hz ELF-MF or 1800 MHz RF-EMF at a specific absorption rate of 4.7 W/kg. Gene expression was analyzed by microarray screening and confirmed using real-time reverse transcription-polymerase chain reaction (RT-PCR). We were unable to confirm microarray-detected changes in three of the ELF-MF responsive candidate genes using RT-PCR ($P > 0.05$). On the other hand, out of the 40 potential RF-EMF responsive genes, only the expressions of structural maintenance of chromosomes 3 (SMC3) and aquaporin 2 (AQY2 (m)) were confirmed, while three other genes, that is, halotolerance protein 9 (HAL9), yet another kinase 1 (YAK1) and one function-unknown gene (open reading frame: YJL171C), showed opposite changes in expression compared to the microarray data ($P < 0.05$). In conclusion, the results of this study suggest that the yeast cells did not alter gene expression in response to 50 Hz ELF-MF and that the response to RF-EMF is limited to only a very small number of genes. The possible biological consequences of the gene expression changes induced by RF-EMF await further investigation.

(E) Chen C, Ma Q, Deng P, Lin M, Gao P, He M, Lu Y, Pi H, He Z, Zhou C, Zhang Y, Yu Z, Zhang L 1800 MHz Radiofrequency Electromagnetic Field Impairs Neurite Outgrowth Through Inhibiting EPHA5 Signaling. *Front Cell Dev Biol* 2021 9:657623. (VT, AE, GE)

The increasing intensity of environmental radiofrequency electromagnetic fields (RF-EMF) has increased public concern about its health effects. Of particular concern are the influences of RF-EMF exposure on the development of the brain. The mechanisms of how RF-EMF acts on the developing brain are not fully understood. Here, based on high-throughput RNA sequencing techniques, we revealed that transcripts related to neurite development were significantly influenced by 1800 MHz RF-EMF exposure during neuronal differentiation. Exposure to RF-EMF remarkably decreased the total length of neurite and the number of branch points in neural stem cells-derived neurons and retinoic acid-induced Neuro-2A cells. The expression of Eph receptors 5 (EPHA5), which is required for neurite outgrowth, was inhibited remarkably after RF-EMF exposure. Enhancing EPHA5 signaling rescued the inhibitory effects of RF-EMF on neurite outgrowth. Besides, we identified that cAMP-response element-binding protein (CREB) and RhoA were critical downstream factors of EPHA5 signaling in mediating the inhibitory effects of RF-EMF on neurite outgrowth. Together, our finding revealed that RF-EMF exposure impaired neurite outgrowth through EPHA5 signaling. This finding explored the effects and key mechanisms of how RF-EMF exposure impaired neurite outgrowth and also provided a new clue to understanding the influences of RF-EMF on brain development.

(NE) Choi J, Min K, Jeon S, Kim N, Paek JK, Song K. Continuous exposure to 1.7 GHz LTE electromagnetic fields increases intracellular reactive oxygen species to decrease human cell proliferation and induce senescence. Sci Rep. 10(1):9238, 2020. (VT, AE, GT, OX)

Due to the rapid development of mobile phone technology, we are continuously exposed to 1.7 GHz LTE radio frequency electromagnetic fields (RF-EMFs), but their biological effects have not been clarified. Here, we investigated the non-thermal cellular effects of these RF-EMFs on human cells, including human adipose tissue-derived stem cells (ASCs), Huh7 and Hep3B liver cancer stem cells (CSCs), HeLa and SH-SY5Y cancer cells, and normal fibroblast IMR-90 cells. When continuously exposed to 1.7 GHz LTE RF-EMF for 72 h at 1 and 2 SAR, cell proliferation was consistently decreased in all the human cells. The anti-proliferative effect was higher at 2 SAR than 1 SAR and was less severe in ASCs. The exposure to RF-EMF for 72 h at 1 and 2 SAR did not induce DNA double strand breaks or apoptotic cell death, but did trigger a slight delay in the G1 to S cell cycle transition. Cell senescence was also clearly observed in ASC and Huh7 cells exposed to RF-EMF at 2 SAR for 72 h. Intracellular ROS increased in these cells and the treatment with an ROS scavenger recapitulated the anti-proliferative effect of RF-EMF. These observations strongly suggest that 1.7 GHz LTE RF-EMF decrease proliferation and increase senescence by increasing intracellular ROS in human cells.

(NE) Ciaravino V, Meltz ML, Erwin DN, Absence of a synergistic effect between moderate power radio-frequency electromagnetic radiation and adriamycin on cell-cycle progression and sister-chromatid exchange. Bioelectromagnetics 12(5):289-298, 1991.(VT, AE, GT)

In our laboratories we are conducting investigations of potential interactions between radio-frequency electromagnetic radiation (RFR) and chemicals that are toxic by different mechanisms to mammalian cells. The RFR is being tested at frequencies in the microwave range and at different power levels. We report here on the 1) ability of simultaneous RFR exposures to alter the distribution of cells in first and second mitoses from that after treatment by adriamycin alone, and 2) on the ability of simultaneous RFR exposure to alter the extent of sister chromatid exchanges (SCEs) induced by adriamycin alone. This chemical was selected because of its reported mechanism of action and because it is of interest in the treatment of cancer. In our studies, Chinese hamster ovary (CHO) cells were exposed for 2 h simultaneously to adriamycin and pulsed RFR at a frequency of 2,450 MHz and a specific absorption rate of 33.8 W/Kg. The maximal temperature (in the tissue-culture medium) was 39.7 +/- 0.2 degrees C. The experiments were controlled for chemical and RFR exposures, as well as for temperature. Verified statistically, the data indicate that the RFR did not affect changes in cell progression caused by adriamycin, and the RFR did not change the number of SCEs that were induced by the adriamycin, which adriamycin is known to affect cells by damaging their membranes and DNA.

***(E) Colciago A, Audano M, Bonalume V, Melfi V, Mohamed T, Reid AJ, Faroni A, Greer PA, Mitro N, Magnaghi V Transcriptomic Profile Reveals Deregulation of Hearing-**

Loss Related Genes in Vestibular Schwannoma Cells Following Electromagnetic Field Exposure. Cells 2021 Jul 20;10(7):1840. (VT, LE, GE)

Hearing loss (HL) is the most common sensory disorder in the world population. One common cause of HL is the presence of vestibular schwannoma (VS), a benign tumor of the VIII cranial nerve, arising from Schwann cell (SC) transformation. In the last decade, the increasing incidence of VS has been correlated to electromagnetic field (EMF) exposure, which might be considered a pathogenic cause of VS development and HL. Here, we explore the molecular mechanisms underlying the biologic changes of human SCs and/or their oncogenic transformation following EMF exposure. Through NGS technology and RNA-Seq transcriptomic analysis, we investigated the genomic profile and the differential display of HL-related genes after chronic EMF. We found that chronic EMF exposure modified the cell proliferation, in parallel with intracellular signaling and metabolic pathways changes, mostly related to translation and mitochondrial activities. Importantly, the expression of HL-related genes such as NEFL, TPRN, OTOGL, GJB2, and REST appeared to be deregulated in chronic EMF exposure. In conclusion, we suggest that, at a preclinical stage, EMF exposure might promote the transformation of VS cells and contribute to HL.

(E) Costantini E, Aielli L, Serra F, De Dominicis L, Falasca K, Di Giovanni P, Reale M. Evaluation of Cell Migration and Cytokines Expression Changes under the Radiofrequency Electromagnetic Field on Wound Healing In Vitro Model. Int J Mol Sci. 2022 Feb 17;23(4):2205. (VT, AE, GE)

Wound healing (WH) proceeds through four distinct phases: hemostasis, inflammation, proliferation, and remodeling. Impaired WH may be the consequence of the alteration of one of these phases and represents a significant health and economic burden to millions of individuals. Thus, new therapeutic strategies are the topics of intense research worldwide. Although radiofrequency electromagnetic field (RF-EMF) has many medical applications in rehabilitation, pain associated with musculoskeletal disorders, and degenerative joint disorders, its impact on WH is not fully understood. The process of WH begins just after injury and continues during the inflammatory and proliferative phases. A thorough understanding of the mechanisms by which RF-EMF can improve WH is required before it can be used as a non-invasive, inexpensive, and easily self-applicable therapeutic strategy. Thus, the aim of this study is to explore the therapeutic potential of different exposure setups of RF-EMF to drive faster healing, evaluating the keratinocytes migration, cytokines, and matrix metalloproteinases (MMPs) expression. The results showed that RF-EMF treatment promotes keratinocytes' migration and regulates the expression of genes involved in healing, such as MMPs, tissue inhibitors of metalloproteinases, and pro/anti-inflammatory cytokines, to improve WH.

(E) Czyn J, Guan K, Zeng Q, Nikolova T, Meister A, Schönborn F, Schuderer J, Kuster N, Wobus AM. High frequency electromagnetic fields (GSM signals) affect gene expression levels in tumor suppressor p53-deficient embryonic stem cells. Bioelectromagnetics 25(4):296-307, 2004. (VT, AE, GE)

Effects of electromagnetic fields (EMF) simulating exposure to the Global System for Mobile Communications (GSM) signals were studied using pluripotent embryonic stem (ES) cells in vitro. Wild-type ES cells and ES cells deficient for the tumor suppressor p53 were exposed to pulse modulated EMF at 1.71 GHz, lower end of the uplink band of GSM 1800, under standardized and controlled conditions, and transcripts of regulatory genes were analyzed during in vitro differentiation. Two dominant GSM modulation schemes (GSM-217 and GSM-Talk), which generate temporal changes between GSM-Basic (active during talking phases) and GSM-DTX (active during listening phases thus simulating a typical conversation), were applied to the cells at and below the basic safety limits for local exposures as defined for the general public by the International Commission on Nonionizing Radiation Protection (ICNIRP). GSM-217 EMF induced a significant upregulation of mRNA levels of the heat shock protein, hsp70 of p53-deficient ES cells differentiating in vitro, paralleled by a low and transient increase of c-jun, c-myc, and p21 levels in p53-deficient, but not in wild-type cells. No responses were observed in either cell type after EMF exposure to GSM-Talk applied at similar slot-averaged specific absorption rates (SAR), but at lower time-averaged SAR values. Cardiac differentiation and cell cycle characteristics were not affected in embryonic stem and embryonic carcinoma cells after exposure to GSM-217 EMF signals. Our data indicate that the genetic background determines cellular responses to GSM modulated EMF.

(E) D'ambrosio G, Lioi MB, Massa R, Scarfi MR, Zeni O. Genotoxic Effects of amplitude-modulated microwaves on human lymphocytes exposed in vitro under controlled conditions. Electro- and Magnetiobiology 14:157-164, 1995. (VT, AE, GT, WS)

Peripheral human blood from 23 healthy donors aged between 23 and 95 years was exposed to continuous wave (CW) or 50 Hz amplitude modulated (AM) microwave radiation and was cultured for 72 h. Other exposure parameters were: frequency 9 GHz, specific absorption rate (SAR) 90 mW/g, exposure duration 10 min. The possible genotoxic effect was evaluated by means of cytokinesis-block micronucleus method. A significant ($p < 0.05$) increase in micronuclei was found following AM microwave exposure

(E) d'Ambrosio G, Massa R, Scarfi MR, Zeni O, Cytogenetic damage in human lymphocytes following GSMK phase modulated microwave exposure. Bioelectromagnetics 23:7-13, 2002. (VT, AE, GT, WS)

The present study investigated, using in vitro experiments on human lymphocytes, whether exposure to a microwave frequency used for mobile communication, either unmodulated or in presence of phase only modulation, can cause modification of cell proliferation kinetics and/or genotoxic effects, by evaluating the cytokinesis block proliferation index and the micronucleus frequency. In the GSM 1800 mobile communication systems the field is both phase (Gaussian minimum shift keying, GSMK) and amplitude (time domain multiple access, TDMA) modulated. The present study investigated only the effects of phase modulation, and no amplitude modulation was applied. Human peripheral blood cultures were exposed to 1.748 GHz, either continuous wave (CW) or phase only modulated wave (GSMK), for 15 min. The maximum specific absorption rate (~ 5 W/kg) was higher than that occurring in the head of mobile phone users; however, no changes were found in cell proliferation kinetics after exposure to either CW

or GMSK fields. As far as genotoxicity is concerned, the micronucleus frequency result was not affected by CW exposure; however, a statistically significant micronucleus effect was found following exposure to phase modulated field. These results would suggest a genotoxic power of the phase modulation per se.

(NE) Dawe AS, Nylund R, Leszczynski D, Kuster N, Reader T, De Pomerai DI. Continuous wave and simulated GSM exposure at 1.8 W/kg and 1.8 GHz do not induce hsp16-1 heat-shock gene expression in *Caenorhabditis elegans*. *Bioelectromagnetics*. 29(2):92-99, 2008. (VO, AE, GE)

Recent data suggest that there might be a subtle thermal explanation for the apparent induction by radiofrequency (RF) radiation of transgene expression from a small heat-shock protein (hsp16-1) promoter in the nematode, *Caenorhabditis elegans*. The RF fields used in the *C. elegans* study were much weaker (SAR 5-40 mW kg⁻¹) than those routinely tested in many other published studies (SAR approximately 2 W kg⁻¹). To resolve this disparity, we have exposed the same transgenic hsp16-1::lacZ strain of *C. elegans* (PC72) to higher intensity RF fields (1.8 GHz; SAR approximately 1.8 W kg⁻¹). For both continuous wave (CW) and Talk-pulsed RF exposures (2.5 h at 25 degrees C), there was no indication that RF exposure could induce reporter expression above sham control levels. Thus, at much higher induced RF field strength (close to the maximum permitted exposure from a mobile telephone handset), this particular nematode heat-shock gene is not up-regulated. However, under conditions where background reporter expression was moderately elevated in the sham controls (perhaps as a result of some unknown co-stressor), we found some evidence that reporter expression may be reduced by approximately 15% following exposure to either Talk-pulsed or CW RF fields.

(E) De Amicis A, Sanctis SD, Cristofaro SD, Franchini V, Lista F, Regalbuto E, Giovenale E, Gallerano GP, Nenzi P, Bei R, Fantini M, Benvenuto M, Masuelli L, Coluzzi E, Cicia C, Sgura A. Biological effects of in vitro THz radiation exposure in human foetal fibroblasts. *Mutat Res Genet Toxicol Environ Mutagen*. 793:150-160, 2015. (VT, AE, GT)

In recent years, terahertz (THz) radiation has been widely used in a variety of applications: medical, security, telecommunications and military areas. However, few data are available on the biological effects of this type of electromagnetic radiation and the reported results, using different genetic or cellular assays, are quite discordant. This multidisciplinary study focuses on potential genotoxic and cytotoxic effects, evaluated by several end-points, associated with THz radiation. For this purpose, in vitro exposure of human foetal fibroblasts to low frequency THz radiation (0.1-0.15THz) was performed using a Compact Free Electron Laser. We did not observe an induction of DNA damage evaluated by Comet assay, phosphorylation of H2AX histone or telomere length modulation. In addition, no induction of apoptosis or changes in pro-survival signalling proteins were detected. Moreover, our results indicated an increase in the total number of micronuclei and centromere positive micronuclei induction evaluated by CREST

analysis, indicating that THz radiation could induce aneugenic rather than clastogenic effects, probably leading to chromosome loss. Furthermore, an increase of actin polymerization observed by ultrastructural analysis after THz irradiation, supports the hypothesis that an abnormal assembly of spindle proteins could lead to the observed chromosomal malsegregation.

(NE) Danese E, Lippi G, Buonocore R, Benati M, Bovo C, Bonaguri C, Salvagno GL, Brocco G, Roggenbuck D, Martina Montagnana M. Mobile phone radiofrequency exposure has no effect on DNA double strand breaks (DSB) in human lymphocytes. Ann Transl Med 2017 Jul;5(13):272. (VT, AE, GT) (See also: [Mortazavi SMJ](#). Comment on "Mobile phone radiofrequency exposure has no effect on DNA double strand breaks (DSB) in human lymphocytes" Ann Transl Med 5(21):441, 2017.)

Background: The use of mobile phones has been associated with an increased risk of developing certain type of cancer, especially in long term users. Therefore, this study was aimed to investigate the potential genotoxic effect of mobile phone radiofrequency exposure on human peripheral blood mononuclear cells *in vitro*. **Methods:** The study population consisted in 14 healthy volunteers. After collection of two whole blood samples, the former was placed in a plastic rack, 1 cm from the chassis of a commercial mobile phone (900 MHz carrier frequency), which was activated by a 30-min call. The second blood sample was instead maintained far from mobile phones or other RF sources. The influence of mobile phone RF on DNA integrity was assessed by analyzing γ -H2AX foci in lymphocytes using immunofluorescence staining kit on AKLIDES. **Results:** No measure of γ -H2AX foci was significantly influenced by mobile phone RF exposure, nor mobile phone exposure was associated with significant risk of genetic damages *in vitro* (odds ratio comprised between 0.27 and 1.00). **Conclusions:** The results of this experimental study demonstrate that exposure of human lymphocytes to a conventional 900 MHz RF emitted by a commercial mobile phone for 30 min does not significantly impact DNA integrity.

(E) Daniells C, I Duce, D Thomas, P Sewell, J Tattersall, D de Pomerai Transgenic nematodes as biomonitors of microwave-induced stress. Mutat Res 399(1):55-64, 1998. (VO, AE, GE)

Transgenic nematodes (*Caenorhabditis elegans* strain PC72), carrying a stress-inducible reporter gene (*Escherichia coli* beta-galactosidase) under the control of a *C. elegans* hsp16 heat-shock promoter, have been used to monitor toxicant responses both in water and soil. Because these transgenic nematodes respond both to heat and toxic chemicals by synthesising an easily detectable reporter product, they afford a useful preliminary screen for stress responses (whether thermal or non-thermal) induced by microwave radiation or other electromagnetic fields. We have used a transverse electromagnetic (TEM) cell fed from one end by a source and terminated at the other end by a matched load. Most studies were conducted using a frequency of 750 MHz, at a nominal power setting of 27 dBm. The TEM cell was held in an incubator at 25 degrees C inside a shielded room; corresponding controls were shielded and placed in the same 25 degrees C incubator; additional baseline controls were held at 15 degrees C (worm growth temperature). Stress responses were measured in terms of beta-galactosidase (reporter) induction above control

levels. The time-course of response to continuous microwave radiation showed significant differences from 25 degrees C controls both at 2 and 16 h, but not at 4 or 8 h. Using a 5 x 5 multiwell plate array exposed for 2 h, the 25 microwaved samples showed highly significant responses compared with a similar control array. The wells most strongly affected were those in the rows closest to the source, whereas the most distant row did not rise above control levels, suggesting a shadow effect. These differential responses are difficult to reconcile with general heating effects, although localised power absorption affords a possible explanation. Experiments in which the frequency and/or power settings were varied suggested a greater response at 21 than at 27 dBm, both at 750 and 300 MHz, although extremely variable responses were observed at 24 dBm and 750 MHz. Thus, lower power levels tended, if anything, to induce larger responses (with the above-mentioned exception), which is opposite to the trend anticipated for any simple heating effect. These results are reproducible and data acquisition is both rapid and simple. The evidence accrued to date suggests that microwave radiation causes measurable stress to transgenic nematodes, presumably reflecting increased levels of protein damage within cells (the common signal thought to trigger hsp gene induction). The response levels observed are comparable to those observed with moderate concentrations (ppm) of metal ions such as Zn²⁺ and Cu²⁺. We conclude that this approach deserves further and more detailed investigation, but that it has already demonstrated clear biological effects of microwave radiation in terms of the activation of cellular stress responses (hsp gene induction).

(E) Dasdag S, Akdag MZ, Erdal ME, Erdal N, Ay OI, Ay ME, Yilmaz SG, Tasdelen B, Yegin K. Long term and excessive use of 900 MHz radiofrequency radiation alter microRNA expression in brain. Int J Radiat Biol 91(4):306-311, 2015. (VO, LE, GE)

Purpose: We still do not have any information on the interaction between radiofrequency radiation (RF) and miRNA, which play paramount role in growth, differentiation, proliferation and cell death by suppressing one or more target genes. The purpose of this study was to bridge this gap by investigating effects of long-term 900 MHz mobile phone exposure on some of the miRNA in brain tissue. **Materials and methods:** The study was carried out on 14 Wistar Albino adult male rats by dividing them into two groups: Sham (n = 7) and exposure (n = 7). Rats in the exposure group were exposed to 900 MHz RF radiation for 3 h per day (7 days a week) for 12 months (one year). The same procedure was applied to the rats in the sham group except the generator was turned off. Immediately after the last exposure, rats were sacrificed and their brains were removed. rno-miR-9-5p, rno-miR-29a-3p, rno-miR-106b-5p, rno-miR-107 and rno-miR-125a-3p in brain were investigated in detail. **Results:** Results revealed that long-term exposure of 900 MHz RF radiation only decreased rno-miR107 (adjP* = 0.045) value where the whole body (rms) SAR value was 0.0369 W/kg. However, our results indicated that other microRNA evaluated in this study was not altered by 900 MHz RF radiation. **Conclusion:** 900 MHz RF radiation can alter some of the miRNA, which, in turn, may lead to adverse effects. Therefore, further studies should be performed.

(E) Dasdag S, Akdag MZ, Erdal ME, Erdal N, Ay OI, Ay ME, Yilmaz SG, Tasdelen B, Yegin K. Effects of 2.4 GHz radiofrequency radiation emitted from Wi-Fi equipment on microRNA expression in brain tissue. Int J Radiat Biol 91(7):555-561, 2015. (VO, LE, GE)

Purpose: MicroRNAs (miRNA) play a paramount role in growth, differentiation, proliferation and cell death by suppressing one or more target genes. However, their interaction with radiofrequencies is still unknown. The aim of this study was to investigate the long-term effects of radiofrequency radiation emitted from a Wireless Fidelity (Wi-Fi) system on some of the miRNA in brain tissue. **Materials and methods:** The study was carried out on 16 Wistar Albino adult male rats by dividing them into two groups such as sham (n = 8) and exposure (n = 8). Rats in the exposure group were exposed to 2.4 GHz radiofrequency (RF) radiation for 24 hours a day for 12 months (one year). The same procedure was applied to the rats in the sham group except the Wi-Fi system was turned off. Immediately after the last exposure, rats were sacrificed and their brains were removed. miR-9-5p, miR-29a-3p, miR-106b-5p, miR-107, miR-125a-3p in brain were investigated in detail. **Results:** The results revealed that long-term exposure of 2.4 GHz Wi-Fi radiation can alter expression of some of the miRNAs such as miR-106b-5p (adj p* = 0.010) and miR-107 (adj p* = 0.005). We observed that miR-107 expression is 3.3 times and miR-106b-5p expression is 3.65 times lower in the exposure group than in the control group. However, miR-9-5p, miR-29a-3p and miR-125a-3p levels in brain were not altered. **Conclusion:** Long-term exposure of 2.4 GHz RF may lead to adverse effects such as neurodegenerative diseases originated from the alteration of some miRNA expression and more studies should be devoted to the effects of RF radiation on miRNA expression levels.

(E) Dasdag S, Erdal ME, Erdal N, Tasdelen B, Kiziltug MT, Yegin K, Akdag MZ. 900 MHz Radiofrequency Radiation Has Potential to Increase the Expression of rno-miR-145-5p in Brain. J Int Dent Med Res 2019; 12(4): 1652-1658, 2019. (VO, LE, GE)

Interaction between radiofrequency radiation (RFR) and miRNA which plays paramount role in growth, differentiation, proliferation, and cell death by suppressing one or more target genes, is still unknown. Therefore, the purpose of this study is to investigate the effects of long term 900 MHz mobile phone exposure on some of the miRNA in brain tissues (sham: 7; Exposure : 7)., which were kept in appropriate laboratory conditions for the second phase of our previous study [5]. It is remembered that rats in the exposure group were exposed to 900 MHz RFR for 3 h per day (7 days a week) for one year. For the aim of this study, expression of miRNAs such as rno-miR-22-3p, rnomiR-24-1-3p, rno-miR-132-3p, rno-miR-145-5p, rno-miR-181a-5p, rno-miR-186-5p, rno-miR-195-5p, rno-miR-219a-5p, rno-miR-221-3p and rno-miR-222-3p were investigated. Results indicated that long-term exposure of 900 MHz RF radiation increased only expression of rno-miR-145-5p (adj P * = 0.047) value where 1g average SAR value in brain was 0.198 W/kg. Our results indicated that chronic exposure of 900 MHz RFR has potential to increase expression of rno-miR-145-5p. Therefore, further studies are necessary to understand

the relation between 900 MHz mobile phone exposure and diseases related to the expression of rno-miR-145-5p.

(E) Dasdag S, Akdag MZ, Bashan M, Kizmaz V, Erdal N, Erdal ME, Kiziltug MT, Yegin K. (2022) Role of 2.4 GHz radiofrequency radiation emitted from Wi-Fi on some miRNA and fatty acids composition in brain, Electromagnetic Biology and Medicine, DOI: [10.1080/15368378.2022.2065682](https://doi.org/10.1080/15368378.2022.2065682) (VO, LE, GE)

The purpose of this study is to investigate the effects of 2.4 GHz Wi-Fi exposure, which is continuously used in the internet connection by mobile phones, computers and other wireless equipment, on microRNA and membrane and depot fatty acid composition of brain cells. Sixteen Wistar Albino rats were divided equally into two groups such as sham and exposure. The rats in the experimental group (n = 8) were exposed to 2.4 GHz RFR emitted from a Wi-Fi generator for 24 h/day for one year. The animals in the control group (n = 8) were kept under the same conditions as the experimental group, but the Wi-Fi generator was turned off. At the end of the study, rats were sacrificed and brains were removed to analyze miRNA expression and membrane and depot fatty acids of brain cells. We analyzed the situation of ten different miRNA expressions and nineteen fatty acid patterns in this study. We observed that long-term and excessive exposure of 2.4 GHz Wi-Fi radiation increased rno-miR-181a-5p, phosphatidylserine (PS) and triacylglycerol (TAG) in the brain. In conclusion, 2.4 GHz Wi-Fi exposure has the potential to alter rno-miR-181a-5p expression and the fatty acid percentage of some membrane lipids such as phospholipid (PL), phosphatidylserine (PS) and triacylglycerol (TAG), which are depot fats in the brain. However, the uncontrolled use of RFRs, whose use and diversity have reached incredible levels with each passing day and which are increasing in the future, may be paving the way for many diseases that we cannot connect with today.

(E) Dasgupta S, Leong C, Simonich MT, Truong L, Liu H, Tanguay RL. Transcriptomic and Long-Term Behavioral Deficits Associated with Developmental 3.5 GHz Radiofrequency Radiation Exposures in Zebrafish. Environ Sci Technol Lett 2022 9(4):327-332. (VO, AE, GE, DE)

The rapid deployment of the fifth-generation (5G) spectrum by the telecommunication industry is intended to promote better connectivity and data integration among various industries. However, concerns among the public about the safety and health effects of radiofrequency radiations (RFRs) emitted from the newer-generation cell phone frequencies remain, partly due to the lack of robust scientific data. Previously, we used developmental zebrafish to model the bioactivity of 3.5 GHz RFR, a frequency used by 5G-enabled cell phones, in a novel RFR exposure chamber. With RFR exposures from 6 h post-fertilization (hpf) to 48 hpf, we observed that, despite no teratogenic effects, embryos showed subtle hypoactivity in a startle response behavior assay, suggesting abnormal sensorimotor behavior. This study builds upon the previous one by investigating the transcriptomic basis of RFR-associated behavior effects and their persistence into adulthood. Using mRNA sequencing, we found a modest transcriptomic disruption at 48 hpf, with 28 differentially expressed genes. KEGG pathway analysis showed

that biochemical pathways related to metabolism were significantly perturbed. Embryos were grown to adulthood, and then a battery of behavioral assays suggested subtle but significant abnormal responses in RFR-exposed fish across the different assays evaluated that suggest potential long-term behavioral effects. Overall, our study suggests the impacts of RFRs on the developing brain, behavior, and the metabolome should be further explored.

(E) De Iuliis GN, Newey RJ, King BV, Aitken RJ. Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. PLoS One 4:e6446, 2009. (VT, AE, GT, OX, RP)

BACKGROUND: In recent times there has been some controversy over the impact of electromagnetic radiation on human health. The significance of mobile phone radiation on male reproduction is a key element of this debate since several studies have suggested a relationship between mobile phone use and semen quality. The potential mechanisms involved have not been established, however, human spermatozoa are known to be particularly vulnerable to oxidative stress by virtue of the abundant availability of substrates for free radical attack and the lack of cytoplasmic space to accommodate antioxidant enzymes. Moreover, the induction of oxidative stress in these cells not only perturbs their capacity for fertilization but also contributes to sperm DNA damage. The latter has, in turn, been linked with poor fertility, an increased incidence of miscarriage and morbidity in the offspring, including childhood cancer. In light of these associations, we have analyzed the influence of RF-EMR on the cell biology of human spermatozoa in vitro. PRINCIPAL FINDINGS: Purified human spermatozoa were exposed to radio-frequency electromagnetic radiation (RF-EMR) tuned to 1.8 GHz and covering a range of specific absorption rates (SAR) from 0.4 W/kg to 27.5 W/kg. In step with increasing SAR, motility and vitality were significantly reduced after RF-EMR exposure, while the mitochondrial generation of reactive oxygen species and DNA fragmentation were significantly elevated ($P < 0.001$). Furthermore, we also observed highly significant relationships between SAR, the oxidative DNA damage bio-marker, 8-OH-dG, and DNA fragmentation after RF-EMR exposure. CONCLUSIONS: RF-EMR in both the power density and frequency range of mobile phones enhances mitochondrial reactive oxygen species generation by human spermatozoa, decreasing the motility and vitality of these cells while stimulating DNA base adduct formation and, ultimately DNA fragmentation. These findings have clear implications for the safety of extensive mobile phone use by males of reproductive age, potentially affecting both their fertility and the health and wellbeing of their offspring.

(NE) de Oliveira FM, Carmona AM, Ladeira C. Is mobile phone radiation genotoxic? An analysis of micronucleus frequency in exfoliated buccal cells. Mutat Res. 822:41-46, 2017. (HU, CE, GT)

Electromagnetic fields (EMF) are classified as "possibly carcinogenic" by the International Agency for Research on Cancer (IARC). Some publications have reported associations between EMF exposure and DNA damage, but many other studies contradict such findings. Cytomorphological changes, such as micronuclei (MN), indicative of genomic damage, are biomarkers of genotoxicity. To test whether mobile phone-associated EMF exposure affects the MN frequency in exfoliated buccal cells, we obtained cells smears from the left and right inner

cheeks of healthy mobile phone users, aged 18-30 (n=86), who also completed a characterization survey. MN frequencies were tested for potential confounding factors and for duration of phone use and preferential side of mobile phone use. No relationship was observed between MN frequency and duration of mobile phone use in daily calls. Cells ipsilateral to mobile phone use did not present a statistically significantly higher MN frequency, compared to cells contralateral to exposure. A highly statistically significant ($p < 0.0001$) increase in MN frequency was found in subjects reporting regular exposure to genotoxic agents. Therefore, our results suggest that mobile phone-associated EMF do not induce MN formation in buccal cells at the observed exposure levels.

(E) Del Re B, Bersani F, Giorgi G. Effect of electromagnetic field exposure on the transcription of repetitive DNA elements in human cells. *Electromagn Biol Med.* 38(4):262-270, 2019. (VT, AE, GE, CS)

Repetitive DNA (RE-DNA) was long thought to be silent and inert; only recent research has shown that it can be transcribed and that transcription alteration can be induced by environmental stress conditions, causing human pathological effects. The aim of this study was to determine whether exposure to radiofrequency electromagnetic fields (RF-EMF) could affect the transcription of RE-DNA. To this purpose, three different human cell lines (HeLa, BE(2)C and SH-SY5Y) were exposed to 900 MHz GSM-modulated RF-EMF at specific absorption rate of 1 W/kg or to sham. After exposure, mRNA levels of RE-DNA were evaluated through quantitative real-time PCR. The following RE-DNA types were investigated: Long Interspersed nucleotide Element 1, DNA alpha satellite and Human Endogenous Retroviruses-like sequences. When comparing cells exposed to RF-EMF versus control samples, different results were found for the three cell lines evaluated, indicating that RF-EMF exposure can significantly affect RE-DNA transcription and that the effects strongly depend on the cellular context and the tissue type. Further studies are needed to elucidate which molecular mechanisms could be involved.

(E) Del Vecchio G, Giuliani A, Fernandez M, Mesirca P, Bersani F, Pinto R, Ardoino L, Lovisolò GA, Giardino L, Calzà L. Continuous exposure to 900MHz GSM-modulated EMF alters morphological maturation of neural cells. *Neurosci Lett.* 455(3):173-177, 2009. (VT, AE, GE, DE)

The effects of radiofrequency electromagnetic field (RF-EMF) exposure on neuronal phenotype maturation have been studied in two different in vitro models: murine SN56 cholinergic cell line and rat primary cortical neurons. The samples were exposed at a dose of 1W/kg at 900 MHz GSM modulated. The phenotype analysis was carried out at 48 and 72 h (24 and 48 h of SN56 cell line differentiation) or at 24, 72, 120 h (2, 4 and 6 days in vitro for cortical neurons) of exposure, on live and immunolabeled neurons, and included the morphological study of neurite emission, outgrowth and branching. Moreover, cortical neurons were studied to detect alterations in the expression pattern of cytoskeleton regulating factors, e.g. beta-thymosin, and of early genes, e.g. c-Fos and c-Jun through real-time PCR on mRNA extracted after 24h exposure to EMF. We found that RF-EMF exposure reduced the number of neurites generated by both cell systems, and this alteration correlates to increased expression of beta-thymosin mRNA.

(E) Demsia G, Vlastos D, Matthopoulos DP. Effect of 910-MHz electromagnetic field on rat bone marrow. ScientificWorld Journal 2004 Oct 20;4 Suppl 2:48-54.(VO, LE, GT)

Aiming to investigate the possibility of electromagnetic fields (EMF) developed by nonionizing radiation to be a noxious agent capable of inducing genotoxicity to humans, in the current study we have investigated the effect of 910-MHz EMF in rat bone marrow. Rats were exposed daily for 2 h over a period of 30 consecutive days. Studying bone marrow smears from EMF-exposed and sham-exposed animals, we observed an almost threefold increase of micronuclei (MN) in polychromatic erythrocytes (PCEs) after EMF exposure. An induction of MN was also observed in polymorphonuclear cells. The induction of MN in female rats was less than that in male rats. The results indicate that 910-MHz EMF could be considered as a noxious agent capable of producing genotoxic effects.

(E) Deshmukh PS, Megha K, Banerjee BD, Ahmed RS, Chandna S, Abegaonkar MP, Tripathi AK. Detection of low level microwave radiation induced deoxyribonucleic acid damage vis-à-vis genotoxicity in brain of Fischer rats. Toxicol Int. 20(1):19-24, 2013. (VO, LE, GT, LI)

BACKGROUND: Non-ionizing radiofrequency radiation has been increasingly used in industry, commerce, medicine and especially in mobile phone technology and has become a matter of serious concern in present time. OBJECTIVE: The present study was designed to investigate the possible deoxyribonucleic acid (DNA) damaging effects of low-level microwave radiation in brain of Fischer rats. MATERIALS AND METHODS: Experiments were performed on male Fischer rats exposed to microwave radiation for 30 days at three different frequencies: 900, 1800 and 2450 MHz. Animals were divided into 4 groups: Group I (Sham exposed): Animals not exposed to microwave radiation but kept under same conditions as that of other groups, Group II: Animals exposed to microwave radiation at frequency 900 MHz at specific absorption rate (SAR) 5.953×10^{-4} W/kg, Group III: Animals exposed to 1800 MHz at SAR 5.835×10^{-4} W/kg and Group IV: Animals exposed to 2450 MHz at SAR 6.672×10^{-4} W/kg. At the end of the exposure period animals were sacrificed immediately and DNA damage in brain tissue was assessed using alkaline comet assay. RESULTS: In the present study, we demonstrated DNA damaging effects of low level microwave radiation in brain. CONCLUSION: We concluded that low SAR microwave radiation exposure at these frequencies may induce DNA strand breaks in brain tissue.

(E) Deshmukh PS, Nasare N, Megha K, Banerjee BD, Ahmed RS, Singh D, Abegaonkar MP, Tripathi AK, Mediratta PK. Cognitive Impairment and Neurogenotoxic Effects in Rats Exposed to Low-Intensity Microwave Radiation. Int J Toxicol. 34(3):24-290, 2015. (VO, LE, GT, LI)

The health hazard of microwave radiation (MWR) has become a recent subject of interest as a result of the enormous increase in mobile phone usage. The present study aimed to investigate the effects of chronic low-intensity microwave exposure on cognitive function, heat shock protein 70 (HSP70), and DNA damage in rat brain. Experiments were performed on male Fischer rats exposed to MWR for 180 days at 3 different frequencies, namely, 900, 1800 MHz, and 2450

MHz. Animals were divided into 4 groups: group I: sham exposed; group II: exposed to MWR at 900 MHz, specific absorption rate (SAR) 5.953×10^{-4} W/kg; group III: exposed to 1800 MHz, SAR 5.835×10^{-4} W/kg; and group IV: exposed to 2450 MHz, SAR 6.672×10^{-4} W/kg. All the rats were tested for cognitive function at the end of the exposure period and were subsequently sacrificed to collect brain. Level of HSP70 was estimated by enzyme-linked immunotarget assay and DNA damage was assessed using alkaline comet assay in all the groups. The results showed declined cognitive function, elevated HSP70 level, and DNA damage in the brain of microwave-exposed animals. The results indicated that, chronic low-intensity microwave exposure in the frequency range of 900 to 2450 MHz may cause hazardous effects on the brain.

(E) Deshmukh PS, Megha K, Nasare N, Banerjee BD, Ahmed RS, Abegaonkar MP, Tripathi AK, Mediratta PK. Effect of low level subchronic microwave radiation on rat brain. Biomed Environ Sci. 29(12):858-867, 2016. (VO, LE, GT, LI)

OBJECTIVE: The present study was designed to investigate the effects of subchronic low level microwave radiation (MWR) on cognitive function, heat shock protein 70 (HSP70) level and DNA damage in brain of Fischer rats. METHODS: Experiments were performed on male Fischer rats exposed to microwave radiation for 90 days at three different frequencies: 900, 1800, and 2450 MHz. Animals were divided into 4 groups: Group I: Sham exposed, Group II: animals exposed to microwave radiation at 900 MHz and specific absorption rate (SAR) 5.953×10^{-4} W/kg, Group III: animals exposed to 1800 MHz at SAR 5.835×10^{-4} W/kg and Group IV: animals exposed to 2450 MHz at SAR 6.672×10^{-4} W/kg. All the animals were tested for cognitive function using elevated plus maze and Morris water maze at the end of the exposure period and subsequently sacrificed to collect brain tissues. HSP70 levels were estimated by ELISA and DNA damage was assessed using alkaline comet assay. RESULTS: Microwave exposure at 900-2450 MHz with SAR values as mentioned above lead to decline in cognitive function, increase in HSP70 level and DNA damage in brain. CONCLUSION: The results of the present study suggest that low level microwave exposure at frequencies 900, 1800, and 2450 MHz may lead to hazardous effects on brain.

(E) Detlavs I., L Dombrovska, A Turauska, B Shkirmante, L Slutskii Experimental study of the effects of radiofrequency electromagnetic fields on animals with soft tissue wounds. Sci Total Environ 180(1):35-42, 1996. (VO, LE, GE)

The effect of radio frequency electromagnetic fields (RF EMF) was studied on Wistar rats with excised full-thickness dermal wounds in the interscapular region. The wounded regions of experimental animals were subjected to EMF for 30 min daily during the first 5 days after wound infliction. Control animals received no treatment. We used RF EMF with (1) frequency 53.53 GHz without modulation; (2) frequency 42.19 GHz without modulation; (3) frequency 42.19 GHz, but with a frequency modulation band 200-MHz wide. On the 7th day the animals were terminated and the granulation-fibrous tissue (GFT) developed in the wounds was subjected to complex quantitative biochemical analysis. RF EMF without frequency modulation decreased the amounts of glycoprotein macromolecules, diminishing the inflammatory exudation. In striking contrast, under the influence of RF EMF with frequency modulation, hexoses and

especially sialic acid concentrations were significantly elevated ($P < 0.001$). This indicated intensification of exudative phenomena. As a consequence of inflammation inhibition in the treatment without frequency modulation, the total collagen accumulation was lowered. However, when frequency was modulated, the inflammatory phenomena were intensified, and pronounced accumulation of collagenous proteins was noted. Thus, our experiments confirm the effects of non-thermal EMF on the reparative-proliferative processes of animals with soft tissue wounds.

(E) Diem E, Schwarz C, Adlkofer F, Jahn O, Rudiger H. Non-thermal DNA breakage by mobile-phone radiation (1800MHz) in human fibroblasts and in transformed GFSH-R17 rat granulosa cells in vitro. Mutat Res. 583:178-183, 2005. (VT, AE, GT, WS)

Cultured human diploid fibroblasts and cultured rat granulosa cells were exposed to intermittent and continuous radiofrequency electromagnetic fields (RF-EMF) used in mobile phones, with different specific absorption rates (SAR) and different mobile-phone modulations. DNA strand breaks were determined by means of the alkaline and neutral comet assay. RF-EMF exposure (1800MHz; SAR 1.2 or 2W/kg; different modulations; during 4, 16 and 24h; intermittent 5min on/10min off or continuous wave) induced DNA single- and double-strand breaks. Effects occurred after 16h exposure in both cell types and after different mobile-phone modulations. The intermittent exposure showed a stronger effect in the comet assay than continuous exposure. Therefore we conclude that the induced DNA damage cannot be based on thermal effects.

(E) Ding Z, Xiang X, Li J, Wu S. Molecular Mechanism of Malignant Transformation of Balb/c-3T3 Cells Induced by Long-Term Exposure to 1800 MHz Radiofrequency Electromagnetic Radiation (RF-EMR). Bioengineering (Basel) 2022, 9(2):43. (VT, LE, GE)

Purpose: We aimed to investigate RF-EMR-induced cell malignant transformation.

Methods: We divided Balb/c-3T3 cells into sham and expo groups. The expo groups were exposed to a 1800 MHz RF continuous wave for 40 and 60 days, for 4 h per day. The sham group was sham-exposed. Cells were harvested for a cell transformation assay, transplantation in severe combined immune deficient (SCID) mice, soft agar clone formation detection, and a transwell assay. The mRNA microarray assay was used to declare key genes and pathways.

Results: The exposed Balb/c-3T3 cells showed a strong increase in cell proliferation and migration. Malignant transformation was observed in expo Balb/c-3T3 cells exposed for 40 days and 60 days, which was symbolized with visible foci and clone formation. Expo Balb/c-3T3 cells that were exposed for 40 days and 60 days produced visible tumors in the SCID mice. Lipid metabolism was the key biological process and pathway involved. The mevalonate (MVA) pathway was the key metabolic pathway. The interacted miRNAs could be further research targets to examine the molecular mechanism of the carcinogenic effects of long-term exposure.

Conclusion: Exposure for 40 and 60 days to 1800 MHz RF-EMR induced malignant transformation in Balb/c-3T3 cells at the SAR of 8.0 W/kg. We declared that lipid metabolism was the pivotal biological process and pathway. The MVA pathway was the key metabolic pathway.

(E) Duan W, Liu C, Zhang L, He M, Xu S, Chen C, Pi H, Gao P, Zhang Y, Zhong M, Yu Z, Zhou Z. Comparison of the genotoxic effects induced by 50 Hz extremely low-frequency electromagnetic fields and 1800 MHz radiofrequency electromagnetic fields in GC-2 cells. Radiat Res. 183(3):305-314, 2015. (VT, AE, GT, OX, WS, RP)

Extremely low-frequency electromagnetic fields (ELF-EMF) and radiofrequency electromagnetic fields (RF-EMF) have been considered to be possibly carcinogenic to humans. However, their genotoxic effects remain controversial. To make experiments controllable and results comparable, we standardized exposure conditions and explored the potential genotoxicity of 50 Hz ELF-EMF and 1800 MHz RF-EMF. A mouse spermatocyte-derived GC-2 cell line was intermittently (5 min on and 10 min off) exposed to 50 Hz ELF-EMF at an intensity of 1, 2 or 3 mT or to RF-EMF in GSM-Talk mode at the specific absorption rates (SAR) of 1, 2 or 4 W/kg. After exposure for 24 h, we found that neither ELF-EMF nor RF-EMF affected cell viability using Cell Counting Kit-8. Through the use of an alkaline comet assay and immunofluorescence against γ -H2AX foci, we found that ELF-EMF exposure resulted in a significant increase of DNA strand breaks at 3 mT, whereas RF-EMF exposure had insufficient energy to induce such effects. Using a formamidopyrimidine DNA glycosylase (FPG)-modified alkaline comet assay, we observed that RF-EMF exposure significantly induced oxidative DNA base damage at a SAR value of 4 W/kg, whereas ELF-EMF exposure did not. Our results suggest that both ELF-EMF and RF-EMF under the same experimental conditions may produce genotoxicity at relative high intensities, but they create different patterns of DNA damage. Therefore, the potential mechanisms underlying the genotoxicity of different frequency electromagnetic fields may be different.

(NE) Durdik M, Kosik P, Markova E, Somsedikova A, Gajdosechova B, Nikitina E, Horvathova E, Kozics K, Davis D, Belyaev I. Microwaves from mobile phone induce reactive oxygen species but not DNA damage, preleukemic fusion genes and apoptosis in hematopoietic stem/progenitor cells. Sci Rep. 9(1):16182, 2019. (VT, AE, GT)

Exposure to electromagnetic fields (EMF) has been associated with the increased risk of childhood leukemia, which arises from mutations induced within hematopoietic stem cells often through preleukemic fusion genes (PFG). In this study we investigated whether exposure to microwaves (MW) emitted by mobile phones could induce various biochemical markers of cellular damage including reactive oxygen species (ROS), DNA single and double strand breaks, PFG, and apoptosis in umbilical cord blood (UCB) cells including CD34+ hematopoietic stem/progenitor cells. UCB cells were exposed to MW pulsed signals from GSM900/UMTS test-mobile phone and ROS, apoptosis, DNA damage, and PFG were analyzed using flow cytometry, automated fluorescent microscopy, imaging flow cytometry, comet assay, and RT-qPCR. In general, no persisting difference in DNA damage, PFG and apoptosis between exposed and sham-exposed samples was detected. However, we found increased ROS level after 1 h of UMTS exposure that was not evident 3 h post-exposure. We also found that the level of ROS rise with the higher degree of cellular differentiation. Our data show that UCB cells exposed to pulsed MW developed transient increase in ROS that did not result in sustained DNA damage and apoptosis.

(E) Eker ED, Arslan B, Yildirim M, Akar A, Aras N. The effect of exposure to 1800 MHz radiofrequency radiation on epidermal growth factor, caspase-3, Hsp27 and p38MAPK gene expressions in the rat eye. Bratisl Lek Listy. 119(9):588-592, 2018. (VO, LE, LI, GE)

Radiofrequency electromagnetic fields (RF-EMF) may induce DNA damage and oxidative stress in human lens epithelial cells (LECs). We aimed to investigate the expression levels of heat shock protein 27 (Hsp27), p38 mitogen-activated protein kinase (p38MAPK), epidermal growth factor receptor (EGFR) and caspase-3 gene expression levels in rat eye that was exposed to 1800 MHz RF-EMF. METHODS: Thirty-seven female Wistar albino rats were divided into three groups. The rats in the study group (n = 9) were exposed to 1800 MHz RF-EMF at an electric field 6.8 ± 0.1 V/m and 0.06 W/kg specific absorption rate (SAR) for 2 hours per day for eight weeks. Sham group (n = 9) was kept under similar conditions as the exposed group without exposure to RF-EMF. The rats in all three groups were sacrificed and their eyes were removed. Hsp27, p38MAPK, EGFR, caspase-3 gene expression levels were investigated in detail with real-time polymerase chain reactions (Real-Time PCR). RESULTS: caspase-3 and p38MAPK gene expression were significantly upregulated in the ocular tissues following exposure to RF-EMF ($p < 0.05$). CONCLUSION: According to our findings, eye cells recognize EMF as a stress factor, and in response, activate caspase-3 and p38MAPK gene expressions. These results confirm that RF-EMF can cause cellular damage in rat ocular cells (Tab. 2, Fig. 3, Ref. 37).

(E) Engelmann JC, Deeken R, Müller T, Nimtz G, Roelfsema MR, Hedrich R. Is gene activity in plant cells affected by UMTS-irradiation? A whole genome approach. Adv Appl Bioinform Chem. 1:71-83, 2008. (VT, AE, GE)

Mobile phone technology makes use of radio frequency (RF) electromagnetic fields transmitted through a dense network of base stations in Europe. Possible harmful effects of RF fields on humans and animals are discussed, but their effect on plants has received little attention. In search for physiological processes of plant cells sensitive to RF fields, cell suspension cultures of *Arabidopsis thaliana* were exposed for 24 h to a RF field protocol representing typical microwave exposition in an urban environment. mRNA of exposed cultures and controls was used to hybridize Affymetrix-ATH1 whole genome microarrays. Differential expression analysis revealed significant changes in transcription of 10 genes, but they did not exceed a fold change of 2.5. Besides that 3 of them are dark-inducible, their functions do not point to any known responses of plants to environmental stimuli. The changes in transcription of these genes were compared with published microarray datasets and revealed a weak similarity of the microwave to light treatment experiments. Considering the large changes described in published experiments, it is questionable if the small alterations caused by a 24 h continuous microwave exposure would have any impact on the growth and reproduction of whole plants.

(E) Ergun DD, Ozsobaci NP, Yilmaz T, Ozcelik D, Kalkan MT. Zinc affects nuclear factor kappa b and DNA methyltransferase activity in C3H cancer fibroblast cells induced by a 2100 MHz electromagnetic field. Electromagn Biol Med 2022 Jan 7;1-8. doi: 10.1080/15368378.2021.2019760. Online ahead of print. (VT, AE, GE, IX, EP)

The use of mobile phones is becoming widespread with the development of technology, and as a result, its effects on human health are becoming more and more important every day. Studies

have reported that the electromagnetic field (EMF) emitted by mobile phones may have adverse effects on the biological systems. In order to evaluate the effect of zinc (Zn) on C3H cancer fibroblast cells exposed to 2100 MHz EMF, we analyzed cell viability%, nuclear factor kappa b (NF-κB) and DNA methyltransferase (DNMT) activities. Cells were divided to following groups: Control, sham control, 2100 MHz EMF, 50 μM Zn + 2100 MHz EMF, 100 μM Zn + 2100 MHz EMF, and 200 μM Zn + 2100 MHz EMF for 2 h. We measurement cell viability, NF-κB and DNMT activities. There was increased cell viability % in the 2100 MHz EMF group compared to the control group, while the cell viability % was decreased in the 50, 100 and 200 μM Zn + 2100 MHz EMF groups compared to 2100 MHz EMF. NF-κB and DNMT activities were a significant increase in the 2100 MHz EMF group compared to the control group, although were statistically decreased in the 50, 100 and 200 μM Zn + 2100 MHz EMF groups compared to the 2100 MHz EMF group. Our results demonstrate that 2100 MHz EMF exposure in cancer fibroblast cells induce NF-κB and DNMT activities, whereas zinc supplementation reduce NF-κB and DNMT activities-induced 2100 MHz EMF.

(E) Esmekaya MA, Aytakin E, Ozgur E, Güler G, Ergun MA, Omeroğlu S, Seyhan N. Mutagenic and morphologic impacts of 1.8GHz radiofrequency radiation on human peripheral blood lymphocytes (hPBLs) and possible protective role of pre-treatment with Ginkgo biloba (EGb 761). Sci Total Environ. 410-411:59-64, 2011. (VT, AE, GT, OX)

The mutagenic and morphologic effects of 1.8GHz Global System for Mobile Communications (GSM) modulated RF (radiofrequency) radiation alone and in combination with Ginkgo biloba (EGb 761) pre-treatment in human peripheral blood lymphocytes (hPBLs) were investigated in this study using Sister Chromatid Exchange (SCE) and electron microscopy. Cell viability was assessed with 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) reduction assay. The lymphocyte cultures were exposed to GSM modulated RF radiation at 1.8GHz for 6, 8, 24 and 48h with and without EGb 761. We observed morphological changes in pulse-modulated RF radiated lymphocytes. Longer exposure periods led to destruction of organelle and nucleus structures. Chromatin change and the loss of mitochondrial crista occurred in cells exposed to RF for 8h and 24h and were more pronounced in cells exposed for 48h. Cytoplasmic lysis and destruction of membrane integrity of cells and nuclei were also seen in 48h RF exposed cells. There was a significant increase ($p < 0.05$) in SCE frequency in RF exposed lymphocytes compared to sham controls. EGb 761 pre-treatment significantly decreased SCE from RF radiation. RF radiation also inhibited cell viability in a time dependent manner. The inhibitory effects of RF radiation on the growth of lymphocytes were marked in longer exposure periods. EGb 761 pre-treatment significantly increased cell viability in RF+EGb 761 treated groups at 8 and 24h when compared to RF exposed groups alone. The results of our study showed that RF radiation affects cell morphology, increases SCE and inhibits cell proliferation. However, EGb 761 has a protective role against RF induced mutagenity. We concluded that RF radiation induces chromosomal damage in hPBLs but this damage may be reduced by EGb 761 pre-treatment.

(NE) Falzone N, Huyser C, Franken DR, Leszczynski D. Mobile phone radiation does not induce pro-apoptosis effects in human spermatozoa. Radiat Res 174:169-176, 2010. (VT, AE, GT, OX)

Recent reports suggest that mobile phone radiation may diminish male fertility. However, the effects of this radiation on human spermatozoa are largely unknown. The present study examined effects of the radiation on induction of apoptosis-related properties in human spermatozoa. Ejaculated, density-purified, highly motile human spermatozoa were exposed to mobile phone radiation at specific absorption rates (SARs) of 2.0 and 5.7 W/kg. At various times after exposure, flow cytometry was used to examine caspase 3 activity, externalization of phosphatidylserine (PS), induction of DNA strand breaks, and generation of reactive oxygen species. Mobile phone radiation had no statistically significant effect on any of the parameters studied. This suggests that the impairment of fertility reported in some studies was not caused by the induction of apoptosis in spermatozoa.

(E) Ferreira AR, Knakievicz T, de Bittencourt Pasquali MA, Gelain DP, Dal-Pizzol F, Fernandez CE, de Almeida de Salles AA, Ferreira HB, Moreira JC. Ultra high frequency-electromagnetic field irradiation during pregnancy leads to an increase in erythrocytes micronuclei incidence in rat offspring. Life Sci 80: 43-50, 2006. (VO, LE, GT, OX, DE)

Mobile telephones and their base stations are an important ultra high frequency-electromagnetic field (UHF-EMF) source and their utilization is increasing all over the world. Epidemiological studies suggested that low energy UHF-EMF emitted from a cellular telephone may cause biological effects, such as DNA damage and changes on oxidative metabolism. An *in vivo* mammalian cytogenetic test, the micronucleus (MN) assay, was used to investigate the occurrence of chromosomal damage in erythrocytes from rat offspring exposed to a non-thermal UHF-EMF from a cellular phone during their embryogenesis; the irradiated group showed a significant increase in MN occurrence. In order to investigate if UHF-EMF could also alter oxidative parameters in the peripheral blood and in the liver - an important hematopoietic tissue in rat embryos and newborns - we also measured the activity of antioxidant enzymes, quantified total sulfhydryl content, protein carbonyl groups, thiobarbituric acid-reactive species and total non-enzymatic antioxidant defense. No significant differences were found in any oxidative parameter of offspring blood and liver. The average number of pups in each litter has also not been significantly altered. Our results suggest that, under our experimental conditions, UHF-EMF is able to induce a genotoxic response in hematopoietic tissue during the embryogenesis through an unknown mechanism.

(NE) Figueiredo ABS; Alves RN; Ramalho AT. Cytogenetic analysis of the effects of 2.5 and 10.5 GHz microwaves on human lymphocytes. Genet. Mol. Biol. 27: 460-466, 2004. (VT, AE, GT)

The biological effects of microwaves on living organisms remain highly controversial. Although some reports have suggested that microwaves may be directly or indirectly genotoxic, a direct action is unlikely because the low energy of microwave photons makes them unable to cause single-strand breaks in DNA. In this work, we examined the possible clastogenic properties of microwaves (2.5 and 10.5 GHz) on blood lymphocytes *in vitro* by monitoring the frequency of chromosomal aberrations. We also investigated whether blood cells showed increased radiosensitivity or radioresistance when pretreated with the microwaves and then irradiated with gamma radiation. There was no significant difference in the frequency of chromosomal aberrations between cells which had or had not been treated with microwaves. Control cells had

a mean frequency of 0.013 aberrations per cell compared to 0.010 and 0.011 aberrations per cell in the microwave-exposed samples. Nor was there any alteration in the radiosensitivity of cells pretreated with microwaves. Gamma irradiated cells showed a mean frequency of 0.279 aberrations per cell compared to 0.343 and 0.310 aberrations per cell in samples pretreated with microwaves. However, cell mortality increased markedly after exposure to microwaves. The results suggest that microwaves do not interact directly or indirectly with chromosomes, although they may target other cell structures, such as cell membranes

(NE) Finnie JW, Cai Z, Blumbergs PC, Manavis J, Kuchel TR. Expression of the immediate early gene, c-fos, in fetal brain after whole of gestation exposure of pregnant mice to global system for mobile communication microwaves. Pathology. 38(4):333-335, 2006. (VO, LE, GE, DE)

AIMS: To study immediate early gene, c-fos, expression as a marker of neural stress after whole of gestation exposure of the fetal mouse brain to mobile telephone-type radiofrequency fields. METHODS: Using a purpose-designed exposure system at 900 MHz, pregnant mice were given a single, far-field, whole body exposure at a specific absorption rate of 4 W/kg for 60 min/day from day 1 to day 19 of gestation. Pregnant control mice were sham-exposed or freely mobile in a cage without further restraint. Immediately prior to parturition on gestational day 19, fetal heads were collected, fixed in 4% paraformaldehyde and paraffin embedded. Any stress response in the brain was detected by c-fos immunohistochemistry in the cerebral cortex, basal ganglia, thalamus, hippocampus, midbrain, cerebellum and medulla. RESULTS: c-fos expression was of limited, but consistent, neuroanatomical distribution and there was no difference in immunoreactivity between exposed and control brains. CONCLUSION: In this animal model, no stress response was detected in the fetal brain using c-fos immunohistochemistry after whole of gestation exposure to mobile telephony.

(E) Fragopoulou AF, Polyzos A, Papadopoulou MD, Sansone A, Manta AK, Balafas E, Kostomitsopoulos N, Skouroliakou A, Chatgililoglu C, Georgakilas A, Stravopodis DJ, Ferreri C, Thanos D, Margaritis LH. Hippocampal lipidome and transcriptome profile alterations triggered by acute exposure of mice to GSM 1800 MHz mobile phone radiation: An exploratory study. Brain Behav. 8(6):e01001, 2018. (VO, AE, GE)

Background: The widespread use of wireless devices during the last decades is raising concerns about adverse health effects of the radiofrequency electromagnetic radiation (RF-EMR) emitted from these devices. Recent research is focusing on unraveling the underlying mechanisms of RF-EMR and potential cellular targets. The "omics" high-throughput approaches are powerful tools to investigate the global effects of RF-EMR on cellular physiology. **Methods:** In this work, C57BL/6 adult male mice were whole-body exposed ($n_{\text{Exp}} = 8$) for 2 hr to GSM 1800 MHz mobile phone radiation at an average electric field intensity range of 4.3-17.5 V/m or sham-exposed ($n_{\text{SE}} = 8$), and the RF-EMR effects on the hippocampal lipidome and transcriptome profiles were assessed 6 hr later. **Results:** The data analysis of the phospholipid fatty acid residues revealed that the levels of four fatty acids [16:0, 16:1 (6c + 7c), 18:1 9c, eicosapentaenoic acid omega-3 (EPA, 20:5 ω 3)] and the two fatty acid sums of saturated and monounsaturated fatty acids (SFA and MUFA) were significantly altered ($p < 0.05$) in the exposed group. The observed changes indicate a membrane remodeling response of the tissue

phospholipids after nonionizing radiation exposure, reducing SFA and EPA, while increasing MUFA residues. The microarray data analysis demonstrated that the expression of 178 genes changed significantly ($p < 0.05$) between the two groups, revealing an impact on genes involved in critical biological processes, such as cell cycle, DNA replication and repair, cell death, cell signaling, nervous system development and function, immune system response, lipid metabolism, and carcinogenesis. **Conclusions:** This study provides preliminary evidence that mobile phone radiation induces hippocampal lipidome and transcriptome changes that may explain the brain proteome changes and memory deficits previously shown by our group.

(E) Franchini V, Regalbuto E, De Amicis A, De Sanctis S, Di Cristofaro S, Coluzzi E, Marinaccio J, Sgura A, Ceccuzzi S, Doria A, Gallerano GP, Giovenale E, Ravera GL, Bei R, Benvenuto M, Modesti A, Masuelli L, Lista F. Genotoxic effects in human fibroblasts exposed to microwave radiation. Health Phys. 115(1):126-139, 2018a. (VT, AE, GT)

In the last decades, technological development has led to an increasing use of devices and systems based on microwave radiation. The increased employment of these devices has elicited questions about the potential long-term health consequences associated with microwave radiation exposure. From this perspective, biological effects of microwave radiation have been the focus of many studies, but the reported scientific data are unclear and contradictory. The aim of this study is to evaluate the potential genotoxic and cellular effects associated with in vitro exposure of human fetal and adult fibroblasts to microwave radiation at the frequency of 25 GHz. For this purpose, several genetic and biological end points were evaluated. Results obtained from comet assay, phosphorylation of H2AX histone, and antikinetochore antibody (CREST)-negative micronuclei frequency excluded direct DNA damage to human fetal and adult fibroblasts exposed to microwaves. No induction of apoptosis or changes in prosurvival signalling proteins were detected. Moreover, CREST analysis showed for both the cell lines an increase in the total number of micronuclei and centromere positive micronuclei in exposed samples, indicating aneuploidy induction due to chromosome loss.

(E) Franchini V, De Sanctis S, Marinaccio J, De Amicis A, Coluzzi E, Di Cristofaro S, Lista F, Regalbuto E, Doria A, Giovenale E, Gallerano GP, Bei R, Benvenuto M, Masuelli L, Udroui I, Sgura A. Study of the effects of 0.15 terahertz radiation on genome integrity of adult fibroblasts. Environ Mol Mutagen. 59(6):476-487, 2018b. (VT, AE, GT)

The applications of Terahertz (THz) technologies have significantly developed in recent years, and the complete understanding of the biological effects of exposure to THz radiation is becoming increasingly important. In a previous study, we found that THz radiation induced genomic damage in fetal fibroblasts. Although these cells demonstrated to be a useful model, exposure of human foetuses to THz radiation is highly improbable. Conversely, THz irradiation of adult dermal tissues is cause of possible concern for some professional and nonprofessional categories. Therefore, we extended our study to the investigation of the effects of THz radiation on adult fibroblasts (HDF). In this work, the effects of THz exposure on HDF cells genome integrity, cell cycle, cytological ultrastructure and proteins expression were assessed. Results of centromere-negative micronuclei frequencies, phosphorylation of H2AX histone, and telomere length modulation indicated no induction of DNA damage.

Concordantly, no changes in the expression of proteins associated with DNA damage sensing and repair were detected. Conversely, our results showed an increase of centromere-positive micronuclei frequencies and chromosomal nondisjunction events, indicating induction of aneuploidy. Therefore, our results indicate that THz radiation exposure may affect genome integrity through aneugenic effects, and not by DNA breakage. Our findings are compared to published studies, and possible biophysical mechanisms are discussed.

(E) Franzellitti S, Valbonesi P, Contin A, Biondi C, Fabbri E. HSP70 expression in human trophoblast cells exposed to different 1.8 Ghz mobile phone signals. Radiat Res. 170(4):488-497, 2008. (VT, AE, GE)

The heat-shock proteins (HSPs) are important cellular stress markers and have been proposed as candidates to infer biological effects of high-frequency electromagnetic fields (EMFs). In the current study, HSP70 gene and protein expression were evaluated in cells of the human trophoblast cell line HTR-8/SVneo after prolonged exposure (4 to 24 h) to 1.8 GHz continuous-wave (CW) and different GSM signals (GSM-217Hz and GSM-Talk) to assess the possible effects of time and modulation schemes on cell responses. Inducible HSP70 protein expression was not modified by high-frequency EMFs under any condition tested. The inducible HSP70A, HSP70B and the constitutive HSC70 transcripts did not change in cells exposed to high-frequency EMFs with the different modulation schemes. Instead, levels of the inducible HSP70C transcript were significantly enhanced after 24 h exposure to GSM-217Hz signals and reduced after 4 and 16 h exposure to GSM-Talk signals. As in other cell systems, in HTR-8/SVneo cells the response to high-frequency EMFs was detected at the mRNA level after exposure to amplitude-modulated GSM signals. The present results suggest that the expression analysis for multiple transcripts, though encoding the same or similar protein products, can be highly informative and may account for subtle changes not detected at the protein level.

(E) Franzellitti S, Valbonesi P, Ciancaglini N, Biondi C, Contin A, Bersani F, Fabbri E. Transient DNA damage induced by high-frequency electromagnetic fields (GSM 1.8 GHz) in the human trophoblast HTR-8/SVneo cell line evaluated with the alkaline comet assay. Mutat Res 683(1-2):35-42, 2010. (VT, AE, GT, WS)

One of the most controversial issue regarding high-frequency electromagnetic fields (HF-EMF) is their putative capacity to affect DNA integrity. This is of particular concern due to the increasing use of HF-EMF in communication technologies, including mobile phones. Although epidemiological studies report no detrimental effects on human health, the possible disturbance generated by HF-EMF on cell physiology remains controversial. In addition, the question remains as to whether cells are able to compensate their potential effects. We have previously reported that a 1-h exposure to amplitude-modulated 1.8 GHz sinusoidal waves (GSM-217 Hz, SAR=2 W/kg) largely used in mobile telephony did not cause increased levels of primary DNA damage in human trophoblast HTR-8/SVneo cells. Nevertheless, further investigations on trophoblast cell responses after exposure to GSM signals of different types and durations were considered of interest. In the present work, HTR-8/SVneo cells were exposed for 4, 16 or 24h to

1.8 GHz continuous wave (CW) and different GSM signals, namely GSM-217 Hz and GSM-Talk (intermittent exposure: 5 min field on, 10 min field off). The alkaline comet assay was used to evaluate primary DNA damages and/or strand breaks due to uncompleted repair processes in HF-EMF exposed samples. The amplitude-modulated signals GSM-217 Hz and GSM-Talk induced a significant increase in comet parameters in trophoblast cells after 16 and 24h of exposure, while the un-modulated CW was ineffective. However, alterations were rapidly recovered and the DNA integrity of HF-EMF exposed cells was similar to that of sham-exposed cells within 2h of recovery in the absence irradiation. Our data suggest that HF-EMF with a carrier frequency and modulation scheme typical of the GSM signal may affect the DNA integrity.

(NE) Fritze K, Wiessner C, Kuster N, Sommer C, Gass P, Hermann DM, Kiessling M, Hossmann KA. Effect of global system for mobile communication microwave exposure on the genomic response of the rat brain. Neuroscience. 81(3):627-639, 1997. (VO, AE, GE)

The acute effect of global system for mobile communication (GSM) microwave exposure on the genomic response of the central nervous system was studied in rats by measuring changes in the messenger RNAs of hsp70, the transcription factor genes c-fos and c-jun and the glial structural gene GFAP using in situ hybridization histochemistry. Protein products of transcription factors, stress proteins and marker proteins of astroglial and microglial activation were assessed by immunocytochemistry. Cell proliferation was evaluated by bromodeoxyuridine incorporation. A special GSM radiofrequency test set, connected to a commercial cellular phone operating in the discontinuous transmission mode, was used to simulate GSM exposure. The study was conducted at time averaged and brain averaged specific absorption rates of 0.3 W/kg (GSM exposure), 1.5 W/kg (GSM exposure) and 7.5 W/kg (continuous wave exposure), respectively. Immediately after exposure, in situ hybridization revealed slight induction of hsp70 messenger RNA in the cerebellum and hippocampus after 7.5 W/kg exposure, but not at lower intensities. A slightly increased expression of c-fos messenger RNA was observed in the cerebellum, neocortex and piriform cortex of all groups subjected to immobilization, but no differences were found amongst different exposure conditions. C-jun and GFAP messenger RNAs did not increase in any of the experimental groups. 24 h after exposure, immunocytochemical analysis of FOS and JUN proteins (c-FOS, FOS B, c-JUN JUN B, JUN D), of HSP70 or of KROX-20 and -24 did not reveal any alterations. Seven days after exposure, neither increased cell proliferation nor altered expression of astroglial and microglial marker proteins were observed. In conclusion, acute high intensity microwave exposure of immobilized rats may induce some minor stress response but does not result in lasting adaptive or reactive changes of the brain.

(E) Fucic A, Garaj-Vrhovac V, Skara M, Dimitrovic B, X-rays, microwaves and vinyl chloride monomer: their clastogenic and aneugenic activity, using the micronucleus assay on human lymphocytes. Mutat Res 282(4):265-271, 1992. (VT, AE, GT)

Chromosome aberration assays, sister-chromatid exchange techniques and micronucleus assays are commonly used methods for biomonitoring genetic material damaged by chemical or physical agents. On the other hand, their aneugenic activity, which can lead to hypoploidy and may also be associated with carcinogenesis, has not been thoroughly investigated. In our study we chose the micronucleus assay with a new mathematical approach to separate clastogenic

from aneugenic activity of three well-known mutagens (vinyl chloride monomer, X-rays and microwaves) on the genome of human somatic cells. The comparison of frequencies of size distribution of micronuclei in the lymphocytes of humans exposed to each of these three mutagens showed that X-rays and microwaves were preferentially clastogens while vinyl chloride monomer showed aneugenic activity as well. Microwaves possess some mutagenic characteristics typical of chemical mutagens

(E) Furtado-Filho OV, Borba JB, Dallegrave A, Pizzolato TM, Henriques JA, Moreira JC, Saffi J. Effect of 950 MHz UHF electromagnetic radiation on biomarkers of oxidative damage, metabolism of UFA and antioxidants in the livers of young rats of different ages. Int J Radiat Biol. 90(2):159-168, 2014. (VO, LE, GT, OX)

Purpose: To assess the effect of 950 MHz ultra-high-frequency electromagnetic radiation (UHF EMR) on biomarkers of oxidative damage, as well as to verify the concentration of unsaturated fatty acids (UFA) and the expression of the catalase in the livers of rats of different ages. Materials and methods: Twelve rats were equally divided into two groups as controls (CR) and exposed (ER), for each age (0, 6, 15 and 30 days). Radiation exposure lasted half an hour per day for up to 51 days (21 days of gestation and 6, 15 or 30 days of life outside the womb). The specific absorption rate (SAR) ranged from 1.3-1.0 W/kg. The damage to lipids, proteins and DNA was verified by thiobarbituric acid reactive substances (TBARS), protein carbonyls and comets, respectively. UFA were determined by gas chromatography with a flame ionization detector. The expression of catalase was by Western blotting. Results: The neonates had low levels of TBARS and concentrations of UFA after exposure. There was no age difference in the accumulation of protein carbonyls for any age. The DNA damage of ER 15 or 30 days was different. The exposed neonates exhibited lower expression of catalase. Conclusions: 950 MHz UHF EMR does not cause oxidative stress (OS), and it is not genotoxic to the livers of neonates or those of 6 and 15 day old rats, but it changes the concentrations of polyunsaturated fatty acid (PUFA) in neonates. For rats of 30 days, no OS, but it is genotoxic to the livers of ER to total body irradiation.

(NE) Furtado-Filho OV, Borba JB, Maraschin T, Souza LM, Jose JA, Moreira CF, Saffi J. Effects of chronic exposure to 950 MHz ultra-high-frequency electromagnetic radiation on reactive oxygen species metabolism in the right and left cerebral cortex of young rats of different ages. Int J Radiat Biol. 91(11):891-897, 2015. (VO, LE, GT)

PURPOSE: To assess the effect of 950 MHz ultra-high-frequency electromagnetic radiation (UHF-EMR) on biomarkers of oxidative damage to DNA, proteins and lipids in the left cerebral cortex (LCC) and right cerebral cortex (RCC) of neonate and 6-day-old rats. MATERIALS AND METHODS: Twelve rats were equally divided into two groups as controls (CR) and exposed (ER), for each age (0 and 6 days). The LCC and RCC were examined in ER and CR after exposure. Radiation exposure lasted half an hour per day for up to 27 days (throughout pregnancy and 6 days postnatal). The specific absorption rate ranged from 1.32 - 1.14 W/kg. The damage to lipids, proteins and DNA was verified by thiobarbituric acid reactive substances, carbonylated

proteins (CP) and comets, respectively. The concentration of glucose in the peripheral blood of the rats was measured by the Accu-Chek Active Kit due to increased CP in RCC. RESULTS: In neonates, no modification of the biomarkers tested was detected. On the other hand, there was an increase in the levels of CP in the RCC of the 6-day-old ER. Interestingly, the concentration of blood glucose was decreased in this group. CONCLUSIONS: Our results indicate that there is no genotoxicity and oxidative stress in neonates and 6 days rats. However, the RCC had the highest concentration of CP that do not seem to be a consequence of oxidative stress. This study is the first to demonstrate the use of UHF-EMR causes different damage responses to proteins in the LCC and RCC.

(E) Gadhia PK, Shah T, Mistry A, Pithawala M, Tamakuwala D. A preliminary study to assess possible chromosomal damage among users of digital mobile phones. Electromag Biol Med 22:149-159, 2003. (HU, LE, GT)

In a preliminary study to examine possible lymphocyte chromosomal damage, we have tested two cytogenetic endpoints, namely, chromosomal aberrations (CA) and sister chromatid exchange frequencies (SCE), in 24 mobile phone users (12 nonsmoker–nonalcoholic subjects and 12 smoker–alcoholics), who used digital mobile phones for at least 2 years, employing Gaussian Minimum Shift Keying modulations with uplink frequencies at 935–960 MHz. and downlinks at 890–915 MHz. For comparison, the control study group included another 24 individuals, matched according to their age, sex, drinking and smoking habits, as well as similar health status, working habits, and professional careers; but did not use mobile phones. Blood samples of 12 mobile users (6 smoker–alcoholic and 6 nonsmoker–nonalcoholic) and 12 controls (identical to mobile users in every respect) were further treated with a known mutagen Mitomycin-C (MMC) to find out comutagenic/synergistic effect. A complete blood picture for each individual was assessed with an automatic particle cell counter. There was a significant increase ($P < 0.05$) in dicentric chromosomes among mobile users who were smoker–alcoholic as compared to nonsmoker–nonalcoholic; the same held true for controls of both types. After MMC treatment, there was a significant increase in dicentrics ($P < 0.05$) and ring chromosomes ($P < 0.001$) in both smoker–alcoholic and nonsmoker–nonalcoholic mobile users when compared with the controls. Although SCEs showed a significant increase among mobile users, no change in cell cycle progression was noted. The hematological picture showed only minor variations between mobile users and controls.

(E) Gajski G, Garaj-Vrhovac V. Radioprotective effects of honeybee venom (Apismellifera) against 915-MHz microwave radiation-induced DNA damage in wistar rat lymphocytes: in vitro study. Int J Toxicol 28:88-98, 2009. (VT, AE, GT, OX, IX)

The aim of this study is to investigate the radioprotective effect of bee venom against DNA damage induced by 915-MHz microwave radiation (specific absorption rate of 0.6 W/kg) in Wistar rats. Whole blood lymphocytes of Wistar rats are treated with 1 microg/mL bee venom 4 hours prior to and immediately before irradiation. Standard and formamidopyrimidine-DNA glycosylase (Fpg)-modified comet assays are used to assess basal and oxidative DNA damage produced by reactive oxygen species. Bee venom shows a decrease in DNA damage compared

with irradiated samples. Parameters of Fpg-modified comet assay are statistically different from controls, making this assay more sensitive and suggesting that oxidative stress is a possible mechanism of DNA damage induction. Bee venom is demonstrated to have a radioprotective effect against basal and oxidative DNA damage. Furthermore, bee venom is not genotoxic and does not produce oxidative damage in the low concentrations used in this study.

(E) Gandhi G, Anita, Genetic damage in mobile phone users: some preliminary findings. Ind J Hum Genet 11:99-104, 2005. (HU, LE, GT)

BACKGROUND: The impact of microwave (MW)/radio frequency radiation (RFR) on important biological parameters is probably more than a simply thermal one. Exposure to radio frequency (RF) signals generated by the use of cellular telephones have increased dramatically and reported to affect physiological, neurological, cognitive and behavioural changes and to induce, initiate and promote carcinogenesis. Genotoxicity of RFR has also been reported in various test systems after in vitro and/or in vivo exposure but none in mobile phone users. AIMS: In the present study, DNA and chromosomal damage investigations were carried out on the peripheral blood lymphocytes of individuals using mobile phones, being exposed to MW frequency ranging from 800 to 2000 MHz. METHODS: DNA damage was assessed using the single cell gel electrophoresis assay and aneugenic and clastogenic damage by the in vivo capillary blood micronucleus test (MNT) in a total of 24 mobile phone users. RESULTS: Mean comet tail length (26.76 ± 0.054 mm; 39.75% of cells damaged) in mobile phone users was highly significant from that in the control group. The in vivo capillary blood MNT also revealed highly significant (0.25) frequency of micronucleated (MNd) cells. CONCLUSIONS: These results highlight a correlation between mobile phone use (exposure to RFR) and genetic damage and require interim public health actions in the wake of widespread use of mobile telephony.

(E) Gandhi G, Singh P. Cytogenetic damage in mobile phone users: preliminary data. Int J Hum Genet 5:259-265, 2005. (HU, LE, GT)

Mobile telephones, sometimes called cellular (cell) phones or handies, are now an integral part of modern life. The mobile phone handsets are low-powered radiofrequency transmitters, emitting maximum powers in the range of 0.2 to 0.6 watts. Scientific concerns have increased sufficiently over the possible hazard to health from using cell phones. The reported adverse health effects include physiological, behavioural and cognitive changes as well as tumour formation and genetic damage. However findings are controversial and no consensus exists. Genotoxicity has been observed either in lower organisms or in vitro studies. The aim of the present study hence was to detect any cytogenetic damage in mobile phone users by analysing short term peripheral lymphocyte cultures for chromosomal aberrations and the buccal mucosal cells for micronuclei (aneugenicity and clastogenicity). The results revealed increased number of micronucleated buccal cells and cytological abnormalities in cultured lymphocytes indicating the genotoxic response from mobile phone use.

(E) Gandhi G, Kaur G, Nisar U. A cross-sectional case control study on genetic damage in individuals residing in the vicinity of a mobile phone base station. Electromagn Biol Med. 34(4):344-354, 2015a. (HU, LE, GT)

Mobile phone base stations facilitate good communication, but the continuously emitting radiations from these stations have raised health concerns. Hence in this study, genetic damage using the single cell gel electrophoresis (comet) assay was assessed in peripheral blood leukocytes of individuals residing in the vicinity of a mobile phone base station and comparing it to that in healthy controls. The power density in the area within 300 m from the base station exceeded the permissive limits and was significantly ($p = 0.000$) higher compared to the area from where control samples were collected. The study participants comprised 63 persons with residences near a mobile phone tower, and 28 healthy controls matched for gender, age, alcohol drinking and occupational sub-groups. Genetic damage parameters of DNA migration length, damage frequency (DF) and damage index were significantly ($p = 0.000$) elevated in the sample group compared to respective values in healthy controls. The female residents ($n = 25$) of the sample group had significantly ($p = 0.004$) elevated DF than the male residents ($n = 38$). The linear regression analysis further revealed daily mobile phone usage, location of residence and power density as significant predictors of genetic damage. The genetic damage evident in the participants of this study needs to be addressed against future disease-risk, which in addition to neurodegenerative disorders, may lead to cancer.

(E) Gandhi, G., Singh P, Kaur G. Perspectives revisited - The buccal cytome assay in mobile phone users. Int. J. Hum. genet. 15:173–182, 2015b. (HU, LE, GT)

Buccal cell preparations previously scored for micronuclei were re-investigated for genomic instability and other biomarkers to assess DNA damage, cell-proliferation and cell-death in healthy mobile phone users ($n=25$; $30.96 \pm 2.09y$) using mobile phones for 3-5y and the non-mobile phones users ($n=25$; $32.28 \pm 2.01y$) according to the buccal micronucleus cytome (BMCyt) assay which was then not available. The frequency of micronuclei (13.66x), nuclear buds (2.57x), basal (1.34x), karyorrhectic (1.26x), karyolytic (2.44x), pyknotic (1.77x) and condensed chromatin (2.08x) cells were highly significantly ($p=0.000$) increased in mobile phone users whereas the binucleated cells (4.03x) and repair index (8.36x) showed significant decrease ($p=0.000$). DNA damage and nuclear anomalies scored in BMCyt assay are indicative of genetic damage that has not been repaired and this may predispose the mobile phone users to malignancy and cytotoxicity ramifications. Therefore, despite the benefits of communication technology, measures need to be taken so that better connectivity is not at expense of health.

(E) Gapeyev AB, Lukyanova NA, Gudkov SV. Hydrogen peroxide induced by modulated electromagnetic radiation protects the cells from DNA damage. Central European Journal of Biology 9, 915–921(2014) (VT, AE, GT, IX, WS)

It is believed that non-ionizing electromagnetic radiation (EMR) and low-level hydrogen peroxide (H_2O_2) may change nonspecific resistance and modify DNA damage caused by ionizing radiation. To check this assumption, the combined effects of extremely high-frequency EMR (EHF EMR) and X-rays on induction of DNA damage in mouse whole blood leukocytes were studied. The cells were exposed to X-rays with or without preliminary treatment with EHF EMR or low-level H_2O_2 . With the use of enhanced chemiluminescence, it was shown for the first time that pulse-modulated EHF EMR (42.2 GHz, incident power density of 0.1 mW/cm^2 , exposure duration of 20 min, modulation frequency of 1 Hz) induced H_2O_2 at a concentration of 4.6 ± 0.3

nM L⁻¹ in physiological saline. With the use of an alkaline comet assay, it was found that the exposure of cells to the pulse-modulated EHF EMR, 25 min prior to treatment with X-rays at a dose of 4 Gy reduced the level of ionizing radiation-induced DNA damage. Continuous EHF EMR was inefficient. In turn, it was shown that low-level H₂O₂ (30–500 nM L⁻¹) protected the cells against X-irradiation. Thus, the mechanisms of radiation protective effect of EHF EMR are connected with the induction of the adaptive response by nanomolar concentrations of reactive oxygen species formed by pulse-modulated EHF EMR.

(E) Garaj-Vrhovac V, Horvat D, Koren Z, The effect of microwave radiation on the cell genome. Mutat Res 243(2):87-93, 1990. (VT, AE, GT)

Cultured V79 Chinese hamster cells were exposed to continuous radiation, frequency 7.7 GHz, power density 30 mW/cm² for 15, 30, and 60 min. The parameters investigated were the incorporation of [3H]thymidine and the frequency of chromosome aberrations. Data obtained by 2 methods (the incorporation of [3H]thymidine into DNA and autoradiography) showed that the inhibition of [3H]thymidine incorporation took place by complete prevention of DNA from entering into the S phase. The normal rate of incorporation of [3H]thymidine was recovered within 1 generation cycle of V79 cells. Mutagenic tests performed concurrently showed that even DNA macromolecules were involved in the process. In comparison with the control samples there was a higher frequency of specific chromosome lesions in cells that had been irradiated. Results discussed in this study suggest that microwave radiation causes changes in the synthesis as well as in the structure of DNA molecules.

(E) Garaj-Vrhovac V, Horvat D, Koren Z, The relationship between colony-forming ability, chromosome aberrations and incidence of micronuclei in V79 Chinese hamster cells exposed to microwave radiation. Mutat Res 263(3):143-149, 1991. (VT, AE, GT)

Cultured V79 Chinese hamster fibroblast cells were exposed to continuous radiation, frequency 7.7 GHz, power density 0.5 mW/cm² for 15, 30 and 60 min. The effect of microwave radiation on cell survival and on the incidence and frequency of micronuclei and structural chromosome aberrations was investigated. The decrease in the number of irradiated V79 cell colonies was related to the power density applied and to the time of exposure. In comparison with the control samples there was a significantly higher frequency of specific chromosome aberrations such as dicentric and ring chromosomes in irradiated cells. The presence of micronuclei in irradiated cells confirmed the changes that had occurred in chromosome structure. These results suggest that microwave radiation can induce damage in the structure of chromosomal DNA.

(E) Garaj-Vrhovac V, Fucic A, Horvat D, The correlation between the frequency of micronuclei and specific chromosome aberrations in human lymphocytes exposed to microwave radiation in vitro. Mutat Res 281(3):181-186, 1992. (VT, AE, GT)

Human whole-blood samples were exposed to continuous microwave radiation, frequency 7.7 GHz, power density 0.5, 10 and 30 mW/cm² for 10, 30 and 60 min. A

correlation between specific chromosomal aberrations and the incidence of micronuclei after in vitro exposure was observed. In all experimental conditions, the frequency of all types of chromosomal aberrations was significantly higher than in the control samples. In the irradiated samples the presence of dicentric and ring chromosomes was established. The incidence of micronuclei was also higher in the exposed samples. The results of the structural chromosome aberration test and of the micronucleus test were comparatively analyzed. The values obtained showed a positive correlation between micronuclei and specific chromosomal aberrations (acentric fragments and dicentric chromosomes). The results of the study indicate that microwave radiation causes changes in the genome of somatic human cells and that the applied tests are equally sensitive for the detection of the genotoxicity of microwaves.

(E) Garaj-Vrhovac V, Fucic A. The rate of elimination of chromosomal aberrations after accidental exposure to microwave radiation. Bioelectrochem. Bioenerg. 1993; 30: 319-25. (HU, AE, GT)

Analysis of structural chromosome aberrations was performed in a group of radar station personnel who were engaged in repairing radar devices a couple of days earlier. Test results showed a major decline from the values recorded by regular mutagenic monitoring in terms of a significantly increased number of chromosome breaks, acentric fragments, dicentric and polycentric chromosomes with accompanying fragments, ring chromosomes and chromatid interchange. Multiply repeated mutagenic testing demonstrated for all subjects a fall in the total number of chromosome aberrations as a function of time. During a 30-week-long follow-up study a decrease in the total number of chromosome aberrations was observed. In the same period the presence of unstable aberrations such as dicentrics and ring chromosomes persisted, together with a relatively unchanged incidence of stable aberrations.

(E) Garaj-Vrhovac, V, Micronucleus assay and lymphocyte mitotic activity in risk assessment of occupational exposure to microwave radiation. Chemosphere 39(13):2301-2312, 1999. (HU, LE, GT)

The effects of radiofrequency electromagnetic radiation (RFR) on the cell kinetics and genome damages in peripheral blood lymphocytes were determined in lymphocytes of 12 subjects occupationally exposed to microwave radiation. Results showed an increase in frequency of micronuclei (MN) as well as disturbances in the distribution of cells over the first, second and third mitotic division in exposed subjects compared to controls. According to previous reports micronucleus assay can serve as a suitable indicator for the assessment of exposure to genotoxic agents (such as RFR) and the analysis of mitotic activity as an additional parameter for the efficient biomonitoring.

(E) Garaj-Vrhovac V, Orescanin V. Assessment of DNA sensitivity in peripheral blood leukocytes after occupational exposure to microwave radiation: the alkaline comet assay and chromatid breakage assay. Cell Biol Toxicol. 25(1):33-43, 2009. (HU, LE, GT)

DNA sensitivity in peripheral blood leukocytes of radar-facility workers daily exposed to microwave radiation and an unexposed control subjects was investigated. The study was carried out on clinically healthy male workers employed on radar equipment and antenna system service within a microwave field of 10 $\mu\text{W}/\text{cm}^2$ -20 mW/cm^2 with frequency range of 1,250-1,350 MHz. The control group consisted of subjects of similar age. The evaluation of DNA damage and sensitivity was performed using alkaline comet assay and chromatid breakage assay (bleomycin-sensitivity assay). The levels of DNA damage in exposed subjects determined by alkaline comet assay were increased compared to control group and showed inter-individual variations. After short exposure of cultured lymphocytes to bleomycin cells of subjects occupationally exposed to microwave (MW) radiation responded with high numbers of chromatid breaks. Almost three times higher number of bleomycin-induced chromatid breaks in cultured peripheral blood lymphocytes were determined in comparison with control group. The difference in break per cell (b/c) values recorded between smokers and non-smokers was statistically significant in the exposed group. Regression analyses showed significant positive correlation between the results obtained with two different methods. Considering the correlation coefficients, the number of metaphase with breaks was a better predictor of the comet assay parameters compared to b/c ratio. The best correlation was found between tail moment and number of chromatid with breaks. Our results indicate that MW radiation represents a potential DNA-damaging hazard using the alkaline comet assay and chromatid breakage assay as sensitive biomarkers of individual cancer susceptibility.

(E) Garaj-Vrhovac V, Gajski G, Trošić I, Pavčić I. Evaluation of basal DNA damage and oxidative stress in Wistar rat leukocytes after exposure to microwave radiation. Toxicology. 259(3):107-112, 2009. (VO, LE, GT, OX)

The aim of this study was to assess whether microwave-induced DNA damage is basal or it is also generated through reactive oxygen species (ROS) formation. After having irradiated Wistar rats with 915MHz microwave radiation, we assessed different DNA alterations in peripheral leukocytes using standard and formamidopyrimidine DNA-glycosylase (Fpg)-modified comet assay. The first is a sensitive tool for detecting primary DNA damage, and the second is much more specific for detecting oxidative damage. The animals were irradiated for 1h a day for 2 weeks at a field power density of 2.4W/m², and the whole-body average specific absorption rate (SAR) of 0.6W/kg. Both the standard and the Fpg-modified comet assay detected increased DNA damage in blood leukocytes of the exposed rats. The significant increase in Fpg-detected DNA damage in the exposed rats suggests that oxidative stress is likely to be responsible. DNA damage detected by the standard comet assay indicates that some other mechanisms may also be involved. In addition, both methods served proved sensitive enough to measure basal and oxidative DNA damage after long-term exposure to 915MHz microwave radiation in vivo.

(E) Garaj-Vrhovac V, Gajski G, Pažanin S, Sarolić A, Domijan AM, Flajs D, Peraica M. Assessment of cytogenetic damage and oxidative stress in personnel occupationally exposed to the pulsed microwave radiation of marine radar equipment. Int J Hyg Environ Health. 4(1):59-65, 2011. (HU, LE, GT, LI, OX)

Due to increased usage of microwave radiation, there are concerns of its adverse effect in today's society. Keeping this in view, study was aimed at workers occupationally exposed to pulsed microwave radiation, originating from marine radars. Electromagnetic field strength was measured at assigned marine radar frequencies (3 GHz, 5.5 GHz and 9.4 GHz) and corresponding specific absorption rate values were determined. Parameters of the comet assay and micronucleus test were studied both in the exposed workers and in corresponding unexposed subjects. Differences between mean tail intensity (0.67 vs. 1.22) and moment (0.08 vs. 0.16) as comet assay parameters and micronucleus test parameters (micronuclei, nucleoplasmic bridges and nuclear buds) were statistically significant between the two examined groups, suggesting that cytogenetic alterations occurred after microwave exposure. Concentrations of glutathione and malondialdehyde were measured spectrophotometrically and using high performance liquid chromatography. The glutathione concentration in exposed group was significantly lower than in controls (1.24 vs. 0.53) whereas the concentration of malondialdehyde was significantly higher (1.74 vs. 3.17), indicating oxidative stress. Results suggests that pulsed microwaves from working environment can be the cause of genetic and cell alterations and that oxidative stress can be one of the possible mechanisms of DNA and cell damage.

(NE) Garson OM, McRobert TL, Campbell LJ, Hocking BA, Gordon I. A chromosomal study of workers with long-term exposure to radio-frequency radiation. Med J Aust 155(5):289-292, 1991. (HU, LE, GT)

OBJECTIVE: To examine whether an increased level of chromosome damage occurs in the stimulated lymphocytes of radio-linemen after long-term but intermittent exposure to radio-frequency radiation (RFR) during the course of their work. DESIGN AND PARTICIPANTS: Chromosome studies were performed on blood samples from 38 radio-linemen matched by age with 38 controls, all of whom were employed by Telecom Australia. The radio-linemen had all worked with RFR in the range 400 kHz-20 GHz with exposures at or below the Australian occupational limits, and the controls were members of the clerical staff who had no exposure to RFR. Two hundred metaphases from each subject were studied and chromosome damage was scored by an observer who was blind to the status of the subjects. RESULTS: The ratio of the rate of aberrant cells in the radio-linemen group to that in the control group was 1.0 (95% confidence interval, 0.8-1.3). There were no statistically significant differences in the types of aberrations that were scored. CONCLUSION: Exposure to RFR at or below the described limits did not appear to cause any increase in chromosomal damage in circulating lymphocytes.

(E) Ghandehari M, Sadri D, Farhadi S. Micronucleus Assay in Cell Phone Users: Importance of Oral Mucosa Screening. Int J Prev Med. 2021 Sep 29;12:125. (HU, LE, GT)

Background: One of the concerns of cell phone users is prolonged exposure to harmful and potentially carcinogenic waves. This study was aimed to investigate the correlation between amount of cell phone use and related factors with percentage of micronucleus containing cells. Methods: This descriptive study was conducted on selected patients referring to Islamic Azad

University Faculty of Dentistry using cell phones regarding related inclusion and exclusion criteria. Papanicolaou staining method was approached for mucosal smears of samples and frequency of micronucleus containing cells and also, frequency of micronucleus in each cell were recorded for each sample; then, correlation of these findings with amount of daily cell phone usage was statistically analyzed using the calculation of Pearson correlation coefficient and preparation of regression analysis (backward) with significant level of lower than 0.05. Results: Of 100 samples, the frequency of micronucleus containing cells was $2.94\% \pm 1.89\%$ and the frequency of micronucleus in each cell was $1.02\% \pm 1.68\%$. The amount of cell phone usage was significantly correlated with the frequency of micronucleus containing cells ($r = 0.70$, $P = 0.0001$) and also with the frequency of micronucleus in each cell ($r = 0.57$, $P = 0.0001$). Also, age and sex were not significantly correlated with the frequency of micronucleus containing cells ($P = 0.47$ and 0.32) and also with the frequency of micronucleus in each cell, respectively ($P = 0.16$ and 0.27). Conclusions: The present study showed that the increased amount of cell phone usage had a strong and significant correlation with the higher frequency of the micronucleus containing cells and the higher frequency of micronucleus in each cell in the buccal mucosa. Also, the related factors as age and sex were not significantly correlated with the frequency of micronucleus containing buccal mucosa cells.

(E) Ghatei N, Nabavi AS, Toosi MHB, Azimian H, Homayoun M, Targhi RG, Haghiri H. Evaluation of *bax*, *bcl-2*, *p21* and *p53* genes expression variations on cerebellum of BALB/c mice before and after birth under mobile phone radiation exposure. Iran J Basic Med Sci. 20(9):1037-1043, 2017. (VO, LE, GE, DE)

Objectives: The increasing rate of over using cell phones has been considerable in youths and pregnant women. We examined the effect of mobile phones radiation on genes expression variation on cerebellum of BALB/c mice before and after of the birth. **Materials and methods:** In this study, a mobile phone jammer, which is an instrument to prevent receiving signals between cellular phones and base transceiver stations (two frequencies 900 and 1800 MHz) for exposure was used and twelve pregnant mice (BALB/c) divided into two groups ($n=6$), first group irradiated in pregnancy period (19th day), the second group did not irradiate in pregnancy period. After childbirth, offspring were classified into four groups ($n=4$): Group 1: control, Group 2: B1 (Irradiated after birth), Group 3: B2 (Irradiated in pregnancy period and after birth), Group 4: B3 (Irradiated in pregnancy period). When maturity was completed (8-10 weeks old), mice were dissected and cerebellum was isolated. The expression level of *bax*, *bcl-2*, *p21* and *p53* genes examined by real-time reverse transcription polymerase chain reaction (Real-Time RT-PCR). **Results:** The data showed that mobile phone radio waves were ineffective on the expression level of *bcl-2* and *p53* genes ($P>0.05$). Also gene expression level of *bax* decreased and gene expression level of *p21* increased comparing to the control group ($P<0.05$). **Conclusion:** From the obtained data it could be concluded that the mobile phone radiations did not induce apoptosis in cells of the cerebellum and the injured cells can be repaired by cell cycle arrest.

(NE) Gläser K, Rohland M, Kleine-Ostmann T, Schrader T, Stopper H, Hintzsche H. Effect of Radiofrequency Radiation on Human Hematopoietic Stem Cells. Radiat Res. 2016 Nov;186(5):455-465. (VT, AE, GT)

Exposure to electromagnetic fields in the radiofrequency range is ubiquitous, mainly due to the worldwide use of mobile communication devices. With improving technologies and affordability, the number of cell phone subscriptions continues to increase. Therefore, the potential effect on biological systems at low-intensity radiation levels is of great interest. While a number of studies have been performed to investigate this issue, there has been no consensus reached based on the results. The goal of this study was to elucidate the extent to which cells of the hematopoietic system, particularly human hematopoietic stem cells (HSC), were affected by mobile phone radiation. We irradiated HSC and HL-60 cells at frequencies used in the major technologies, GSM (900 MHz), UMTS (1,950 MHz) and LTE (2,535 MHz) for a short period (4 h) and a long period (20 h/66 h), and with five different intensities ranging from 0 to 4 W/kg specific absorption rate (SAR). Studied end points included apoptosis, oxidative stress, cell cycle, DNA damage and DNA repair. In all but one of these end points, we detected no clear effect of mobile phone radiation; the only alteration was found when quantifying DNA damage. Exposure of HSC to the GSM modulation for 4 h caused a small but statistically significant decrease in DNA damage compared to sham exposure. To our knowledge, this is the first published study in which putative effects (e.g., genotoxicity or influence on apoptosis rate) of radiofrequency radiation were investigated in HSC. Radiofrequency electromagnetic fields did not affect cells of the hematopoietic system, in particular HSC, under the given experimental conditions.

(E) Gökçek-Saraç C, Akçay G, Karakurt S, Ateş K, Özen S, Derin N. Possible effects of different doses of 2.1 GHz electromagnetic radiation on learning, and hippocampal levels of cholinergic biomarkers in Wistar rats. Electromagn Biol Med 40:179-190, 2021. (VO, LE, GE)

The present study evaluated whether short-term exposure to different doses of 2.1 GHz radiofrequency electromagnetic radiation (RF-EMR) has different effects on rats' behaviour and hippocampal levels of central cholinergic biomarkers. Animals were divided into three equal groups namely; group 1 was sham-exposed group, group 2-3 were exposed to 45 V/m and 65 V/m doses of 2.1 GHz frequency for 1 week respectively. Numerical dosimetry simulations were carried out. Object location and Y-maze were used as behavioural tasks. The protein and mRNA expression levels of AChE, ChAT, and VACHT, in the hippocampus were tested using Western Blotting and Real-Time PCR. The impairment performance of rats subjected to 65 V/m dose of 2.1 GHz RF-EMR in both object location and Y-maze tasks was observed. The hippocampal levels of AChE, ChAT, and VACHT, were significantly lower in rats exposed to 65 V/m dose of 2.1 GHz RF-EMR than others. The stronger effect of "65 V/m" dose on both rat's hippocampal-dependent behavioural performances and hippocampal levels of cholinergic biomarkers may be due to the stronger effect of "65 V/m" dose where rats' snouts were located at the nearest distance from the monopole antenna. Furthermore, the simulated SAR values were high for 65

V/m electric-field strengths. For the first time, we report the potential dose-dependent effects of short-term exposure to 2.1 GHz radiation on rat's behavioural performances as well as hippocampal levels of cholinergic biomarkers. Further studies are needed to understand the mechanisms by which RF-EMR influences the function of the central cholinergic system in the brain.

(NE) Görlitz B-D, Müller M, Ebert S, Hecker H, Kuster N, Dasenbrock C. Effects of 1-week and 6-week exposure to GSM/DCS radiofrequency radiation on micronucleus formation in B6C3F1 mice. Radiat Res 2005 Oct;164(4 Pt 1):431-439. (VO, LE, GT)

The aim of this study was to examine the possible induction of micronuclei in erythrocytes of the peripheral blood and bone marrow and in keratinocytes and spleen lymphocytes of mice exposed to radiofrequency (RF) radiation for 2 h per day over periods of 1 and 6 weeks, respectively. The applied signal simulated the exposure from GSM900 and DCS1800 handsets, including the low-frequency amplitude-modulation components as they occur during speaking (GSM Basic), listening (DTX) and moving within the environment (handovers, power control). The carrier frequency was set to the center of the system's uplink band, i.e., 902 MHz for GSM and 1747 MHz for DCS. Uniform whole-body exposure was achieved by restraining the mice in tubes at fixed positions in the exposure setup. Mice were exposed to slot-averaged whole-body SARs of 33.2, 11.0, 3.7 and 0 mW/g during the 1-week study and 24.9, 8.3, 2.8 and 0 mW/g during the 6-week study. Exposure levels for the 1- and 6-week studies were determined in a pretest to confirm that no thermal effect was present that could influence the genotoxic end points. During both experiments and for both frequencies, no clinical abnormalities were detected in the animals. Cells of the bone marrow from the femur (1-week study), erythrocytes of the peripheral blood (6-week study), keratinocytes from the tail root, and lymphocytes from the spleen (both studies) were isolated on slides and stained for micronucleus analysis. Two thousand cells per animal were scored in erythrocyte and keratinocyte samples. In spleen lymphocytes, 1000 binucleated lymphocytes were scored for each animal. The RF-field exposure had no influence on the formation of red blood cells. After 1 week of exposure, the ratio of polychromatic to normochromatic erythrocytes was unchanged in the treated groups compared to the sham-exposed groups. Furthermore, the RF-field exposure of mice did not induce an increase in the number of micronuclei in erythrocytes of the bone marrow or peripheral blood, in keratinocytes, or in spleen lymphocytes compared to the sham-treated control.

(E) Gorpichenko I, Nikitin O, Banyra O, Shulyak A. The influence of direct mobile phone radiation on sperm quality. Cent European J Urol. 67(1):65-71, 2014. (VT, AE, GT, RP)

INTRODUCTION: It is impossible to imagine a modern socially-active man who does not use mobile devices and/or computers with Wi-Fi function. The effect of mobile phone radiation on male fertility is the subject of recent interest and investigations. The aim of this study was to investigate the direct in vitro influence of mobile phone radiation on sperm DNA fragmentation and motility parameters in healthy subjects with normozoospermia. **MATERIAL AND**

METHODS: 32 healthy men with normal semen parameters were selected for the study. Each sperm sample was divided into two equal portions (A and B). Portions A of all involved men were placed for 5 hours in a thermostat, and portions B were placed into a second thermostat for the same period of time, where a mobile phone in standby/talk mode was placed. After 5 hours of incubation the sperm samples from both thermostats were re-evaluated regarding basic motility parameters. The presence of DNA fragmentation in both A and B portions of each sample was determined each hour using a standard sperm chromatin dispersion test. **RESULTS:** The number of spermatozoa with progressive movement in the group, influenced by electromagnetic radiation, is statistically lower than the number of spermatozoa with progressive movement in the group under no effect of the mobile phone. The number of non-progressive movement spermatozoa was significantly higher in the group, which was influenced by cell phone radiation. The DNA fragmentation was also significantly higher in this group. **CONCLUSIONS:** A correlation exists between mobile phone radiation exposure, DNA-fragmentation level and decreased sperm motility.

(E) Gulati S, Yadav A, Kumar N, Kanupriya, Aggarwal NK, Kumar R, Gupta R. Effect of GSTM1 and GSTT1 Polymorphisms on Genetic Damage in Humans Populations Exposed to Radiation From Mobile Towers. Arch Environ Contam Toxicol. 70(3): 615-625, 2016. (HU, LE, GT)

All over the world, people have been debating about associated health risks due to radiation from mobile phones and mobile towers. The carcinogenicity of this nonionizing radiation has been the greatest health concern associated with mobile towers exposure until recently. The objective of our study was to evaluate the genetic damage caused by radiation from mobile towers and to find an association between genetic polymorphism of GSTM1 and GSTT1 genes and DNA damage. In our study, 116 persons exposed to radiation from mobile towers and 106 control subjects were genotyped for polymorphisms in the GSTM1 and GSTT1 genes by multiplex polymerase chain reaction method. DNA damage in peripheral blood lymphocytes was determined using alkaline comet assay in terms of tail moment (TM) value and micronucleus assay in buccal cells (BMN). There was a significant increase in BMN frequency and TM value in exposed subjects (3.65 ± 2.44 and 6.63 ± 2.32) compared with control subjects (1.23 ± 0.97 and 0.26 ± 0.27). However, there was no association of GSTM1 and GSTT1 polymorphisms with the level of DNA damage in both exposed and control groups.

(E) Gulati S, Yadav A, Kumar N, Priya K, Aggarwal NK, Gupta R. Phenotypic and genotypic characterization of antioxidant enzyme system in human population exposed to radiation from mobile towers. Mol Cell Biochem. 440(1-2):1-9, 2018. (HU, LE, GE)

In the present era, cellular phones have changed the life style of human beings completely and have become an essential part of their lives. The number of cell phones and cell towers are increasing in spite of their disadvantages. These cell towers transmit radiation continuously without any interruption, so people living within 100s of meters from the tower receive 10,000 to 10,000,000 times stronger signal than required for mobile communication. In the present study, we have examined superoxide dismutase (SOD) enzyme activity, catalase (CAT) enzyme activity, lipid peroxidation assay, and effect of functional polymorphism of SOD and CAT

antioxidant genes against mobile tower-induced oxidative stress in human population. From our results, we have found a significantly lower mean value of manganese superoxide dismutase (MnSOD) enzyme activity, catalase (CAT) enzyme activity, and a high value of lipid peroxidation assay in exposed as compared to control subjects. Polymorphisms in antioxidant MnSOD and CAT genes significantly contributed to its phenotype. In the current study, a significant association of genetic polymorphism of antioxidant genes with genetic damage has been observed in human population exposed to radiations emitted from mobile towers.

(E) Gulati, S, Kosik, P, Durdik, M, Skorvaga, M., Jakl, L., Markova, E., Belyaev, I. (2020). Effects of different mobile phone UMTS signals on DNA, apoptosis and oxidative stress in human lymphocytes. Environ Pollut. 267:115632. doi: 10.1016/j.envpol.2020.115632. (VT, AE, GT,WS)

Different scientific reports suggested a link between exposure to radiofrequency radiation (RF) from mobile communications and induction of reactive oxygen species (ROS) and DNA damage while other studies have not found such a link. However, the available studies are not directly comparable because they were performed at different parameters of exposure, including carrier frequency of RF signal, which was shown to be critical for appearance of the RF effects. For the first time, we comparatively analyzed genotoxic effects of UMTS signals at different frequency channels used by 3G mobile phones (1923, 1947.47, and 1977 MHz). Genotoxicity was examined in human lymphocytes exposed to RF for 1 h and 3 h using complimentary endpoints such as induction of ROS by imaging flow cytometry, DNA damage by alkaline comet assay, mutations in TP53 gene by RSM assay, preleukemic fusion genes (PFG) by RT-qPCR, and apoptosis by flow cytometry. No effects of RF exposure on ROS, apoptosis, PFG, and mutations in TP53 gene were revealed regardless of the UMTS frequency while inhibition of a bulk RNA expression was found. On the other hand, we found relatively small but statistically significant induction of DNA damage in dependence on UMTS frequency channel with maximal effect at 1977.0 MHz. Our data support a notion that each specific signal used in mobile communication should be tested in specially designed experiments to rule out that prolonged exposure to RF from mobile communication would induce genotoxic effects and affect the health of the human population

(E) Guler G, Tomruk A, Ozgur E, Seyhan N. The effect of radiofrequency radiation on DNA and lipid damage in non-pregnant and pregnant rabbits and their newborns. Gen Physiol Biophys 29:59-66, 2010. (VO, LE, LI, GT, OX, DE)

The concerns of people on possible adverse health effects of radiofrequency radiation (RFR) generated from mobile phones as well as their supporting transmitters (base stations) have increased markedly. RFR effect on oversensitive people, such as pregnant women and their developing fetuses, and older people is another source of concern that should be considered. In this study, oxidative DNA damage and lipid peroxidation levels in the brain tissue of pregnant and non-pregnant New Zealand White rabbits and their newborns exposed to RFR were investigated. Thirteen-month-old rabbits were studied in four groups as non-pregnant-control, non-pregnant-RFR exposed, pregnant-control and pregnant-RFR exposed. They were exposed to RFR (1800 MHz GSM; 14 V/m as reference level) for 15 min/day during 7 days. Malondialdehyde (MDA) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels were analyzed.

MDA and 8-OHdG levels of non-pregnant and pregnant-RFR exposed animals significantly increased with respect to controls ($p < 0.001$, Mann-Whitney test). No difference was found in the newborns ($p > 0.05$, Mann-Whitney). There exist very few experimental studies on the effects of RFR during pregnancy. It would be beneficial to increase the number of these studies in order to establish international standards for the protection of pregnant women from RFR.

(E) Güler G, Tomruk A, Ozgur E, Sahin D, Sepici A, Altan N, Seyhan N. The effect of radiofrequency radiation on DNA and lipid damage in female and male infant rabbits. Int J Radiat Biol. 88(4):367-373, 2012. (VO, LE, GT, OX, DE)

PURPOSE: We aimed to design a prolonged radiofrequency (RF) radiation exposure and investigate in an animal model, possible bio-effects of RF radiation on the ongoing developmental stages of children from conception to childhood. **MATERIALS AND METHODS:** A total of 72 New Zealand female and male white rabbits aged one month were used. Females were exposed to RF radiation for 15 min/day during 7 days, whereas males were exposed to the same level of radiation for 15 min/day during 14 days. Thirty-six female and 36 male infant rabbits were randomly divided into four groups: Group I [Intrauterine (IU) exposure (-); Extrauterine (EU) exposure (-)]: Sham exposure which means rabbits were exposed to 1800 MHz Global System for Mobile Telecommunication (GSM)-like RF signals neither in the IU nor in the EU periods. Group II [IU exposure (-); EU exposure (+)]: Infant rabbits were exposed to 1800 MHz GSM-like RF signals when they reached one month of age. Group III [IU exposure (+); EU exposure (-)]: Infant rabbits were exposed to 1800 MHz GSM-like RF signals in the IU period (between 15th and 22nd days of the gestational period). Group IV [IU exposure (+); EU exposure (+)]: Infant rabbits were exposed to 1800 MHz GSM-like RF signals both in the IU period (between 15th and 22nd days of the gestational period) and in the EU period when they reached one month of age. Biochemical analysis for lipid peroxidation and DNA damage were carried out in the livers of all rabbits. **RESULTS:** Lipid peroxidation levels in the liver tissues of female and male infant rabbits increased under RF radiation exposure. Liver 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels of female rabbits exposed to RF radiation were also found to increase when compared with the levels of non-exposed infants. However, there were no changes in liver 8-OHdG levels of male rabbits under RF exposure. **CONCLUSION:** Consequently, it can be concluded that GSM-like RF radiation may induce biochemical changes by increasing free radical attacks to structural biomolecules in the rabbit as an experimental animal model.

***(E) Gunes M, Ates K, Yalcin B, Akkurt S, Ozen S, Kaya B An Evaluation of the Genotoxic Effects of Electromagnetic Radiation at 900 MHz, 1800 MHz, and 2100 MHz Frequencies with a SMART Assay in *Drosophila melanogaster*. Electromagn Biol Med 2021 Feb 24;1-10. (VO, CE, GT)**

With the development of today's technology, the electromagnetic radiation spread by mobile phones and base stations is also rapidly increasing, and this causes serious concerns about the environment and human health. The *Drosophila* model organism is widely used in genetic toxicology studies because its genome is highly similar to the genes identified in human diseases. In this study, the genotoxic effects of radiofrequency electromagnetic radiation were evaluated by the wing Somatic Mutation and Recombination Test (SMART) in *Drosophila melanogaster* at 900 MHz, 1800 MHz, and 2100 MHz. The SMART method is based on the

observation of genetic changes occurring in the trichomes of the *Drosophila* wings appearing as mutant clones under the microscope. Throughout the study, total clone parameters were evaluated by exposing the *Drosophila* larvae to electromagnetic fields for two, four, and six hours per day for two days. As a result of the study, it was observed that the number of mutant clones was statistically increased according to the negative control group in all applications except for the six-hour application at 1800 MHz.

(NE) Gurbuz N, Sirav B, Yuvaci HU, Turhan N, Coskun ZK, Seyhan N. Is there any possible genotoxic effect in exfoliated bladder cells of rat under the exposure of 1800 MHz GSM-like modulated radio frequency radiation (RFR)? Electromagn Biol Med. 29(3):98-104, 2010. (VO, LE, GT)

People are exposed to many carcinogenic and mutagenic chemicals in their everyday lives. These include antineoplastic drugs, Polycyclic aromatic hydrocarbons (PAH)s, aromatic amines, nitrosamines, metals, and electromagnetic radiation. Based on the state of knowledge acquired during the last 50 years of research on possible biological effects of electromagnetic fields (EMF), the majority of the scientific community is convinced that exposure to EMF below the existing security limits does not cause a risk to the health of the general public. However, this position is questioned by others, who are of the opinion that the available research data are contradictory or inconsistent and, therefore, unreliable. In this study, we aimed to investigate if there is any effect of 1800 MHz GSM modulated radio frequency radiation (RFR) on the number of micronucleus in exfoliated bladder cells of rat which will be informative about the genotoxic damage. Exposure period was 20 min/day, 5 days/week during a month. Six female Wistar rats were used for two groups: Group I (n=6): controls; Group II (n=6): 1.8 GHz exposed animals. 1800 MHz RFR did not showed a significant MN frequencies in rat bladder cells when compared with the control group ($p>0.05$). 1800 MHz RFR-exposed animals did not produce any genotoxic effect when compared with the control group ($p>0.05$). Kinetic studies are important for any biomarker, especially those in which tissue differentiation and maturation processes will heavily influence the time between induction of damage and collection of damaged cells for micronucleus analysis.

(NE) Gurbuz N, Sirav B, Colbay M, Yetkin I, Seyhan N. No genotoxic effect in exfoliated bladder cells of rat under the exposure of 1800 and 2100-MHz radio frequency radiation. Electromagn Biol Med. 33(4):296-301, 2014. (VO, LE, GT)

In this study, we aimed to investigate the effects of 1800 and 2100 MHz Radio Frequency (RF) radiation on the number of micronucleus (MN) in exfoliated bladder cells of rat which shows the genotoxic damage. Exposure period was 30 min/day, 6 days/week for a month and two months exposure periods. Thirty male wistar albino rats were used for five groups: Group I (n = 6): 1800 MHz RF exposed animals for one month, Group II (n = 6): 2100 MHz RF exposed animals for one month, Group III (n = 6): 2100 MHz RF exposed for two months, Group IV (n = 6): control group for one month, Group V (n = 6): control group for two months. Rats of the control groups were housed in their home cages during the entire experimental period without subjecting to any experimental manipulation. 1800 and 2100 MHz RF exposures did not result in any significant MN frequencies in rat bladder cells with respect to the control groups ($p > 0.05$).

There was no statistically significant difference between 2100 MHz RF exposed groups, either. Further studies are needed to demonstrate if there is any genotoxic effect, micronucleus formation in other tissues of rats.

(NE) Gurbuz N, Sirav B, Kuzay D, Ozer C, Seyhan N. Does radio frequency radiation induce micronuclei frequency in exfoliated bladder cells of diabetic rats? Endocr Regul 2015 Jul;49(3):126-130.(VT, LE, GT)

Objective: For many years there has been a discussion among both experts and the general public regarding the effects of radio frequency (RF) radiation on the human organism. The purpose of the present study was to evaluate the relationship of micronuclei (MN) frequency and RF radiation in exfoliated bladder cells of non-diabetic and diabetic rats. **Methods:** Three groups were used in the experiment: Group I (n=6): diabetic group without RF exposure; Group II (n=6): diabetic group exposed 2100 MHz RF radiation and Group III (n=6): control animals (non-diabetic group, no RF exposure). RF exposure in the experiment resulted in a whole body average SAR of 0.24 W/kg with an ERMS field of 17.5 V/m in non-thermal levels.

Result: Results showed that there was no statistically important differences between non-RF exposed diabetes group and control group; Group I and Group III ($p>0.05$). There was no statistically important differences between diabetes group and diabetes+RF exposed group (Group I and Group II) ($p>0.05$). RF exposure did not result in increased MN frequencies in exfoliated bladder cells of diabetic rats with respect to control animals (Group II and Group III), either and this result found no statistically important ($p>0.05$). **Conclusions:** This study suggested no possible genotoxic effects of RF radiation among human beings especially with chronic disorders, such as diabetes.

(NE) Gurisik E, Warton K, Martin DK, Valenzuela SM. An in vitro study of the effects of exposure to a GSM signal in two human cell lines: monocytic U937 and neuroblastoma SK-N-SH. Cell Biol Int 30(10):793-759, 2006. (VT, AE, GE)

The use of mobile phones is increasing, which also increases the population's exposure to global system of mobile communications (GSM) signals. Questions of safety and possible biological effects are of concern and to date, remain largely unanswered. In order to examine possible biological effects of a GSM-like signal at a cellular level, we exposed two human cell lines (one of neuronal (SK-N-SH) and the other of monocytoid (U937) origin) to a 900 MHz RF signal, pulsed at 217 Hz, producing a specific absorption rate (SAR) of 0.2 W/kg. Putative effects were assessed by comparing radiofrequency-exposed cells to sham-exposed cells using a variety of assay techniques. For the cell line SK-N-SH, effects were specifically assessed by gene microarray, followed by real-time PCR of the genes of interest, Western blot analysis was used to measure heat shock protein levels, and flow cytometry to measure cell cycle distributions and apoptosis. Effects of radiofrequency on the cell line U937 were assessed by cell viability and cell cycle analysis. From our study of these two cell lines, we found no significant difference between sham-exposed versus radiofrequency-exposed cells in any of the assays or conditions examined.

(E) Gürler HS, Bilgici B, Akar AK, Tomak L, Bedir A. Increased DNA oxidation (8-OHdG) and protein oxidation (AOPP) by Low level electromagnetic field (2.45 GHz) in rat brain and protective effect of garlic. Int J Radiat Biol. 90(10):892-896, 2014. (VO, LE, LI, GT, OX, IX)

Purpose: To investigate the oxidative damage and protective effect of garlic on rats exposed to low level of electromagnetic fields (EMF) at 2.45 GHz Microwave radiation (MWR). Methods: Thirty six Wistar rats were divided into three groups. Group I was the control group and not exposed to EMF. Group II and III were exposed to low level EMF (3.68 ± 0.36 V/m) at 2.45 GHz MWR for 1 hour/day for 30 consecutive days. Daily 500 mg/kg garlic was given to Group III during the study period. At the end of the study, thiobarbituric acid reactive substances (TBARS), advanced oxidation protein products (AOPP) and 8-hydroxydeoxyguanosine (8-OHdG) levels were investigated in brain tissue and blood samples. Results: Exposure to low level of EMF increased 8-OHdG level in both plasma and brain tissue whereas it increased AOPP level only in plasma. Garlic prevented the increase of 8-OHdG level in brain tissue and plasma AOPP levels. Conclusions: It may be concluded that low level EMF at 2.45 GHz MWR increases the DNA damage in both brain tissues and plasma of the rats whereas it increases protein oxidation only in plasma. It may also be argued that the use of garlic decreases these effects.

(E) Gustavino B, Carboni G, Petrillo R, Paoluzzi G, Santovetti E, Rizzoni M. Exposure to 915 MHz radiation induces micronuclei in Vicia faba root tips. Mutagenesis. 31(2):187-192, 2016. (VO, AE, GT)

The increasing use of mobile phones and wireless networks raised a great debate about the real carcinogenic potential of radiofrequency-electromagnetic field (RF-EMF) exposure associated with these devices. Conflicting results are reported by the great majority of in vivo and in vitro studies on the capability of RF-EMF exposure to induce DNA damage and mutations in mammalian systems. Aimed at understanding whether less ambiguous responses to RF-EMF exposure might be evidenced in plant systems with respect to mammalian ones, in the present work the mutagenic effect of RF-EMF has been studied through the micronucleus (MN) test in secondary roots of *Vicia faba* seedlings exposed to mobile phone transmission in controlled conditions, inside a transverse electro magnetic (TEM) cell. Exposure of roots was carried out for 72h using a continuous wave (CW) of 915 MHz radiation at three values of equivalent plane wave power densities (23, 35 and 46W/m²). The specific absorption rate (SAR) was measured with a calorimetric method and the corresponding values were found to fall in the range of 0.4-1.5W/kg. Results of three independent experiments show the induction of a significant increase of MN frequency after exposure, ranging from a 2.3-fold increase above the sham value, at the lowest SAR level, up to a 7-fold increase at the highest SAR. These findings are in agreement with the limited number of data on cytogenetic effects detected in other plant systems exposed to mobile phone RF-EMF frequencies and clearly show the capability of radiofrequency exposure to induce DNA damage in this eukaryotic cell system.

(E) Habauzit D, Le Quément C, Zhadobov M, Martin C, Aubry M, Sauleau R, Le Dréan Y. Transcriptome analysis reveals the contribution of thermal and the specific effects in

cellular response to millimeter wave exposure. PLoS One. 9(10):e109435, 2014. (VT, AE, GE)

Radiofrequency radiations constitute a new form of environmental pollution. Among them, millimeter waves (MMW) will be widely used in the near future for high speed communication systems. This study aimed therefore to evaluate the biocompatibility of MMW at 60 GHz. For this purpose, we used a whole gene expression approach to assess the effect of acute 60 GHz exposure on primary cultures of human keratinocytes. Controls were performed to dissociate the electromagnetic from the thermal effect of MMW. Microarray data were validated by RT-PCR, in order to ensure the reproducibility of the results. MMW exposure at 20 mW/cm², corresponding to the maximum incident power density authorized for public use (local exposure averaged over 1 cm²), led to an increase of temperature and to a strong modification of keratinocyte gene expression (665 genes differentially expressed). Nevertheless, when temperature is artificially maintained constant, no modification in gene expression was observed after MMW exposure. However, a heat shock control did not mimic exactly the MMW effect, suggesting a slight but specific electromagnetic effect under hyperthermia conditions (34 genes differentially expressed). By RT-PCR, we analyzed the time course of the transcriptomic response and 7 genes have been validated as differentially expressed: ADAMTS6, NOG, IL7R, FADD, JUNB, SNAI2 and HIST1H1A. Our data evidenced a specific electromagnetic effect of MMW, which is associated to the cellular response to hyperthermia. This study raises the question of co-exposures associating radiofrequencies and other environmental sources of cellular stress.

(NE) Habauzit D, Nogue G, Bourbon F, Martin C, Del Vecchio F, Maunoir-Regimbal S, Poyot T, Valente M, Jaoui R, Crouzier D, Le Dréan Y, Debouzy JC. Evaluation of the Effect of Chronic 94 GHz Exposure on Gene Expression in the Skin of Hairless Rats *In Vivo*. Radiat Res. 193(4):351-358, 2020. (VO, LE, GE)

Millimeter waves (MMW) are broadband frequencies that have recently been used in several applications in wireless communications, medical devices and nonlethal weapons [i.e., the nonlethal weapon, Active Denial Systems, (ADS) operating at 94-95 GHz, CW]. However, little information is available on their potential effects on humans. These radio-frequencies are absorbed and stopped by the first layer of the skin. In this study, we evaluated the effects of 94 GHz on the gene expression of skin cells. Two rat populations consisting of 17 young animals and 14 adults were subjected to chronic long-term 94 GHz MMW exposure. Each group of animals was divided into exposed and sham subgroups. The two independent exposure experiments were conducted for 5 months with rats exposed 3 h per day for 3 days per week to an incident power density of 10 mW/cm², which corresponded to twice the ICNIRP limit of occupational exposure for humans. At the end of the experiment, skin explants were collected and RNA was extracted. Then, the modifications to the whole gene expression profile were analyzed with a gene expression microarray. Without modification of the animal's temperature, long-term chronic 94 GHz-MMW exposure did not significantly modify the gene expression of the skin on either the young or adult rats.

(E) Haider T, Knasmueller S, Kundi M, Haider M. Clastogenic effects of radiofrequency Radiations on chromosomes of Tradescantia. Mutat Res 324(1-2):65-68, 1994. (VO, AE, GT)

The clastogenicity of electromagnetic fields (EMF) has so far been studied only under laboratory conditions. We used the Tradescantia-micronucleus (Trad-MCN) bioassay in an in situ experiment to find out whether short-wave electromagnetic fields used for broadcasting (10-21 MHz) may show genotoxic effects. Plant cuttings bearing young flower buds were exposed (30 h) on both sides of a slewable curtain antenna (300/500 kW, 40-170 V/m) and 15 m (90 V/m) and 30 m (70 V/m) distant from a vertical cage antenna (100 kW) as well as at the neighbors living near the broadcasting station (200 m, 1-3 V/m). The exposure at both sides of the slewable curtain antenna was performed simultaneously within cages, one of the Faraday type shielding the field and one non-shielding mesh cage. Laboratory controls were maintained for comparison. Higher MCN frequencies than in laboratory controls were found for all exposure sites in the immediate vicinity of the antennae, where the exposure standards of the electric field strength of the International Radiation Protection Association (IRPA) were exceeded. The results at all exposure sites except one were statistically significant. Since the parallel exposure in a non-shielding and a shielding cage also revealed significant differences in MCN frequencies (the latter showing no significant differences from laboratory controls), the clastogenic effects are clearly attributable to the short-wave radiation from the antennae.

(E) Hamann W, Abou-Sherif S, Thompson S, Hall S. Pulsed radiofrequency applied to dorsal root ganglia causes a selective increase in ATF3 in small neurons. Eur J Pain. 10(2):171-176, 2006. (VO, AE, GE)

BACKGROUND: This is a "proof of concept study" to test the hypothesis that pulsed radiofrequency, PRF, produces cell stress at the primary afferent level without signs of overt thermal damage. We assumed that cell stress would result in impairment of normal function, and used the expression of activating transcription factor 3, ATF3, as an indicator of cellular "stress". METHODS: PRF (20ms of 500-kHz RF pulses, delivered at a rate of 2Hz; maximum temperature 42 degrees C) was delivered either to the sciatic nerve of adult rats in mid thigh, or to the L4 anterior primary ramus just distal to the intervertebral foramen. Controls were sham-operated or L4 axotomised. All tissues were examined 14 days after surgery. The percentage of CGRP- or ATF3-positive DRG neuronal somata was calculated using image analysis software (SigmaScan Pro 4). RESULTS: ATF3 expression was upregulated in L4 DRG neuronal cell bodies, irrespective of their size, after axotomy. It was also upregulated significantly ($p < 0.002$) and selectively, in small and medium calibre L4 DRG neurons, when PRF was applied close to the DRG just distal to the intervertebral foramen. PRF did not produce any obvious cellular changes in the nerve or L4 DRG neurons when applied to the sciatic nerve in mid-thigh. CONCLUSION: PRF has a biological effect, unlikely to be related to overt thermal damage. It appears to be selective in that it targets the group of neurons whose axons are the small diameter C and A delta nociceptive fibres.

(E) Hancı H, Odacı E, Kaya H, Aliyazıcıoğlu Y, Turan İ, Demir S, Çolakoğlu S. The effect of prenatal exposure to 900-MHz electromagnetic field on the 21-old-day rat testicle. Reprod Toxicol. 42:203-209, 2013. (VO, LE, GT, OX, RP)

The aim of this study was to investigate the effect of exposure to a 900-MHz electromagnetic field (EMF) in the prenatal term on the 21-old-day rat testicle. Pregnant rats were divided into control (CG) and EMF (EMFG) groups. EMFG was exposed to 900-MHz EMF during days 13-21 of pregnancy. Newborn CG rats were obtained from the CG and newborn EMFG (NEMFG) rats from the EMFG. Testicles were extracted at postnatal day 21. Lipid peroxidation and DNA oxidation levels, apoptotic index and histopathological damage scores were compared. NEMFG rats exhibited irregularities in seminiferous tubule basal membrane and epithelium, immature germ cells in the lumen, and a decreased diameter in seminiferous tubules and thickness of epithelium. Apoptotic index, lipid peroxidation and DNA oxidation were higher in NEMFG rats than in NCG. 21-day-old rat testicles exposed to 900-MHz EMF in the prenatal term may be adversely affected, and this effect persists after birth.

(NE) Hansteen IL, Lågeide L, Clausen KO, Haugan V, Svendsen M, Eriksen JG, Skiaker R, Hauger E, Vistnes AI, Kure EH. Cytogenetic effects of 18.0 and 16.5 GHz microwave radiation on human lymphocytes in vitro. Anticancer Res 29:2885-2892, 2009a. (VT, AE, GT, WS)

BACKGROUND: There are few cell studies on the direct genotoxic effects of microwave radiation. In this study, cytogenetic effects of microwave radiation alone or in combination with mitomycin C (MMC) were investigated. MATERIALS AND METHODS: Lymphocytes from two smoking and four non-smoking donors were exposed for 53 hours in vitro to 1.0 W/m continuous-wave radiation at 18.0 GHz or 10 W/m pulsed-wave at 16.5 GHz, alone or in combination with MMC. DNA synthesis and repair were inhibited in vitro in some cultures. RESULTS: No synergistic effect was observed in cells exposed to combinations of microwave radiation and in vitro exposure to MMC, or to cells pre-exposed in vivo to tobacco smoke. For the 16.5 GHz pulsed exposure, a non-significant trend consisting of an increase in aberration frequencies with microwave radiation was shown for the DNA synthesis and repair inhibited cultures both with and without MMC. CONCLUSION: Neither 18.0 GHz continuous-wave nor 16.5 GHz pulsed-wave exposure to human lymphocytes in vitro induced statistically significant increases in chromosomal aberration frequencies. 16.5 GHz pulsed-wave exposure requires further documentation before a true negative conclusion can be drawn.

(NE) Hansteen IL, Clausen KO, Haugan V, Svendsen M, Svendsen MV, Eriksen JG, Skiaker R, Hauger E, Lågeide L, Vistnes AI, Kure EH. Cytogenetic effects of exposure to 2.3 GHz radiofrequency radiation on human lymphocytes in vitro. Anticancer Res 29:4323-4330, 2009b. (VT, AE, GT, IX)

BACKGROUND: No previous in vitro studies have tested radio frequency radiation for at least one full cell cycle in culture. The aim was to test if exposure used in mobile phones and wireless network technologies would induce DNA damage in cultured human lymphocytes with and without a known clastogen. MATERIALS AND METHODS: Lymphocytes from six donors were exposed to 2.3 GHz, 10 W/m continuous waves, or 2.3 GHz, 10 W/m pulsed waves (200

Hz pulse frequency, 50% duty cycle). Mitomycin C was added to half of the cultures. DNA synthesis and repair were inhibited in one experiment. RESULTS: No statistically significant differences were observed between control and exposed cultures. A weak trend for more chromosomal damage with the interaction of pulsed fields with mitomycin C compared to a constant field was observed. CONCLUSION: Exposure during the whole cell cycle in inhibited cultures did not result in significant differences in chromosomal aberrations as compared to controls.

(E) Hao Y, Yang X, Chen C, Yuan-Wang, Wang X, Li M, Yu Z. STAT3 signalling pathway is involved in the activation of microglia induced by 2.45 GHz electromagnetic fields. . Int J Radiat Biol 86(1):27-36, 2010.(VT, AE, GE)

Purpose: Microglia activation plays a pivotal role in the initiation and progression of central nervous system (CNS) insult. The aim of the present work was to investigate the activation of microglia and involvement of signal transducer and activator of transcription 3 (STAT3) in microglia activation after 2.45 GHz electromagnetic fields (EMF) exposure. **Materials and methods:** In this study, murine N9 microglial cells were exposed to 2.45 GHz EMF, the protein expressions of STAT3, Janus Tyrosine kinase 1 and 2 (JAK1 and JAK2), phosphor-(Try705) STAT3 and DNA binding activity of STAT3 were examined by Western blot analysis and electrophoresis mobility shift assay (EMSA). Levels of the nitric oxide (NO) derivative nitrite were determined in the culture medium by the Griess reaction. The mRNA expression of tumour necrosis factor alpha (TNF-alpha) and inducible nitric oxide synthase (iNOS) were detected by reverse transcription and polymerase chain reaction (RT-PCR). **Results:** A significant increase of STAT3 DNA-binding ability was noted after exposure. Consistent with this, EMF rapidly induced phosphorylation of STAT3 and activated JAK1 and JAK2. In addition, EMF exposure increased transcription levels of the inflammation-associated genes, iNOS and TNF-alpha, which are reported to contain STAT-binding elements in their promoter region. P6, a JAK inhibitor, reduced induction of iNOS and TNF-alpha, nuclear factor binding activity, and activation of STAT3 in EMF-stimulated microglia. **Conclusion:** These results provide evidence that EMF exposure can initiate the activation of microglia cells and STAT3 signalling involves in EMF-induced microglial activation.

(E) Hassanzadeh-Taheri M, Khalili MA, Mohebati AH, Zardast M, Hosseini M, Palmerini MG, Doostabadi MR. The detrimental effect of cell phone radiation on sperm biological characteristics in normozoospermic. Andrologia 2021 Oct 10;e14257.doi: 10.1111/and.14257. Online ahead of print.(VT, AE, GT, RP)

Radiofrequency electromagnetic radiation emitted from cell phone has harmful effects on some organs of the body, such as the brain, heart, and testes. This study aimed to assess the effects of cell phones on sperm parameters, DNA fragmentation, and apoptosis in normozoospermic. Normal sperm samples were divided into two groups of control and case. The samples from the case were placed for 60 min at a distance of approximately 2.5 cm from the cell phone set in the active antenna position. Control samples were exposed to cell phones without active antennas. All specimens were analysed by World Health Organization criteria. Sperm viability, sperm with chromatin abnormality and maturity, DNA fragmentation, and apoptosis were examined. Viability and motility in the case were significantly lower than the control ($p < .001$, $p = .004$

respectively). The percentage of apoptotic sperms and DNA fragmentation were significantly higher in the case when compared with the control ($p = .031$, $p < .001$ respectively). The other parameters studied such as morphology, chromatin abnormality, and maturity showed no significant difference between the case and control groups. Cell phone waves had a detrimental effect on human sperm's biological features. Therefore, it is recommended to keep the cell phone away from the pelvis as much as possible.

(E) He Q, Sun Y, Zong L, Tong J, Cao Y. Induction of Poly(ADP-ribose) Polymerase in Mouse Bone Marrow Stromal Cells Exposed to 900 MHz Radiofrequency Fields: Preliminary Observations. Biomed Res Int 2016; 2016:4918691. (VT, LE, GE, LI)

Background. Several investigators have reported increased levels of poly(ADP-ribose) polymerase-1 (PARP-1), a nuclear enzyme which plays an important role in the repair of damaged DNA, in cells exposed to extremely low dose ionizing radiation which does not cause measurable DNA damage. Objective. To examine whether exposure of the cells to nonionizing radiofrequency fields (RF) is capable of increasing messenger RNA of PARP-1 and its protein levels in mouse bone marrow stromal cells (BMSCs). Methods. BMSCs were exposed to 900 MHz RF at $120 \mu\text{W}/\text{cm}^2$ power intensity for 3 hours/day for 5 days. PARP-1 mRNA and its protein levels were examined at 0, 0.5, 1, 2, 4, 6, 8, and 10 hours after exposure using RT-PCR and Western blot analyses. Sham-exposed (SH) cells and those exposed to ionizing radiation were used as unexposed and positive control cells. Results. BMSCs exposed to RF showed significantly increased expression of PARP-1 mRNA and its protein levels after exposure to RF while such changes were not observed in SH-exposed cells. Conclusion. Nonionizing RF exposure is capable of inducing PARP-1.

(E) He Q, Zong L, Sun Y, Vijayalaxmi, Prihoda TJ, Tong J, Cao Y. Adaptive response in mouse bone marrow stromal cells exposed to 900 MHz radiofrequency fields: Impact of poly (ADP-ribose) polymerase (PARP). Mutat Res 820:19-25, 2017. (VT, LE, GE, LI, IX)

This study examined whether non-ionizing radiofrequency fields (RF) exposure is capable of inducing poly (ADP-ribose) polymerase-1 (PARP-1) in bone marrow stromal cells (BMSCs) and whether it plays a role in RF-induced adaptive response (AR). Bone marrow stromal cells (BMSCs) were exposed to 900MHz RF at $120\mu\text{W}/\text{cm}^2$ power flux density for 3h/day for 5days and then challenged with a genotoxic dose of 1.5Gy gamma-radiation (GR). Some cells were also treated with 3-aminobenzamide (3-AB, 2mM final concentration), a potent inhibitor of PARP-1. Un-exposed and sham (SH)-exposed control cells as well as positive control cells exposed to gamma radiation (GR) were included in the experiments. The expression of PARP-1 mRNA and its protein levels as well as single strand breaks in the DNA and the kinetics of their repair were evaluated at several times after exposures. The results indicated the following. (a) Cells exposed to RF alone showed significantly increased PARP-1 mRNA expression and its protein levels compared with those exposed to SH- and GR alone. (b) Treatment of RF-exposed cells with 3-AB had diminished such increase in PARP-1. (c) Cells exposed to RF+GR showed significantly decreased genetic damage as well as faster kinetics of repair compared with those

exposed to GR alone. (d) Cells exposed to RF+3-AB+GR showed no such decrease in genetic damage. Thus, the overall data suggested that non-ionizing RF exposure was capable of inducing PARP-1 which has a role in RF-induced AR.

(E) Hekmat A, Saboury AA, Moosavi-Movahedi AA. The toxic effects of mobile phone radiofrequency (940MHz) on the structure of calf thymus DNA. Ecotoxicol Environ Saf. 88:35-41, 2013. (VT, AE, GT, LI)

Currently, the biological effects of nonionizing electromagnetic fields (EMFs) including radiofrequency (RF) radiation have been the subject of numerous experimental and theoretical studies. The aim of this study is to evaluate the possible biological effects of mobile phone RF (940MHz, 15V/m and SAR=40mW/kg) on the structure of calf thymus DNA (ct DNA) immediately after exposure and 2h after 45min exposure via diverse range of spectroscopic instruments. The UV-vis and circular dichroism (CD) experiments depict that mobile phone EMFs can remarkably cause disturbance on ct DNA structure. In addition, the DNA samples, immediately after exposure and 2h after 45min exposure, are relatively thermally unstable compared to the DNA solution, which was placed in a small shielded box (unexposed ct DNA). Furthermore, the exposed DNA samples (the DNA samples that were exposed to 940MHz EMF) have more fluorescence emission when compared with the unexposed DNA, which may have occurred attributable to expansion of the exposed DNA structure. The results of dynamic light scattering (DLS) and zeta potential experiments demonstrate that RF-EMFs lead to increment in the surface charge and size of DNA. The structure of DNA immediately after exposure is not significantly different from the DNA sample 2h after 45min exposure. In other words, the EMF-induced conformational changes are irreversible. Collectively, our results reveal that 940MHz can alter the structure of DNA. The displacement of electrons in DNA by EMFs may lead to conformational changes of DNA and DNA disaggregation. Results from this study could have an important implication on the health effects of RF-EMFs exposure. In addition, this finding could proffer a novel strategy for the development of next generation of mobile phone.

(NE) Herrala M, Kumari K, Koivisto H, Luukkonen J, Tanila H, Naarala J, Juutilainen J. Genotoxicity of intermediate frequency magnetic fields in vitro and in vivo. Environ Res 167:759-769, 2018. (VT, VO, LE, AE, GT)

We assessed genotoxic effects of intermediate frequency magnetic fields (MF) in vitro and in vivo. Rat primary astrocytes were exposed for 24 h to a 7.5 kHz MF at a magnetic flux density of 30 or 300 μ T. Male C57BL/6 J mice were exposed continuously for 5 weeks to a 7.5 kHz MF at 12 or 120 μ T, and blood samples were collected for the genotoxicity assays. To evaluate possible co-genotoxicity, the in vitro experiments included combined exposure with menadione (an agent that induces mitochondrial superoxide production and DNA damage) and methyl methanesulfonate (an alkylating agent). DNA damage and DNA repair (in vitro) were measured using the alkaline Comet assay and formation of micronuclei was assessed microscopically (in vivo) or using flow cytometry (in vitro). The results did not support genotoxicity or co-genotoxicity of 7.5 kHz MFs at magnetic flux densities up to 300 μ T in vitro or in vivo. On the contrary, there was some evidence that exposure to 7.5 kHz MFs might reduce the level of genetic damage. Strongest indication of any biological effects was obtained from measurements

of relative cell number, which was significantly and consistently increased after MF exposure in all in vitro experiments. Health implications of this finding are unclear, but it suggests that 7.5 kHz MFs may stimulate cell proliferation or suppress cell death.

(NE) Hintzsche H, Stopper H. Micronucleus frequency in buccal mucosa cells of mobile phone users. Toxicol Lett. 193(1):124-130, 2010. (HU, LE, GT)

Mobile phones are being used extensively throughout the world, with more than four billion accounts existing in 2009. This technology applies electromagnetic radiation in the microwave range. Health effects of this radiation have been subject of debate for a long time, both within the scientific community and within the general public. This study investigated the effect of mobile phone use on genomic instability of the human oral cavity's mucosa cells. 131 Individuals donated buccal mucosa cells extracted by slightly scraping the oral cavity with a cotton swab. Every participant filled out a questionnaire about mobile phone use including duration of weekly use, overall period of exposure and headset usage. 13 Individuals did not use mobile phones at all, 85 reported using the mobile phone for three hours per week or less, and 33 reported use of more than three hours per week. Additionally, information on age, gender, body weight, smoking status, medication and nutrition was retrieved. For staining of the cells a procedure using alpha-tubulin-antibody and chromomycin A(3) was applied. Micronuclei and other markers were evaluated in 1000 cells per individual at the microscope. A second scorer counted another 1000 cells, resulting in 2000 analyzed cells per individual. Mobile phone use did not lead to a significantly increased frequency of micronuclei.

(NE) Hintzsche H, Jastrow C, Kleine-Ostmann T, Schrader T, Stopper H. 900 MHz radiation does not induce micronucleus formation in different cell types. Mutagenesis. 27(4):477-483, 2012a. (VT, AE, GT)

The exposure of the population to non-ionising electromagnetic radiation is still increasing, mainly due to mobile communication. Whether low-intensity electromagnetic fields can cause other effects apart from heating has been a subject of debate. One of the effects, which were proposed to be caused by mobile phone radiation, is the occurrence of mitotic disturbances. The aim of this study was to investigate possible consequences of these mitotic disturbances as manifest genomic damage, i.e. micronucleus induction. Cells were irradiated at a frequency of 900 MHz, which is located in one of the main frequency bands applied for mobile communication. Two cell types were used, HaCaT cells as human cells and A(L) cells (human-hamster hybrid cells), in which mitotic disturbances had been reported to occur. After different post-exposure incubation periods, cells were fixed and micronucleus frequencies were evaluated. Both cell types did not show any genomic damage after exposure. To adapt the protocol for the micronucleus test into the direction of the protocol for mitotic disturbances, the post-exposure incubation period was reduced and exposure time was extended to one cell cycle length. This did not result in any increase of the genomic damage. In conclusion, micronucleus induction was not observed as a consequence of exposure to non-ionising radiation, even though this agent was reported to cause mitotic disturbances under similar experimental conditions.

(NE) Hintzsche H, Jastrow C, Kleine-Ostmann T, Kärst U, Schrader T, Stopper H. Terahertz electromagnetic fields (0.106 THz) do not induce manifest genomic damage in vitro. PLoS One 2012b;7(9):e46397. (VT, AE, GT)

Terahertz electromagnetic fields are non-ionizing electromagnetic fields in the frequency range from 0.1 to 10 THz. Potential applications of these electromagnetic fields include the whole body scanners, which currently apply millimeter waves just below the terahertz range, but future scanners will use higher frequencies in the terahertz range. These and other applications will bring along human exposure to these fields. Up to now, only a limited number of investigations on biological effects of terahertz electromagnetic fields have been performed. Therefore, research is strongly needed to enable reliable risk assessment. Cells were exposed for 2 h, 8 h, and 24 h with different power intensities ranging from 0.04 mW/cm² to 2 mW/cm², representing levels below, at, and above current safety limits. Genomic damage on the chromosomal level was measured as micronucleus formation. DNA strand breaks and alkali-labile sites were quantified with the comet assay. No DNA strand breaks or alkali-labile sites were observed as a consequence of exposure to terahertz electromagnetic fields in the comet assay. The fields did not cause chromosomal damage in the form of micronucleus induction.

(NE) Hirose H, Sakuma N, Kaji N, Suhara T, Sekijima M, Nojima T, Miyakoshi J. Phosphorylation and gene expression of p53 are not affected in human cells exposed to 2.1425 GHz band CW or W-CDMA modulated radiation allocated to mobile radio base stations. Bioelectromagnetics 27:494-504, 2006. (VT, AE, GE)

A large-scale in vitro study focusing on low-level radiofrequency (RF) fields from mobile radio base stations employing the International Mobile Telecommunication 2000 (IMT-2000) cellular system was conducted to test the hypothesis that modulated RF fields induce apoptosis or other cellular stress response that activate p53 or the p53-signaling pathway. First, we evaluated the response of human cells to microwave exposure at a specific absorption rate (SAR) of 80 mW/kg, which corresponds to the limit of the average whole-body SAR for general public exposure defined as a basic restriction by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines. Second, we investigated whether continuous wave (CW) and wideband code division multiple access (W-CDMA) modulated signal RF fields at 2.1425 GHz induced apoptosis or any signs of stress. Human glioblastoma A172 cells were exposed to W-CDMA radiation at SARs of 80, 250, and 800 mW/kg, and CW radiation at 80 mW/kg for 24 or 48 h. Human IMR-90 fibroblasts from fetal lungs were exposed to both W-CDMA and CW radiation at a SAR of 80 mW/kg for 28 h. Under the RF field exposure conditions described above, no significant differences in the percentage of apoptotic cells were observed between the test groups exposed to RF signals and the sham-exposed negative controls, as evaluated by the Annexin V affinity assay. No significant differences in expression levels of phosphorylated p53 at serine 15 or total p53 were observed between the test groups and the negative controls by the bead-based multiplex assay. Moreover, microarray hybridization and real-time RT-PCR analysis showed no noticeable differences in gene expression of the subsequent downstream targets of p53 signaling involved in apoptosis between the test groups and the negative controls. Our results confirm that exposure to low-level RF signals up to 800 mW/kg does not induce p53-dependent apoptosis, DNA damage, or other stress response in human cells.

(NE) Hirose H, Sakuma N, Kaji N, Nakayama K, Inoue K, Sekijima M, Nojima T, Miyakoshi J. Mobile phone base station-emitted radiation does not induce phosphorylation of Hsp27. Bioelectromagnetics 28:99-108, 2007. (VT, AE, GE)

An in vitro study focusing on the effects of low-level radiofrequency (RF) fields from mobile radio base stations employing the International Mobile Telecommunication 2000 (IMT-2000) cellular system was conducted to test the hypothesis that modulated RF fields act to induce phosphorylation and overexpression of heat shock protein hsp27. First, we evaluated the responses of human cells to microwave exposure at a specific absorption rate (SAR) of 80 mW/kg, which corresponds to the limit of the average whole-body SAR for general public exposure defined as a basic restriction in the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines. Second, we investigated whether continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) modulated signal RF fields at 2.1425 GHz induced activation or gene expression of hsp27 and other heat shock proteins (hsps). Human glioblastoma A172 cells were exposed to W-CDMA radiation at SARs of 80 and 800 mW/kg for 2-48 h, and CW radiation at 80 mW/kg for 24 h. Human IMR-90 fibroblasts from fetal lungs were exposed to W-CDMA at 80 and 800 mW/kg for 2 or 28 h, and CW at 80 mW/kg for 28 h. Under the RF field exposure conditions described above, no significant differences in the expression levels of phosphorylated hsp27 at serine 82 (hsp27[pS82]) were observed between the test groups exposed to W-CDMA or CW signal and the sham-exposed negative controls, as evaluated immediately after the exposure periods by bead-based multiplex assays. Moreover, no noticeable differences in the gene expression of hsps were observed between the test groups and the negative controls by DNA Chip analysis. Our results confirm that exposure to low-level RF field up to 800 mW/kg does not induce phosphorylation of hsp27 or expression of hsp gene family.

(NE) Hook GJ, Zhang P, Lagroye I, Li L, Higashikubo R, Moros EG, Straube WL, Pickard WF, Baty JD, Roti Roti JL. Measurement of DNA damage and apoptosis in molt-4 cells after in vitro exposure to radiofrequency radiation. Radiat Res. 161(2): 193-200, 2004. (VT, AE, LI, GT)

To determine whether exposure to radiofrequency (RF) radiation can induce DNA damage or apoptosis, Molt-4 T lymphoblastoid cells were exposed with RF fields at frequencies and modulations of the type used by wireless communication devices. Four types of frequency/modulation forms were studied: 847.74 MHz code-division multiple-access (CDMA), 835.62 MHz frequency-division multiple-access (FDMA), 813.56 MHz iDEN(R) (iDEN), and 836.55 MHz time-division multiple-access (TDMA). Exponentially growing cells were exposed to RF radiation for periods up to 24 h using a radial transmission line (RTL) exposure system. The specific absorption rates used were 3.2 W/kg for CDMA and FDMA, 2.4 or 24 mW/kg for iDEN, and 2.6 or 26 mW/kg for TDMA. The temperature in the RTLs was maintained at 37 degrees C +/- 0.3 degrees C. DNA damage was measured using the single-cell gel electrophoresis assay. The annexin V affinity assay was used to detect apoptosis. No statistically significant difference in the level of DNA damage or apoptosis was observed between sham-treated cells and cells exposed to RF radiation for any frequency, modulation or exposure time. Our results show that exposure of Molt-4 cells to CDMA, FDMA, iDEN or TDMA modulated RF radiation does not

induce alterations in level of DNA damage or induce apoptosis.

(NE) Hou Q, Wang M, Wu S, Ma X, An G, Liu H, Xie F. Oxidative changes and apoptosis induced by 1800-MHz electromagnetic radiation in NIH/3T3 cells. Electromagn Biol Med 2015 Mar;34(1):85-92. (VT, AE, GT)

To investigate the potential adverse effects of mobile phone radiation, we studied reactive oxygen species (ROS), DNA damage and apoptosis in mouse embryonic fibroblasts (NIH/3T3) after intermittent exposure (5 min on/10 min off, for various durations from 0.5 to 8 h) to an 1800-MHz GSM-talk mode electromagnetic radiation (EMR) at an average specific absorption rate of 2 W/kg. A 2',7'-dichlorofluorescein diacetate fluorescence probe was used to detect intracellular ROS levels, immunofluorescence was used to detect γ H2AX foci as a marker for DNA damage, and flow cytometry was used to measure apoptosis. Our results showed a significant increase in intracellular ROS levels after EMR exposure and it reached the highest level at an exposure time of 1 h ($p < 0.05$) followed by a slight decrease when the exposure continued for as long as 8 h. No significant effect on the number of γ H2AX was detected after EMR exposure. The percentage of late-apoptotic cells in the EMR-exposed group was significantly higher than that in the sham-exposed groups ($p < 0.05$). These results indicate that an 1800-MHz EMR enhances ROS formation and promotes apoptosis in NIH/3T3 cells.

(E) Houston BJ, Nixon B, McEwan KE, Martin JH, King BV, Aitken RJ, De Iuliis GN. Whole-body exposures to radiofrequency-electromagnetic energy can cause DNA damage in mouse spermatozoa via an oxidative mechanism. Sci Rep. 9(1):17478, 2019. (VO, LE, GT, RP, OX)

Artificially generated radiofrequency-electromagnetic energy (RF-EME) is now ubiquitous in our environment owing to the utilization of mobile phone and Wi-Fi based communication devices. While several studies have revealed that RF-EME is capable of eliciting biological stress, particularly in the context of the male reproductive system, the mechanistic basis of this biophysical interaction remains largely unresolved. To extend these studies, here we exposed unrestrained male mice to RF-EME generated via a dedicated waveguide (905 MHz, 2.2 W/kg) for 12 h per day for a period of 1, 3 or 5 weeks. The testes of exposed mice exhibited no evidence of gross histological change or elevated stress, irrespective of the RF-EME exposure regimen. By contrast, 5 weeks of RF-EME exposure adversely impacted the vitality and motility profiles of mature epididymal spermatozoa. These spermatozoa also experienced increased mitochondrial generation of reactive oxygen species after 1 week of exposure, with elevated DNA oxidation and fragmentation across all exposure periods. Notwithstanding these lesions, RF-EME exposure did not impair the fertilization competence of spermatozoa nor their ability to support early embryonic development. This study supports the utility of male germ cells as sensitive tools with which to assess the biological impacts of whole-body RF-EME exposure.

(NE) Huang TQ, Lee MS, Oh E, Zhang BT, Seo JS, Park WY. Molecular responses of Jurkat T-cells to 1763 MHz radiofrequency radiation. Int J Radiat Biol 84:734-741, 2008a. (VT, AE, GT, GE)

PURPOSE: The biological effects of exposure to mobile phone emitted radiofrequency (RF) radiation are the subject of intense study, yet the hypothesis that RF exposure is a potential health hazard remains controversial. In this paper, we monitored cellular and molecular changes in Jurkat human T lymphoma cells after irradiating with 1763 MHz RF radiation to understand the effect on RF radiation in immune cells. MATERIALS AND METHODS: Jurkat T-cells were exposed to RF radiation to assess the effects on cell proliferation, cell cycle progression, DNA damage and gene expression. Jurkat cells were exposed to 1763 MHz RF radiation at 10 W/kg specific absorption rate (SAR) and compared to sham exposed cells. RESULTS: RF exposure did not produce significant changes in cell numbers, cell cycle distributions, or levels of DNA damage. In genome-wide analysis of gene expressions, there were no genes changed more than two-fold upon RF-radiation while ten genes change to 1.3 approximately 1.8-fold. Among ten genes, two cytokine receptor genes such as chemokine (C-X-C motif) receptor 3 (CXCR3) and interleukin 1 receptor, type II (IL1R2) were down-regulated upon RF radiation, but they were not directly related to cell proliferation or DNA damage responses. CONCLUSION: These results indicate that the alterations in cell proliferation, cell cycle progression, DNA integrity or global gene expression was not detected upon 1763 MHz RF radiation under 10 W/kg SAR for 24 h to Jurkat T cells.

(NE) Huang TQ, Lee MS, Oh EH, Kalinec F, Zhang BT, Seo JS, Park WY. Characterization of biological effect of 1763 MHz radiofrequency exposure on auditory hair cells. Int J Radiat Biol 84:909-915, 2008b. (VT, AE, GT, GE)

Purpose: Radiofrequency (RF) exposure at the frequency of mobile phones has been reported not to induce cellular damage in in vitro and in vivo models. We chose HEI-OC1 immortalized mouse auditory hair cells to characterize the cellular response to 1763 MHz RF exposure, because auditory cells could be exposed to mobile phone frequencies. Materials and methods: Cells were exposed to 1763 MHz RF at a 20 W/kg specific absorption rate (SAR) in a code division multiple access (CDMA) exposure chamber for 24 and 48 h to check for changes in cell cycle, DNA damage, stress response, and gene expression. Results: Neither of cell cycle changes nor DNA damage was detected in RF-exposed cells. The expression of heat shock proteins (HSP) and the phosphorylation of mitogen-activated protein kinases (MAPK) did not change, either. We tried to identify any alteration in gene expression using microarrays. Using the Applied Biosystems 1700 full genome expression mouse microarray, we found that only 29 genes (0.09% of total genes examined) were changed by more than 1.5-fold on RF exposure. Conclusion: From these results, we could not find any evidence of the induction of cellular responses, including cell cycle distribution, DNA damage, stress response and gene expression, after 1763 MHz RF exposure at an SAR of 20 W/kg in HEI-OC1 auditory hair cells.

(E) Ibitayo AO, Afolabi OB, Akinyemi AJ, Ojiezeh TI, Adekoya KO, Ojewunmi OO. RAPD Profiling, DNA Fragmentation, and Histomorphometric Examination in Brains of Wistar Rats Exposed to Indoor 2.5 Ghz Wi-Fi Devices Radiation. Biomed Res Int. 2017;2017:8653286. (VO, LE, GT)

The advent of Wi-Fi connected high technology devices in executing day-to-day activities is fast evolving especially in developing countries of the world and hence the need to assess its safety among others. The present study was conducted to investigate the injurious effect of radiofrequency emissions from installed Wi-Fi devices in brains of young male rats. Animals were divided into four equal groups; group 1 served as control while groups 2, 3, and 4 were exposed to 2.5 Ghz at intervals of 30, 45, and 60 consecutive days with free access to food and water ad libitum. Alterations in harvested brain tissues were confirmed by histopathological analyses which showed vascular congestion and DNA damage in the brain was assayed using agarose gel electrophoresis. Histomorphometry analyses of their brain tissues showed perivascular congestion and tissue damage as well.

(E) Jeong YJ, Kang GY, Kwon JH, Choi HD, Pack JK, Kim N, Lee YS, Lee HJ. 1950 MHz Electromagnetic Fields Ameliorate A β Pathology in Alzheimer's Disease Mice. *Curr Alzheimer Res.* 12(5):481-492, 2015. (VO, LE, GE)

The involvement of radiofrequency electromagnetic fields (RF-EMF) in the neurodegenerative disease, especially Alzheimer's disease (AD), has received wide consideration, however, outcomes from several researches have not shown consistency. In this study, we determined whether RF-EMF influenced AD pathology in vivo using Tg-5xFAD mice as a model of AD-like amyloid β (A β) pathology. The transgenic (Tg)-5xFAD and wild type (WT) mice were chronically exposed to RF-EMF for 8 months (1950 MHz, SAR 5W/kg, 2 hrs/day, 5 days/week). Notably, chronic RFEMF exposure significantly reduced not only A β plaques, APP, and APP carboxyl-terminal fragments (CTFs) in whole brain including hippocampus and entorhinal cortex but also the ratio of A β 42 and A β 40 peptide in the hippocampus of Tg-5xFAD mice. We also found that parenchymal expression of β -amyloid precursor protein cleaving enzyme 1 (BACE1) and neuroinflammation were inhibited by RF-EMF exposure in Tg-5xFAD. In addition, RF-EMF was shown to rescue memory impairment in Tg-5xFAD. Moreover, gene profiling from microarray data using hippocampus of WT and Tg- 5xFAD following RF-EMF exposure revealed that 5 genes (Tshz2, Gm12695, St3gal1, Isx and Tll1), which are involved in A β , are significantly altered in Tg-5xFAD mice, exhibiting different responses to RF-EMF in WT or Tg-5xFAD mice; RF-EMF exposure in WT mice showed similar patterns to control Tg-5xFAD mice, however, RF-EMF exposure in Tg- 5xFAD mice showed opposite expression patterns. These findings indicate that chronic RF-EMF exposure directly affects A β pathology in AD but not in normal brain. Therefore, RF-EMF has preventive effects against AD-like pathology in advanced AD mice with a high expression of A β , which suggests that RF-EMF can have a beneficial influence on AD.

(NE) Jeong YJ, Son Y, Han NK, Choi HD, Pack JK, Kim N, Lee YS, Lee HJ. Impact of Long-Term RF-EMF on Oxidative Stress and Neuroinflammation in Aging Brains of C57BL/6 Mice. *Int J Mol Sci.* 19(7): 2103, 2018. (VO, CE, GT, OX)

The expansion of mobile phone use has raised questions regarding the possible biological effects of radiofrequency electromagnetic field (RF-EMF) exposure on oxidative stress and brain inflammation. Despite accumulative exposure of humans to radiofrequency electromagnetic fields (RF-EMFs) from mobile phones, their long-term effects on oxidative stress and neuroinflammation in the aging brain have not been studied. In the present study, middle-aged C57BL/6 mice (aged 14 months) were exposed to 1950 MHz electromagnetic fields for 8 months (specific absorption rate (SAR) 5 W/kg, 2 h/day, 5 d/week). Compared with those in the young group, levels of protein (3-nitro-tyrosine) and lipid (4-hydroxy-2-nonenal) oxidative damage markers were significantly increased in the brains of aged mice. In addition, levels of markers for DNA damage (8-hydroxy-2'-deoxyguanosine, p53, p21, γ H2AX, and Bax), apoptosis (cleaved caspase-3 and cleaved poly(ADP-ribose) polymerase 1 (PARP-1)), astrocyte (GFAP), and microglia (Iba-1) were significantly elevated in the brains of aged mice. However, long-term RF-EMF exposure did not change the levels of oxidative stress, DNA damage, apoptosis, astrocyte, or microglia markers in the aged mouse brains. Moreover, long-term RF-EMF exposure did not alter locomotor activity in aged mice. Therefore, these findings indicate that long-term exposure to RF-EMF did not influence age-induced oxidative stress or neuroinflammation in C57BL/6 mice.

(E) Jeong, Y.J., Son, Y., Choi, H-D., Kim, N., Lee, Y-S., Kom, Y-G., Lee, H-J (2020). Behavioral changes and gene profile alterations after chronic 1,950-MHz radiofrequency exposure: An observation in C57BL/6 mice. Brain Behav. 2020 10(11):e01815. (VO, LE, GE)

Introduction: Due to public concerns about deleterious biological consequences of radiofrequency electromagnetic fields (RF-EMF), the potential effects of RF-EMF on the central nervous system have received wide consideration. Methods: Here, two groups of C57BL/6 mice, aged 2 and 12 months, were exposed to 1,950-MHz RF-EMF at a specific absorption rate of 5.0 W/kg for chronic periods (2 hr/day and 5 days/week for 8 months). Behavioral changes were then assessed in the mice at 10 months (sham- or RF-10M) and 20 months (sham- or RF-20M), on the open-field test, the Y-maze test, and an object recognition memory task, while biological effects were analyzed via microarray gene profiling of the hippocampus. Results: Open-field test results showed a decrease in the time duration spent at the center while there was a decrease in enhanced memory shown by the Y-maze test and the novel object recognition test in the RF-20M mice, compared to sham-exposed mice, but no significant changes in the RF-10M group. Based on a 2-fold change cutoff, the microarray data revealed that 15 genes, which are listed as being involved in neurogenesis on Gene Ontology, were altered in both groups. Quantitative real-time PCR for validation showed increased expression of Epha8 and Wnt6 in the hippocampi of RF-20M group mice, although 13 additional genes showed no significant changes following RF-EMF exposure. Conclusion: Therefore, cognitive enhancement following chronic exposure for 8 months to RF-EMF from middle age may be associated with increases in neurogenesis-related signals in the hippocampus of C57BL/6 mice.

(E) Ji S, Oh E, Sul D, Choi JW, Park H, Lee E. DNA Damage of lymphocytes in volunteers after 4 hours use of mobile phone. J Prev Med Public Health. 37(4):373-380, 2004. (HU, AE, GT)

OBJECTIVES: There has been gradually increasing concern about the adverse health effects of electromagnetic radiation originating from cell phones which are widely used in modern life. Cell phone radiation may affect human health by increasing free radicals of human blood cells. This study has been designed to identify DNA damage of blood cells by electromagnetic radiation caused by cell phone use. **METHODS:** This study investigated the health effect of acute exposure to commercially available cell phones on certain parameters such as an indicator of DNA damage for 14 healthy adult volunteers. Each volunteer during the experiment talked over the cell phone with the keypad facing the right side of the face for 4 hours. The single cell gel electrophoresis assay (Comet assay), which is very sensitive in detecting the presence of DNA strand-breaks and alkali-labile damage in individual cells, was used to assess peripheral blood cells (T-cells, B-cells, granulocytes) from volunteers before and after exposure to cell phone radiation. The parameters of Comet assay measured were Olive Tail Moment and Tail DNA %. **RESULTS:** The Olive Tail Moment of B-cells and granulocytes and Tail DNA % of B-cells and granulocytes were increased by a statistically significant extent after 4- hour use of a cell phone compared with controls. **CONCLUSIONS:** It is concluded that cell phone radiation caused the DNA damage during the 4 hours of experimental condition. Nonetheless, this study suggested that cell phone use may increase DNA damage by electromagnetic radiation and other contributing factors.

(E) Ji Y, He Q, Sun Y, Tong J, Cao Y. Adaptive response in mouse bone-marrow stromal cells exposed to 900-MHz radiofrequency fields: Gamma-radiation-induced DNA strand breaks and repair. J Toxicol Environ Health A. 79(9-10):419-426, 2016. (VT, LE, LI, GT, IX) (protective effect on DN damage)

The aim of this study was to examine whether radiofrequency field (RF) preexposure induced adaptive responses (AR) in mouse bone-marrow stromal cells (BMSC) and the mechanisms underlying the observed findings. Cells were preexposed to 900-MHz radiofrequency fields (RF) at 120 $\mu\text{W}/\text{cm}^2$ power intensity for 4 h/d for 5 d. Some cells were subjected to 1.5 Gy γ -radiation (GR) 4 h following the last RF exposure. The intensity of strand breaks in the DNA was assessed immediately at 4 h. Subsequently, some BMSC were examined at 30, 60, 90, or 120 min utilizing the alkaline comet assay and γ -H2AX foci technique. Data showed no significant differences in number and intensity of strand breaks in DNA between RF-exposed and control cells. A significant increase in number and intensity of DNA strand breaks was noted in cells exposed to GR exposure alone. RF followed by GR exposure significantly decreased number of strand breaks and resulted in faster kinetics of repair of DNA strand breaks compared to GR alone. Thus, data suggest that RF preexposure protected cells from damage induced by GR. Evidence indicates that in RF-mediated AR more rapid repair kinetics occurs under conditions of GR-induced damage, which may be attributed to diminished DNA strand breakage.

(E) Jiang B, Nie J, Zhou Z, Zhang J, Tong J, Cao Y. Adaptive response in mice exposed to 900 MHz radiofrequency fields: primary DNA damage. PLoS One. 7(2):e32040, 2012. (VO, LE, GT, IA)

The phenomenon of adaptive response (AR) in animal and human cells exposed to ionizing radiation is well documented in scientific literature. We have examined whether such AR could be induced in mice exposed to non-ionizing radiofrequency fields (RF) used for wireless communications. Mice were pre-exposed to 900 MHz RF at 120 $\mu\text{W}/\text{cm}^2$ power density for 4 hours/day for 1, 3, 5, 7 and 14 days and then subjected to an acute dose of 3 Gy γ -radiation. The primary DNA damage in the form of alkali labile base damage and single strand breaks in the DNA of peripheral blood leukocytes was determined using the alkaline comet assay. The results indicated that the extent of damage in mice which were pre-exposed to RF for 1 day and then subjected to γ -radiation was similar and not significantly different from those exposed to γ -radiation alone. However, mice which were pre-exposed to RF for 3, 5, 7 and 14 days showed progressively decreased damage and was significantly different from those exposed to γ -radiation alone. Thus, the data indicated that RF pre-exposure is capable of inducing AR and suggested that the pre-exposure for more than 4 hours for 1 day is necessary to elicit such AR.

(E) Jiang B, Zong C, Zhao H, Ji Y, Tong J, Cao Y. Induction of adaptive response in mice exposed to 900MHz radiofrequency fields: application of micronucleus assay. Mutat Res 2013 Mar 18;751(2):127-129. (VO, LE, GT, IX)

Adult male ICR mice were pre-exposed to non-ionizing radiofrequency fields (RF), 900MHz at 120 $\mu\text{W}/\text{cm}^2$ power density for 4h/day for 7 days (adaptation dose, AD) and then subjected to an acute whole body dose of 3Gy γ -radiation (challenge dose, CD). The classical micronucleus (MN) assay was used to determine the extent of genotoxicity in immature erythrocytes in peripheral blood and bone marrow. The data obtained in mice exposed to AD+CD were compared with those exposed to CD alone. The results indicated that in both tissues, the MN indices were similar in un-exposed controls and those exposed to AD alone while a significantly increased MN frequency was observed in mice exposed to CD alone. Exposure of mice to AD+CD resulted in a significant decrease in MN indices compared to those exposed to CD alone. Thus, the data suggested that pre-exposure of mice to non-ionizing RF is capable of 'protecting' the erythrocytes in the blood and bone marrow from genotoxic effects of subsequent γ -radiation. Such protective phenomenon is generally described as 'adaptive response' (AR) and is well documented in human and animal cells which were pre-exposed to very low doses of ionizing radiation. It is interesting to observe AR being induced by non-ionizing RF.

***(E) Jin H, Kim K, Park GY, Kim M, Lee H-J, Jeon S, Kim JH, Kim HR, Lim KM, Lee YS. The Protective Effects of EMF-LTE against DNA Double-Strand Break Damage In Vitro and In Vivo. Int J Mol Sci 2021 May 12;22(10):5134. (VT, VO, LE, AE, GT, IX)**

With the rapid growth of the wireless communication industry, humans are extensively exposed to electromagnetic fields (EMF) comprised of radiofrequency (RF). The skin is considered the primary target of EMFs given its outermost location. Recent evidence suggests that extremely low frequency (ELF)-EMF can improve the efficacy of DNA repair in human cell-lines. However, the effects of EMF-RF on DNA damage remain unknown. Here, we investigated the impact of EMF-long term evolution (LTE, 1.762 GHz, 8 W/kg) irradiation on DNA double-

strand break (DSB) using the murine melanoma cell line B16 and the human keratinocyte cell line HaCaT. EMF-LTE exposure alone did not affect cell viability or induce apoptosis or necrosis. In addition, DNA DSB damage, as determined by the neutral comet assay, was not induced by EMF-LTE irradiation. Of note, EMF-LTE exposure can attenuate the DNA DSB damage induced by physical and chemical DNA damaging agents (such as ionizing radiation (IR, 10 Gy) in HaCaT and B16 cells and bleomycin (BLM, 3 μ M) in HaCaT cells and a human melanoma cell line MNT-1), suggesting that EMF-LTE promotes the repair of DNA DSB damage. The protective effect of EMF-LTE against DNA damage was further confirmed by attenuation of the DNA damage marker γ -H2AX after exposure to EMF-LTE in HaCaT and B16 cells. Most importantly, irradiation of EMF-LTE (1.76 GHz, 6 W/kg, 8 h/day) on mice in vivo for 4 weeks reduced the γ -H2AX level in the skin tissue, further supporting the protective effects of EMF-LTE against DNA DSB damage. Furthermore, p53, the master tumor-suppressor gene, was commonly upregulated by EMF-LTE irradiation in B16 and HaCaT cells. This finding suggests that p53 plays a role in the protective effect of EMF-LTE against DNA DSBs. Collectively, these results demonstrated that EMF-LTE might have a protective effect against DNA DSB damage in the skin, although further studies are necessary to understand its impact on human health.

(NE) Juutilainen J, Heikkinen P, Soikkeli H, Mäki-Paakkanen J. Micronucleus frequency in erythrocytes of mice after long-term exposure to radiofrequency radiation. *Int J Radiat Biol.* 83(4):213-220, 2007. (VO, LE, GT)

PURPOSE: The aim of the study was to investigate genotoxicity of long-term exposure to radiofrequency (RF) electromagnetic fields by measuring micronuclei in erythrocytes. The blood samples were collected in two animal studies evaluating possible cocarcinogenic effects of RF fields. **METHODS:** In study A, female CBA/S mice were exposed for 78 weeks (1.5 h/d, 5 d/week) to either a continuous 902.5 MHz signal similar to that emitted by analog NMT (Nordic Mobile Telephone) phones at a whole-body specific absorption rate (SAR) of 1.5 W/kg, or to a pulsed 902.4 MHz signal similar to that of digital GSM (Global System for Mobile Communications) phones at 0.35 W/kg. A third group was sham-exposed, and a fourth group served as cage controls. All but the cage control animals were exposed to 4 Gy of x-rays during three first weeks of the experiment. In study B, female transgenic mice (line K2) and their nontransgenic littermates were exposed for 52 weeks (1.5 h/d, 5 d/week). Two digital mobile phone signals, GSM and DAMPS (Digital Advanced Mobile Phone System), were used at 0.5 W/kg. All but the cage-control animals were exposed 3 times per week to an ultraviolet radiation dose of 1.2 MED (minimum erythema dose). **RESULTS AND CONCLUSIONS:** The results did not show any effects of RF fields on micronucleus frequency in polychromatic or normochromatic erythrocytes. The results were consistent in two mouse strains (and in a transgenic variant of the second strain), after 52 or 78 weeks of exposure, at three SAR levels relevant to human exposure from mobile phones, and for three different mobile signals.

(E) Kao HK, Li Q, Flynn B, Qiao X, Ruberti JW, Murphy GF, Guo L. Collagen synthesis modulated in wounds treated by pulsed radiofrequency energy. *Plast Reconstr Surg.* 131(4):490e-498e, 2013.(VT, AE, GE)

BACKGROUND: Chronic wounds are biochemically complex and are associated with insufficient cell proliferation, angiogenesis, and extracellular matrix remodeling. The mechanisms by which pulsed radiofrequency energy modulates wound healing are still unclear. **METHODS:** Db/db mice were wounded and exposed to pulsed radiofrequency energy. Gross closure, cell proliferation, and morphometric analysis of CD31-stained wound cross-sections were assessed. The mRNA expression of profibrotic factors (transforming growth factor- β and platelet-derived growth factor-A), angiogenic factors (vascular endothelial growth factor and basic fibroblast growth factor), and extracellular matrix components (collagen I and α -smooth muscle actin) were evaluated by quantitative reverse-transcriptase polymerase chain reaction. Collagen protein level of the wound was determined by Western blot analysis. To test the effect of pulsed radiofrequency energy on cell movement in wound healing, cell migration was monitored in monolayer dermal fibroblast cultures. The degree of collagen alignment and gelation time was quantitatively assessed using image analysis techniques. **RESULTS:** Pulsed radiofrequency energy-treated wounds were characterized by dermal cell proliferation and increased collagen synthesis. By contrast, the CD31 density and the mRNA expression of vascular endothelial growth factor and basic fibroblast growth factor showed no significant difference between the pulsed radiofrequency energy-treated wounds and the sham group. The pulsed radiofrequency energy-treated dermal fibroblast cultures expressed a significantly longer gelation time compared with the sham-exposed cultures. **CONCLUSIONS:** Exposing wounds to pulsed radiofrequency accelerated wound healing in this diabetic mouse model by means of significantly increasing dermal cell proliferation and collagen synthesis. A cellular mechanism behind these observations has been proposed.

(E) Karaca E, Durmaz B, Altug H, Yildiz T, Guducu C, Irgi M, Koksall MG, Ozkinay F, Gunduz C, Cogulu O. The genotoxic effect of radiofrequency waves on mouse brain. J Neurooncol 106:53-58, 2012. (VT, LE, GT, GE)

Concerns about the health effects of radiofrequency (RF) waves have been raised because of the gradual increase in usage of cell phones, and there are scientific questions and debates about the safety of those instruments in daily life. The aim of this study is to evaluate the genotoxic effects of RF waves in an experimental brain cell culture model. Brain cell cultures of the mice were exposed to 10.715 GHz with specific absorption rate (SAR) 0.725 W/kg signals for 6 h in 3 days at 25°C to check for the changes in the micronucleus (MNi) assay and in the expression of 11 proapoptotic and antiapoptotic genes. It was found that MNi rate increased 11-fold and STAT3 expression decreased 7-fold in the cell cultures which were exposed to RF. Cell phones which spread RF may damage DNA and change gene expression in brain cells.

***(E) Keleş AI, Süt BB Histopathological and epigenetic alterations in the spinal cord due to prenatal electromagnetic field exposure: An H3K27me3-related mechanism. Toxicol Ind Health 2021 Feb 23;748233721996947. (VO, LE, DE, GP, EP)**

Neural system development is one of the most important stages of embryogenesis. Perturbations in this crucial process due to genetic and environmental risk factors cause neural tube defects and other central nervous system diseases. We investigated the effects of prenatal exposure to 900-MHz electromagnetic field (EMF) on the spinal cord. Pregnant rats were exposed to 900-MHz

EMF for 1 h/day from E13.5 until birth. Six pups from the control and EMF groups were sacrificed at postnatal day 32, and the upper thoracic region of the spine was removed and processed for histological procedures. For histopathological analyses, hematoxylineosin staining and, for stereological analyses and the quantitation of motor neurons, cresyl violet staining was performed. H3K27me3 levels were determined via immunofluorescence staining. Histopathological analysis identified structural alterations of ependymal cells, enlarged central canals, as well as degenerated and shrunken motor neurons in the EMF group, while the control group tissues had normal appearances. We also observed enrichment of H3K27me3 in the ependymal cells and the motor neurons in the spinal cord of the control group rats, while the EMF group had low levels of H3K27me3 staining. Our results suggest that the loss of H3K27me3 signals might correlate with reduced neuronal stem cell potential in the EMF group and result in anatomical and structural differences in the spinal cord. This study provided a comprehensive histopathological analysis of the spinal cord after prenatal EMF exposure and offered an H3K27me3-dependent molecular explanation for the detrimental effects of EMF exposure on the spine.

(NE) Kerbacher JJ, Meltz ML, Erwin DN, Influence of radiofrequency radiation on chromosome aberrations in CHO cells and its interaction with DNA-damaging agents. Radiat Res 123(3):311-319, 1990. (VT, AE, GT, IX)

A limited number of contradictory reports have appeared in the literature about the ability of radiofrequency (rf) radiation to induce chromosome aberrations in different biological systems. The technical documentation associated with such reports is often absent or deficient. In addition, no information is available as to whether any additional genotoxic hazard would result from a simultaneous exposure of mammalian cells to rf radiation and a chemical which (by itself) induces chromosome aberrations. In the work described, we have therefore tested two hypotheses. The first is that rf radiation by itself, at power densities and exposure conditions which are higher than is consistent with accepted safety guidelines, can induce chromosome aberrations in mammalian cells. The second is that, during a simultaneous exposure to a chemical known to be genotoxic, rf radiation can affect molecules, biochemical processes, or cellular organelles, and thus result in an increase or decrease in chromosome aberrations. Mitomycin C (MMC) and Adriamycin (ADR) were selected because they act by different mechanisms, and because they might put normal cells at risk during combined-modality rf radiation (hyperthermia)-chemotherapy treatment of cancer. The studies were performed with suitable 37 degrees C and equivalent convection heating-temperature controls in a manner designed to discriminate between any thermal and possible nonthermal action. Radiofrequency exposures were conducted for 2 h under conditions resulting in measurable heating (a maximum increase of 3.2 degrees C), with pulsed-wave rf radiation at a frequency of 2450 MHz and an average net forward power of 600 W, resulting in an SAR of 33.8 W/kg. Treatments with MMC or ADR were for a total of 2.5 h and encompassed the 2-h rf radiation exposure period. The CHO cells from each of the conditions were subsequently analyzed for chromosome aberrations. In cells exposed to rf radiation alone, and where a maximum temperature of approximately 40 degrees C was achieved in the tissue culture medium, no alteration in the frequency

from 37 degrees C control levels was observed. Relative to the chemical treatment with MMC alone at 37 degrees C, for two different concentrations, no alteration was observed in the extent of chromosome aberrations induced by either simultaneous rf radiation exposure or convection heating to equivalent temperatures. At the ADR concentration that was used, most of the indices of chromosome aberrations which were scored indicated a similar result.

(E) Kesari KK, Behari J. Fifty-gigahertz Microwave exposure effect of radiations on rat brain. Appl Biochem Biotechnol 158:126-139, 2009. (VO, LE, GT, OX)

The object of this study is to investigate the effects of 50-GHz microwave radiation on the brain of Wistar rats. Male rats of the Wistar strain were used in the study. Animals of 60-day age were divided into two groups-group 1, sham-exposed, and group 2, experimental (microwave-exposed). The rats were housed in a temperature-controlled room (25 degrees C) with constant humidity (40-50%) and received food and water ad libitum. During exposure, rats were placed in Plexiglas cages with drilled ventilation holes and kept in an anechoic chamber. The animals were exposed for 2 h a day for 45 days continuously at a power level of 0.86 $\mu\text{W}/\text{cm}^2$ with nominal specific absorption rate 8.0×10^{-4} w/kg. After the exposure period, the rats were killed and homogenized, and protein kinase C (PKC), DNA double-strand break, and antioxidant enzyme activity [superoxides dismutase (SOD), catalase, and glutathione peroxidase (GPx)] were estimated in the whole brain. Result shows that the chronic exposure to these radiations causes DNA double-strand break (head and tail length, intensity and tail migration) and a significant decrease in GPx and SOD activity ($p < 0.05$) in brain cells, whereas catalase activity shows significant increase in the exposed group of brain samples as compared with control ($p = < 0.001$). In addition to these, PKC decreased significantly in whole brain and hippocampus ($p < 0.05$). All data are expressed as mean \pm standard deviation. We conclude that these radiations can have a significant effect on the whole brain.

(E) Kesari KK, Behari J, Kumar S. Mutagenic response of 2.45 GHz radiation exposure on rat brain. Int J Radiat Biol 86:334-343, 2010. (VO, LE, GT, OX)

Purpose: To investigate the effect of 2.45 GHz microwave radiation on rat brain of male wistar strain. Material and methods: Male rats of wistar strain (35 days old with 130 \pm 10 g body weight) were selected for this study. Animals were divided into two groups: Sham exposed and experimental. Animals were exposed for 2 h a day for 35 days to 2.45 GHz frequency at 0.34 mW/cm power density. The whole body specific absorption rate (SAR) was estimated to be 0.11 W/Kg. Exposure took place in a ventilated Plexiglas cage and kept in anechoic chamber in a far field configuration from the horn antenna. After the completion of exposure period, rats were sacrificed and the whole brain tissue was dissected and used for study of double strand DNA (Deoxyribonucleic acid) breaks by micro gel electrophoresis and the statistical analysis was carried out using comet assay (IV-2 version software). Thereafter, antioxidant enzymes and histone kinase estimation was also performed. Results: A significant increase was observed in comet head ($P < 0.002$), tail length ($P < 0.0002$) and in tail movement ($P < 0.0001$) in exposed brain cells. An analysis of antioxidant enzymes glutathione peroxidase ($P < 0.005$), and superoxide dismutase ($P < 0.006$) showed a decrease while an increase in catalase ($P < 0.006$) was observed. A significant decrease ($P < 0.023$) in histone kinase was also recorded in the exposed group as compared to the control (sham-exposed) ones. One-way analysis of variance

(ANOVA) method was adopted for statistical analysis. Conclusion: The study concludes that the chronic exposure to these radiations may cause significant damage to brain, which may be an indication of possible tumour promotion (Behari and Paulraj 2007).

(E) Kesari KK, Kumar S, Behari J. Effects of radiofrequency electromagnetic wave exposure from cellular phones on the reproductive pattern in male Wistar rats. Appl Biochem Biotechnol. 164(4):546-559, 2011. (VO, LE, GT, OX)

The present study investigates the effect of free radical formation due to mobile phone exposure and effect on fertility pattern in 70-day-old male Wistar rats (sham exposed and exposed). Exposure took place in Plexiglas cages for 2 h a day for 35 days to mobile phone frequency. The specific absorption rate was estimated to be 0.9 W/kg. An analysis of antioxidant enzymes glutathione peroxidase ($P < 0.001$) and superoxide dismutase ($P < 0.007$) showed a decrease, while an increase in catalase ($P < 0.005$) was observed. Malondialdehyde ($P < 0.003$) showed an increase and histone kinase ($P = 0.006$) showed a significant decrease in the exposed group. Micronuclei also show a significant decrease ($P < 0.002$) in the exposed group. A significant change in sperm cell cycle of G(0)-G(1) ($P = 0.042$) and G(2)/M ($P = 0.022$) were recorded. Generation of free radicals was recorded to be significantly increased ($P = 0.035$). Our findings on antioxidant, malondialdehyde, histone kinase, micronuclei, and sperm cell cycle are clear indications of an infertility pattern, initiated due to an overproduction of reactive oxygen species. It is concluded that radiofrequency electromagnetic wave from commercially available cell phones might affect the fertilizing potential of spermatozoa.

(E) Kesari KK, Meena R, Nirala J, Kumar J, Verma HN. Effect of 3G cell phone exposure with computer controlled 2-D stepper motor on non-thermal activation of the hsp27/p38MAPK stress pathway in rat brain. Cell Biochem Biophys. 68(2):347-358, 2014. (VO, LE, GT, GE)

Cell phone radiation exposure and its biological interaction is the present concern of debate. Present study aimed to investigate the effect of 3G cell phone exposure with computer controlled 2-D stepper motor on 45-day-old male Wistar rat brain. Animals were exposed for 2 h a day for 60 days by using mobile phone with angular movement up to zero to 30°. The variation of the motor is restricted to 90° with respect to the horizontal plane, moving at a pre-determined rate of 2° per minute. Immediately after 60 days of exposure, animals were sacrificed and numbers of parameters (DNA double-strand break, micronuclei, caspase 3, apoptosis, DNA fragmentation, expression of stress-responsive genes) were performed. Result shows that microwave radiation emitted from 3G mobile phone significantly induced DNA strand breaks in brain. Meanwhile a significant increase in micronuclei, caspase 3 and apoptosis were also observed in exposed group ($P < 0.05$). Western blotting result shows that 3G mobile phone exposure causes a transient increase in phosphorylation of hsp27, hsp70, and p38 mitogen-activated protein kinase (p38MAPK), which leads to mitochondrial dysfunction-mediated cytochrome c release and subsequent activation of caspases, involved in the process of radiation-induced apoptotic cell death. Study shows that the oxidative stress is the main factor which activates a variety of

cellular signal transduction pathways, among them the hsp27/p38MAPK is the pathway of principle stress response. Results conclude that 3G mobile phone radiations affect the brain function and cause several neurological disorders.

(NE) Khalil, A.M., Ashamali, A.M., Gagaa, M. (2011). Detection of oxidative stress induced by mobile phone radiation in tissues of mice using 8-0xo-7, 8-dihydro-2'-deoxyguanosine as a biomarker. Int. J. Biol. Biomol. Agricul. Food Biotech. Eng. 5: 240-245. (VO, LE, GT, OX)

We investigated oxidative DNA damage caused by radio frequency radiation using 8-oxo-7, 8-dihydro-2'- deoxyguanosine (8-oxodG) generated in mice tissues after exposure to 900 MHz mobile phone radio frequency in three independent experiments. The RF was generated by a Global System for Mobile Communication (GSM) signal generator. The radio frequency field was adjusted to 25 V/m. The whole body specific absorption rate (SAR) was 1.0 W/kg. Animals were exposed to this field for 30 min daily for 30 days. 24 h post-exposure, blood serum, brain and spleen were removed and DNA was isolated. Enzyme-linked immunosorbent assay (ELISA) was used to measure 8-oxodG concentration. All animals survived the whole experimental period. The body weight of animals did not change significantly at the end of the experiment. No statistically significant differences observed in the levels of oxidative stress. Our results are not in favor of the hypothesis that 900 MHz RF induces oxidative damage.

(E) Khalil AM, Gagaa M, Alshamali A. 8-Oxo-7, 8-dihydro-2'-deoxyguanosine as a biomarker of DNA damage by mobile phone radiation. Hum ExpToxicol 31(7):734-740, 2012. (VO, AE, GT, OX)

We examined the effect of exposure to mobile phone 1800 MHz radio frequency radiation (RFR) upon the urinary excretion of 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxodG), one major form of oxidative DNA damage, in adult male Sprague-Dawley rats. Twenty-four rats were used in three independent experiments (RFR exposed and control, 12 rats, each). The animals were exposed to RFR for 2 h from Global System for Mobile Communications (GSM) signal generator with whole-body-specific absorption rate of 1.0 W/kg. Urine samples were collected from the rat while housed in a metabolic cage during the exposure period over a 4-h period at 0.5, 1.0, 2.0 and 4.0 h from the beginning of exposure. In the control group, the signal generator was left in the turn-off position. The creatinine-standardized concentrations of 8-oxodG were measured. With the exception of the urine collected in the last half an hour of exposure, significant elevations were noticed in the levels of 8-oxodG in urine samples from rats exposed to RFR when compared to control animals. Significant differences were seen overall across time points of urine collection with a maximum at 1 h after exposure, suggesting repair of the DNA lesions leading to 8-oxodG formation.

(NE) Khalil AM, Khadra KMA, Aljaberi AM, Gagaa MH, Issa HS. Assessment of oxidant/antioxidant status in saliva of cell phone users. Electromagn Biol Med 2014 Jun;33(2):92-97. (HU, AE, GT, OX)

Hazardous health effects resulting from exposure to radiofrequency electromagnetic radiation (RF-EMR) emitted from cell phones have been reported in the literature. However, the cellular and molecular targets of RF-EMR are still controversial. The aim of this study was to examine the oxidant/antioxidant status in saliva of cell phone users. Saliva samples collected before using a cell phone as well as at the end of 15 and 30 min calls were tested for two commonly used oxidative stress biomarkers: malondialdehyde (MDA) and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-Oxo-dG). The 8-oxo-dG levels were determined by enzyme-linked immunosorbent (ELISA) competitive assay, while the MDA levels were measured using the OxiSelect MDA adduct ELISA Kit. The antioxidant capacity of the saliva was evaluated using the oxygen radical absorption capacity (ORAC) and the hydroxyl radical averting capacity (HORAC) assays according to the manufacture instructions. The mean 8-oxo-dG and the Bradford protein concentrations (ng/ml and mg/ml, respectively) peaked at 15 min. The levels of HORAC, ORAC and MDA progressively increased with time and reached maximum at 30 min. However, there was no significant effect of talking time on the levels of 8-OxodG and MDA. Similarly, there was no statistically significant effect of talking time on the oxygen and hydroxyl radicals averting capacities, (ORAC) and (HORAC), respectively. These findings suggest that there is no relationship between exposure to radio frequency radiation (RFR) and changes in the salivary oxidant/antioxidant profile.

(E) Khodamoradi E, Afrashi S, Khoshgard K, Fathi F, Shahasavari S, Azmoonfar R, Najafi M. Simultaneous effect of gamma and Wi-Fi radiation on gamma-H2Ax expression in peripheral blood of rat: A radio-protection note. Biochem Biophys Rep. 30, 101233, 2022. (VO, AE, GT, IX)

Introduction. Nuclear medicine patients are isolated in a room after the injection of a radiopharmaceutical. They may be active Wi-Fi option of its smartphone mobile or other environmental radiofrequency waves. The hypothesis of this study was the evaluation of increased biological effects of the simultaneous exposure to gamma-ray and the Wi-Fi waves by measuring the level of the increased double strand-breaks DNA in peripheral blood lymphocyte in the rat. Materials and methods. Fifty male Wistar rats were exposed for 2, 24, and 72 h only by Wi-Fi, 99m Tc, and simultaneously by Wi-Fi and 99m Tc. The power density levels of Wi-Fi emitter at 15 cm was 4.2nW/ cm². An activity of 100 µCi of 99m Tc was injected intraperitoneally. Blood samples were taken by cardiac puncture following general anesthesia. Mononuclear cells are extraction by Ficoll-Hypaque density gradient centrifugation. The number of gamma-H2AX foci per nucleus was counted by flow cytometry. The statistical differences between experimental groups at 2, 24, and 72 h were determined with a repeated measure's analysis of variance. The significant difference between groups at the same time was analyzed with the Kruskal-Wallis Test. Results The manner of gamma-H2AX expression was not the same for three groups in time. The number of gamma-H2AX foci between the three groups was a significant difference after 72 h. Conclusion Simultaneous Wi-Fi and gamma-ray exposures can increase the number of double-strand break DNA in peripheral blood lymphocytes to exposure of gamma-ray to 72 h after technetium injection in the rat.

(E) Kim HM, S, Yang JY, Sun HJ, Jang M, Kang D, Son KH, Byun K Evaluating Whether Radiofrequency Irradiation Attenuated UV-B-Induced Skin Pigmentation by Increasing Melanosomal Autophagy and Decreasing Melanin Synthesis. Int J Mol Sci 2021 Oct 3;22(19):10724. (VT, LE, GE)

Autophagy is involved in the degradation of melanosomes and the determination of skin color. TLR4 and tumor necrosis factor (TNF) signaling upregulates NF- κ B expression, which is involved in the upregulation of mTOR. The activation of mTOR by UV-B exposure results in decreased autophagy, whereas radiofrequency (RF) irradiation decreases TLR4 and TNF receptor (TNFR) expression. We evaluated whether RF decreased skin pigmentation by restoring autophagy by decreasing the expression of TLR4 or TNFR/NF- κ B/mTOR in the UV-B-irradiated animal model. UV-B radiation induced the expressions of TNFR, TLR, and NF- κ B in the skin, which were all decreased by RF irradiation. RF irradiation also decreased phosphorylated mTOR expression and upregulated autophagy initiation factors such as FIP200, ULK1, ULK2, ATG13, and ATG101 in the UV-B-irradiated skin. Beclin 1 expression and the expression ratio of LC3-I to LC3-II were increased by UV-B/RF irradiation. Furthermore, melanin-containing autophagosomes increased with RF irradiation. Fontana-Masson staining showed that the amount of melanin deposition in the skin was decreased by RF irradiation. This study showed that RF irradiation decreased skin pigmentation by restoring melanosomal autophagy, and that the possible signal pathways which modulate autophagy could be TLR4, TNFR, NF- κ B, and mTOR.

(E) Kim JH, Chung KH, Hwang YR, Park HR, Kim HJ, Kim HG, Kim HR. Exposure to RF-EMF Alters Postsynaptic Structure and Hinders Neurite Outgrowth in Developing Hippocampal Neurons of Early Postnatal Mice. Int J Mol Sci 22(10):5340, 2021. (AS, CS, CE, GE)

Exposure to radiofrequency electromagnetic fields (RF-EMFs) has increased rapidly in children, but information on the effects of RF-EMF exposure to the central nervous system in children is limited. In this study, pups and dams were exposed to whole-body RF-EMF at 4.0 W/kg specific absorption rate (SAR) for 5 h per day for 4 weeks (from postnatal day (P) 1 to P28). The effects of RF-EMF exposure on neurons were evaluated by using both pups' hippocampus and primary cultured hippocampal neurons. The total number of dendritic spines showed statistically significant decreases in the dentate gyrus (DG) but was not altered in the cornu ammonis (CA1) in hippocampal neurons. In particular, the number of mushroom-type dendritic spines showed statistically significant decreases in the CA1 and DG. The expression of glutamate receptors was decreased in mushroom-type dendritic spines in the CA1 and DG of hippocampal neurons following RF-EMF exposure. The expression of brain-derived neurotrophic factor (BDNF) in the CA1 and DG was significantly lower statistically in RF-EMF-exposed mice. The number of post-synaptic density protein 95 (PSD95) puncta gradually increased over time but was significantly decreased statistically at days in vitro (DIV) 5, 7, and 9 following RF-EMF exposure. Decreased BDNF expression was restricted to the soma and was not observed in neurites of hippocampal neurons following RF-EMF exposure. The length of neurite outgrowth and number of branches showed statistically significant decreases, but no changes in the soma size of hippocampal neurons were observed. Further, the memory index showed statistically

significant decreases in RF-EMF-exposed mice, suggesting that decreased synaptic density following RF-EMF exposure at early developmental stages may affect memory function. Collectively, these data suggest that hindered neuronal outgrowth following RF-EMF exposure may decrease overall synaptic density during early neurite development of hippocampal neurons.

(E) Kim JH, Jeon S, Choi H-D, Lee J-H, Bae J-S, Kim N, Kim H-G, Kim K-B, Kim HR. Exposure to long-term evolution radiofrequency electromagnetic fields decreases neuroblastoma cell proliferation via Akt/mTOR-mediated cellular senescence. J Toxicol Environ Health A 84(20):846-857, 2021. (VT, LE, GE)

The aim of this study was to examine the potential effects of long-term evolution (LTE) radiofrequency electromagnetic fields (RF-EMF) on cell proliferation using SH-SY5Y neuronal cells. The growth rate and proliferation of SH-SY5Y cells were significantly decreased upon exposure to 1760 MHz RF-EMF at 4 W/kg specific absorption rate (SAR) for 4 hr/day for 4 days. Cell cycle analysis indicated that the cell cycle was delayed in the G₀/G₁ phase after RF-EMF exposure. However, DNA damage or apoptosis was not involved in the reduced cellular proliferation following RF-EMF exposure because the expression levels of histone H2A.X at Ser139 (γ H2AX) were not markedly altered and the apoptotic pathway was not activated. However, SH-SY5Y cells exposed to RF-EMF exhibited a significant elevation in Akt and mTOR phosphorylation levels. In addition, the total amount of p53 and phosphorylated-p53 was significantly increased. Data suggested that Akt/mTOR-mediated cellular senescence led to p53 activation via stimulation of the mTOR pathway in SH-SY5Y cells. The transcriptional activation of p53 led to a rise in expression of cyclin-dependent kinase (CDK) inhibitors p21 and p27. Further, subsequent inhibition of CDK2 and CDK4 produced a fall in phosphorylated retinoblastoma (pRb at Ser807/811), which decreased cell proliferation. Taken together, these data suggest that exposure to RF-EMF might induce Akt/mTOR-mediated cellular senescence, which may delay the cell cycle without triggering DNA damage in SH-SY5Y neuroblastoma cells.

(E) Kim JY, Hong SY, Lee YM, Yu SA, Koh WS, Hong JR, Son T, Chang SK, Lee M. In vitro assessment of clastogenicity of mobile-phone radiation (835 MHz) using the alkaline comet assay and chromosomal aberration test. Environ Toxicol 23:319-327, 2008. (VT, AE, GT, IX)

Recently we demonstrated that 835-MHz radiofrequency radiation electromagnetic fields (RF-EMF) neither affected the reverse mutation frequency nor accelerated DNA degradation in vitro. Here, two kinds of cytogenetic endpoints were further investigated on mammalian cells exposed to 835-MHz RF-EMF (the most widely used communication frequency band in Korean CDMA mobile phone networks) alone and in combination with model clastogens: in vitro alkaline comet assay and in vitro chromosome aberration (CA) test. No direct cytogenetic effect of 835-MHz RF-EMF was found in the in vitro CA test. The combined exposure of the cells to RF-EMF in the presence of ethylmethanesulfonate (EMS) revealed a weak and insignificant cytogenetic effect when compared to cells exposed to EMS alone in CA test. Also, the comet assay results to evaluate the ability of RF-EMF alone to damage DNA were nearly negative, although showing a small increase in tail moment. However, the applied RF-EMF had potentiation effect in comet assay when administered in combination with model clastogens (cyclophosphamide or 4-

nitroquinoline 1-oxide). Thus, our results imply that we cannot confidently exclude any possibility of an increased risk of genetic damage, with important implications for the possible health effects of exposure to 835-MHz electromagnetic fields.

Kivrak EG, Yurt KK, Kaplan AA, Alkan I, Altun G. Effects of electromagnetic fields exposure on the antioxidant defense system. J Microsc Ultrastruct. 5(4):167-176, 2017. (Review)

Technological devices have become essential components of daily life. However, their deleterious effects on the body, particularly on the nervous system, are well known. Electromagnetic fields (EMF) have various chemical effects, including causing deterioration in large molecules in cells and imbalance in ionic equilibrium. Despite being essential for life, oxygen molecules can lead to the generation of hazardous by-products, known as reactive oxygen species (ROS), during biological reactions. These reactive oxygen species can damage cellular components such as proteins, lipids and DNA. Antioxidant defense systems exist in order to keep free radical formation under control and to prevent their harmful effects on the biological system. Free radical formation can take place in various ways, including ultraviolet light, drugs, lipid oxidation, immunological reactions, radiation, stress, smoking, alcohol and biochemical redox reactions. Oxidative stress occurs if the antioxidant defense system is unable to prevent the harmful effects of free radicals. Several studies have reported that exposure to EMF results in oxidative stress in many tissues of the body. Exposure to EMF is known to increase free radical concentrations and traceability and can affect the radical couple recombination. The purpose of this review was to highlight the impact of oxidative stress on antioxidant systems.

Kocaman A, Altun G, Kaplan AA, Deniz ÖG, Yurt KK, Kaplan S. Genotoxic and carcinogenic effects of non-ionizing electromagnetic fields. Environ Res. 163:71-79, 2018. (review)

New technologies in electronics and communications are continually emerging. An increasing use of these electronic devices such as mobile phone, computer, wireless fidelity connectors or cellular towers is raising questions concerning whether they have an adverse effect on the body. Exposure to electromagnetic fields (EMF) is frequently suggested to have adverse health effects on humans and other organisms. This idea has been reported in many studies. In contrast, the therapeutic effects of EMF on different organs have also been reported. Research findings are inconsistent. This has given rise to very profound discrepancies. The duration and frequency of mobile phone calls and the association observed with various health effects has raised serious concerns due to the frequency with which these devices are used and the way they are held close to the head. The present review assesses the results of in vitro, in vivo, experimental, and epidemiological studies. The purpose of the study is to assess data concerning the carcinogenic and genotoxic effects of non-ionizing EMF. The major genotoxic and carcinogenic effects of EMF, divided into subsections as low frequency effects and radiofrequency effects, were reviewed. The inconsistent results between similar studies and the same research groups have made it very difficult to make any comprehensive interpretation. However, evaluation of current studies suggests that

EMF may represent a serious source of concern and may be hazardous to living organisms.

(NE) Komatsubara Y, Hirose H, Sakurai T, Koyama S, Suzuki Y, Taki M, Miyakoshi J. Effect of high-frequency electromagnetic fields with a wide range of SARs on chromosomal aberrations in murine m5S cells. Mutat Res. 587(1-2):114-119, 2005. (VT, AE, GT) (very high SAR)

To investigate the induction of chromosomal aberrations in mouse m5S cells after exposure to high-frequency electromagnetic fields (HFEMFs) at 2.45GHz, cells were exposed for 2h at average specific absorption rates (SARs) of 5, 10, 20, 50 and 100W/kg with continuous wave-form (CW), or at a mean SAR of 100W/kg (with a maximum of 900W/kg) with pulse wave-form (PW). The effects of HFEMF exposure were compared with those in sham-exposed controls and with mitomycin C (MMC) or X-ray treatment as positive controls. We examined all structural, chromatid-type and chromosome-type changes after HFEMF exposures and treatments with MMC and X-rays. No significant differences were observed following exposure to HFEMFs at SARs from 5 to 100W/kg CW and at a mean SAR of 100W/kg PW (a maximum SAR of 900W/kg) compared with sham-exposed controls, whereas treatments with MMC and X-rays increased the frequency of chromatid-type and chromosome-type aberrations. In summary, HFEMF exposures at 2.45GHz for 2h with up to 100W/kg SAR CW and an average 100W/kg PW (a maximum SAR of 900W/kg) do not induce chromosomal aberrations in m5S cells. Furthermore, there was no difference between exposures to CW and PW HFEMFs.

(E) Korenstein-Ilan A, Barbul A, Hasin P, Eliran A, Gover A, Korenstein R. Terahertz radiation increases genomic instability in human lymphocytes. Radiat Res 2008 Aug;170(2):224-34. (VT, AE, GT)

Terahertz radiation is increasingly being applied in new and evolving technologies applied in areas such as homeland security and medical imaging. Thus a timely assessment of the potential hazards and health effects of occupational and general population exposure to THz radiation is required. We applied continuous-wave (CW) 0.1 THz radiation (0.031 mW/cm²) to dividing lymphocytes for 1, 2 and 24 h and examined the changes in chromosome number of chromosomes 1, 10, 11 and 17 and changes in the replication timing of their centromeres using interphase fluorescence in situ hybridization (FISH). Chromosomes 11 and 17 were most vulnerable (about 30% increase in aneuploidy after 2 and 24 h of exposure), while chromosomes 1 and 10 were not affected. We observed changes in the asynchronous mode of replication of centromeres 11, 17 and 1 (by 40%) after 2 h of exposure and of all four centromeres after 24 h of exposure (by 50%). It is speculated that these effects are caused by radiation-induced low-frequency collective vibrational modes of proteins and DNA. Our results demonstrate that exposure of lymphocytes in vitro to a low power density of 0.1 THz radiation induces genomic instability. These findings, if verified, may suggest that such exposure may result in an increased risk of cancer.

(E) Koyama S, Nakahara T, Wake K, Taki M, Isozumi Y, Miyakoshi J. Effects of high frequency electromagnetic fields on micronucleus formation in CHO-K1 cells. Mutat Res. 2003; 541: 81-89. (VT, AE, GT)

To investigate the effects of high frequency electromagnetic fields (HFEMFs), we assessed the frequency of micronucleus (MN) formation induced by chromosomal breakage or inhibition of spindles during cell division in Chinese hamster ovary (CHO)-K1 cells, using the cytokinesis block micronucleus method. The MN frequency in cells in the inner, middle and outer wells of an annular culture plate was determined for the following four conditions: (1) CHO-K1 cells were exposed to a HFEMF for 18 h at average specific absorption rates (SARs) of 13, 39 and 50 W/kg with input power 7.8 W, and were compared with a sham-exposed control; (2) the cells were also exposed to a HFEMF at SARs of 78 and 100 W/kg with input power 13 W, and were compared with a sham-exposed control; (3) the cells were treated with bleomycin alone or with bleomycin followed by exposure to a HFEMF for 18 h at SARs of 25, 78 and 100 W/kg, and were compared with a bleomycin-treated positive control. The cells treated with bleomycin alone were compared with sham-exposed controls; and (4) As a high temperature control, CHO-K1 cells were incubated at 39 degrees C for 18 h. In study (1), the MN frequency of cells exposed to a HFEMF at a SAR of up to 50 W/kg was not different to that in sham-exposed cells. In study (2), there were statistically significant increases in the MN frequencies of cells in the middle and outer wells of the annular culture plate caused by exposure to a HFEMF at 100 and 78 W/kg, respectively. In study (3), the MN frequencies of cells in the middle (100 W/kg) and outer wells (78 W/kg) of the annular culture plate were statistically higher than that caused by bleomycin-treatment alone. In study (4), there was a statistically significant increase of MN frequency in the cells treated by heat at 39 degrees C. These results indicate that cells exposed to a HFEMF at a SAR of 78 W/kg and higher form MN more frequently than sham-exposed cells, while exposure to a HFEMF at up to 50 W/kg does not induce MN formation. In addition, a HFEMF at a SAR of 78 W/kg and higher may potentiate MN formation induced by bleomycin-treatment.

(E) Koyama S, Isozumi Y, Suzuki Y, Taki M, Miyakoshi J. Effects of 2.45-GHz electromagnetic fields with a wide range of SARs on micronucleus formation in CHO-K1 cells. ScientificWorldJournal 4 Suppl 2:29-40, 2004. (VT, AE, GT) (very high SAR)

There has been considerable discussion about the influence of high-frequency electromagnetic fields (HFEMF) on the human body. In particular, HFEMF used for mobile phones may be of great concern for human health. In order to investigate the properties of HFEMF, we have examined the effects of 2.45-GHz EMF on micronucleus (MN) formation in Chinese hamster ovary (CHO)-K1 cells. MN formation is induced by chromosomal breakage or inhibition of spindles during cell division and leads to cell damage. We also examined the influence of heat on MN formation, since HFEMF exposure causes a rise in temperature. CHO-K1 cells were exposed to HFEMF for 2 h at average specific absorption rates (SARs) of 5, 10, 20, 50, 100, and 200 W/kg, and the effects on these cells were compared with those in sham-exposed control cells. The cells were also treated with bleomycin alone as a positive control or with combined treatment of HFEMF exposure and bleomycin. Heat treatment was performed at temperatures of 37, 38, 39, 40, 41, and 42 degrees C. The MN frequency in cells exposed to HFEMF at a SAR of

lower than 50 W/kg did not differ from the sham-exposed controls, while those at SARs of 100 and 200 W/kg were significantly higher when compared with the sham-exposed controls. There was no apparent combined effect of HFEMF exposure and bleomycin treatment. On heat treatment at temperatures from 38-42 degrees C, the MN frequency increased in a temperature-dependent manner. We also showed that an increase in SAR causes a rise in temperature and this may be connected to the increase in MN formation generated by exposure to HFEMF.

(NE) Koyama S, Narita E, Shimizu Y, Shiina T, Taki M, Shinohara N, et al. Twenty four-hour exposure to a 0.12 THz electromagnetic field does not affect the genotoxicity, morphological changes, or expression of heat shock protein in HCE-T cells. Int. J. Environ. Health Res. Pub. Health. 2016a; 13: 793, 1-9. (VT, AE, GT)

To investigate the cellular effects of terahertz (THz) exposure, human corneal epithelial (HCE-T) cells derived from human eye were exposed to 0.12 THz radiation at 5 mW/cm² for 24 h, then the genotoxicity, morphological changes, and heat shock protein (Hsp) expression of the cells were examined. There was no statistically significant increase in the micronucleus (MN) frequency of cells exposed to 0.12 THz radiation compared with sham-exposed controls and incubator controls, whereas the MN frequency of cells treated with bleomycin for 1 h (positive control) did increase significantly. Similarly, there were no significant morphological changes in cells exposed to 0.12 THz radiation compared to sham-exposed controls and incubator controls, and Hsp expression (Hsp27, Hsp70, and Hsp90 α) was also not significantly different between the three treatments. These results indicate that exposure to 0.12 THz radiation using the present conditions appears to have no or very little effect on MN formation, morphological changes, and Hsp expression in cells derived from human eye.

(NE) Koyama S, Narita E, Shimizu Y, Suzuki Y, Shiina T, Taki M, et al. Effects of long-term exposure to 60 GHz millimeter-wavelength radiation on the genotoxicity and heat shock protein (Hsp) expression of cells derived from human eye. Int. J. Environ. Health Res. Pub. Health. 2016b; 13: 802, 1-9. (VT, AE, GT)

Human corneal epithelial (HCE-T) and human lens epithelial (SRA01/04) cells derived from the human eye were exposed to 60 gigahertz (GHz) millimeter-wavelength radiation for 24 h. There was no statistically significant increase in the micronucleus (MN) frequency in cells exposed to 60 GHz millimeter-wavelength radiation at 1 mW/cm² compared with sham-exposed controls and incubator controls. The MN frequency of cells treated with bleomycin for 1 h provided positive controls. The comet assay, used to detect DNA strand breaks, and heat shock protein (Hsp) expression also showed no statistically significant effects of exposure. These results indicate that exposure to millimeter-wavelength radiation has no effect on genotoxicity in human eye cells.

(E) Kumar A, Kaur S, Chandel S, Singh HP, Batish DR, Kohli RK. Comparative cyto- and genotoxicity of 900 MHz and 1800 MHz electromagnetic field radiations in root meristems of Allium cepa. Ecotoxicol Environ Saf. 188:109786, 2020. (VO, AE, LI, GT, WS)

In the last few decades, tremendous increase in the use of wireless electronic gadgets, particularly the cell phones, has significantly enhanced the levels of electromagnetic field radiations (EMF-r) in the environment. Therefore, it is pertinent to study the effect of these radiations on biological systems including plants. We investigated comparative cytotoxic and DNA damaging effects of 900 and 1800 MHz EMF-r in *Allium cepa* (onion) root meristematic cells in terms of mitotic index (MI), chromosomal aberrations (CAs) and single cell gel electrophoresis (comet assay). Onion bulbs were subjected to 900 and 1800 MHz (at power densities $261 \pm 8.50 \text{ mW m}^{-2}$ and $332 \pm 10.36 \text{ mW m}^{-2}$, respectively) of EMF-r for 0.5 h, 1 h, 2 h, and 4 h. Root length declined by 13.2% and 12.3%, whereas root thickness was increased by 46.7% and 48.3% after 4 h exposure to 900 MHz and 1800 MHz, respectively. Cytogenetic studies exhibited clastogenic effect of EMF-r as depicted by increased CAs and MI. MI increased by 36% and 53% after 2 and 4 h exposure to 900 MHz EMF-r, whereas it increased by 41% and 67% in response to 1800 MHz EMF-r. Aberration index was increased by 41%-266% and 14%-257% during 0.5-4 h of exposure to 900 MHz and 1800 MHz, respectively, over the control. EMF-r exposure decreased % head DNA (DNAH) and increased % tail DNA (DNAT) and olive tail moment (OTM) at both 900 and 1800 MHz EMF-r. In 4 h exposure treatments, head DNA (%) declined by 19% and 23% at 900 MHz and 1800 MHz, respectively. DNAT and OTM were increased by 2.3 and 3.7 fold upon exposure to 900 MHz EMF-r over that in the control, whereas 2.8 and 5.8 fold increase was observed in response to 1800 MHz EMF-r exposure for 4 h and the difference was statistically significant. The study concludes that EMF-r in the communication range (900 and 1800 MHz) adversely affect root meristems in plants and induce cytotoxic and DNA damage. EMF-r induced DNA damage was more pronounced at 1800 MHz than that at 900 MHz.

(NE) Kumar G, Wood AW, Anderson V, McIntosh RL, Chen YY, McKenzie RJ. Evaluation of Hematopoietic System Effects After in Vitro Radiofrequency Radiation Exposure in Rats. Int J Radiat Biol 87(2):231-240, 2011. (VT, AE, GT)

Purpose: This study was designed to investigate the effect of a 900-MHz continuous-wave (CW) radiofrequency radiation (RFR) exposure on the hematopoietic system in the rat. **Materials and methods:** Rat long bones (femur and tibia) were divided into two groups: Sham-exposed and radiofrequency (RF)-exposed. The mean Specific energy Absorption Rate (SAR) at 900-MHz averaged over the bone marrow (calculated by the finite-difference-time-domain (fdtD) method) was 2 W/kg at 16.7 W root mean square (rms) forward power into a Transverse Electromagnetic (TEM) cell. The bones, placed in a Petri dish containing media, were kept in the TEM cell for 30 min duration of sham or RF exposure. After exposure, the bone marrow cells were extracted and the following end points were tested: (a) Proliferation rate of whole bone marrow cells, (b) maturation rate of erythrocytes, (c) proliferation rate of lymphocytes, and (d) DNA damage (strand breaks/alkali labile sites) of lymphocytes. **Results:** Our data did not indicate any significant change in the proliferation rate of bone marrow cells and lymphocytes, erythrocyte maturation rate and DNA damage of lymphocytes. **Conclusion:** Our findings revealed no effect on the hematopoietic system in rats for 900 MHz CW RF exposure at the 2 W/kg localised SAR limit value recommended by the International Commission for Non-Ionising Radiation Protection (ICNIRP) for public exposures.

(NE) Kumar G, McIntosh RL, Anderson V, McKenzie RJ, Wood AW. A genotoxic analysis of the hematopoietic system after mobile phone type radiation exposure in rats. Int J Radiat Biol. 91(8):664-672, 2015. (VT, AE, GT)

PURPOSE: In our earlier study we reported that 900 MHz continuous wave (CW) radiofrequency radiation (RFR) exposure (2 W/kg specific absorption rate [SAR]) had no significant effect on the hematopoietic system of rats. In this paper we extend the scope of the previous study by testing for possible effects at: (i) different SAR levels; (ii) both 900 and 1800 MHz, and; (iii) both CW and pulse modulated (PM) RFR. MATERIALS AND METHODS: Excised long bones from rats were placed in medium and RFR exposed in (i) a Transverse Electromagnetic (TEM) cell or (ii) a waveguide. Finite-difference time-domain (FDTD) numerical analyses were used to estimate forward power needed to produce nominal SAR levels of 2/10 and 2.5/12.4 W/kg in the bone marrow. After exposure, the lymphoblasts were extracted and assayed for proliferation rate, and genotoxicity. RESULTS: Our data did not indicate any significant change in these end points for any combination of CW/PM exposure at 900/1800 MHz at SAR levels of nominally 2/10 W/kg or 2.5/12.4 W/kg. CONCLUSIONS: No significant changes were observed in the hematopoietic system of rats after the exposure of CW/PM wave 900 MHz/1800 MHz RF radiations at different SAR values.

(E) Kumar, R, Deshmukh, P.S., Sharma, S., Banerjee, B.D. Effect of mobile phone signal radiation on epigenetic modulation in the hippocampus of Wistar rat. Environ Res 2021 Jan;192:110297. (VO, CE, GE, LI, EP)

Exponential increase in mobile phone uses, given rise to public concern regarding the alleged deleterious health hazards as a consequence of prolonged exposure. In 2018, the U.S. National toxicology program reported, two year toxicological studies for potential health hazards from exposure to cell phone radiations. Epigenetic modulations play a critical regulatory role in many cellular functions and pathological conditions. In this study, we assessed the dose-dependent and frequency-dependent epigenetic modulation (DNA and Histone methylation) in the hippocampus of Wistar rats. A Total of 96 male Wistar rats were segregated into 12 groups exposed to 900 MHz, 1800 MHz and 2450 MHz RF-MW at a specific absorption rate (SAR) of 5.84×10^{-4} W/kg, 5.94×10^{-4} W/kg and 6.4×10^{-4} W/kg respectively for 2 h per day for 1-month, 3-month and 6-month periods. At the end of the exposure duration, animals were sacrificed to collect the hippocampus. Global hippocampal DNA methylation and histone methylation were estimated by ELISA. However, DNA methylating enzymes, DNA methyltransferase1 (DNMT1) and histone methylating enzymes euchromatic histone methyltransferase1 (EHMT1) expression was evaluated by real-time PCR, as well as further validated with Western blot. Alteration in epigenetic modulation was observed in the hippocampus. Global DNA methylation was decreased and histone methylation was increased in the hippocampus. We observed that microwave exposure led to significant epigenetic modulations in the hippocampus with increasing frequency and duration of exposure. Microwave exposure with increasing frequency and exposure duration brings significant ($p < 0.05$) epigenetic modulations which alters gene expression in the hippocampus.

(E) Kumar S, Kesari KK, Behari J. Evaluation of genotoxic effects in male Wistar rats following microwave exposure. Indian J Exp Biol 48:586-592, 2010. (VO, LE, LI, GT, OX)

Wistar rats (70 days old) were exposed for 2 h a day for 45 days continuously at 10 GHz [power density 0.214 mW/cm², specific absorption rate (SAR) 0.014 W/kg] and 50 GHz (power density 0.86 microW/cm², SAR 8.0 x10⁽⁻⁴⁾ W/kg). Micronuclei (MN), reactive oxygen species (ROS), and antioxidant enzymes activity were estimated in the blood cells and serum. These radiations induce micronuclei formation and significant increase in ROS production. Significant changes in the level of serum glutathione peroxidase, superoxide dismutase and catalase were observed in exposed group as compared with control group. It is concluded that microwave exposure can be affective at genetic level. This may be an indication of tumor promotion, which comes through the overproduction of reactive oxygen species.

(E) Kumar S, Behari J, Sisodia R. Influence of electromagnetic fields on reproductive system of male rats. Int J Radiat Biol. 89: 147-154, 2013. (VO, LE, GT, RP, LI)

Purpose: Reports of declining male fertility have renewed interest in the role of environmental and occupational exposures in the etiology of human infertility. The aim of the present work was to investigate the effect of 10 GHz exposure on the male Wistar rat's reproductive system and to find out the possible causative factors. **Materials and methods:** The study was divided into sham-exposed and exposed groups. Seventy day-old rats were exposed to 10 GHz microwave radiation for 2 h per day for 45 days at power density 0.21 mW/cm² and specific absorption rate (SAR) of 0.014 W/kg. After the end of the experiment, blood samples were collected for the estimation of in vivo chromosomal aberration damage and micronucleus test. Spermatozoa were taken out for estimation of Caspase-3, comet assay, testosterone and electron microscopy and compared with sham-exposed. **Results:** The study of scanning electron microscopic revealed shrinkage of the lumen of the seminiferous tubules. Apoptotic bodies were found in exposed group. A flow cytometry examination showed formation of micronuclei body in lymphocytes of exposed group. Comet assay confirmed DNA (deoxyribonucleic acid) strand break. Testosterone level was found significantly decreased with the shrinkage of testicular size. **Conclusions:** 10 GHz field has an injurious effect on fertility potential of male-exposed animals.

(E) Kumar S, Nirala JP, Behari J, Paulraj R. Effect of electromagnetic irradiation produced by 3G mobile phone on male rat reproductive system in a simulated scenario. Indian J Exp Biol. 52(9):890-897, 2014. (VO, LE, LI, GT, OX, RP)

Reports of declining male fertility have renewed interest in assessing the role of electromagnetic fields (EMFs). Testicular function is particularly susceptible to the radiation emitted by EMFs. Significant decrease in sperm count, increase in the lipid peroxidation damage in sperm cells, reduction in seminiferous tubules and testicular weight and DNA damage were observed following exposure to EMF in male albino rats. The results suggest that mobile phone exposure adversely affects male fertility.

(E) Kundu A, Vangaru S, Bhowmick S, Bhattacharyya S, Mallick AI, Gupta B. One-time Electromagnetic Irradiation Modifies Stress-sensitive Gene Expressions in Rice Plant. Bioelectromagnetics 42(8):649-658, 2021.(VO, AE, GE)

Electromagnetic energy is utilized over multiple frequency bands to provide seamless wireless communication services. Plants can well perceive electromagnetic energy present in open environment due to reasonably high permittivity and electrical conductivity of constituent tissues. Moreover, higher surface-to-volume ratio of plant structure facilitates increased interaction with the incident electromagnetic waves. To date, a few well-designed studies have been conducted inside controlled electromagnetic reverberation chambers to investigate either short duration-low amplitude or long duration-periodic electromagnetic irradiation-induced molecular responses in plants. However, as far as is known, studies investigating molecular responses particularly at the mid-vegetative stage in plants following one-time (hours-long) electromagnetic irradiation have not been reported earlier. Hence, the present study aimed at investigating molecular responses in 40-day-old Swarnaprabha rice plants following one-time 1837.50 MHz, 2.75 mW/m² electromagnetic irradiation of 2 h 30 min duration. Controlled electromagnetic irradiation inside a simple reverberation chamber was ensured to achieve pure electromagnetic environment at 1837.50 MHz with deterministic electromagnetic power density at selected position. Swarnaprabha rice plant was chosen for this investigation since the rice variety is widely cultivated and consumed in the Indian subcontinent. Subsequent alterations in some selected stress-sensitive gene expressions were assayed using real-time quantitative polymerase chain reaction technique-significant upregulation in calmodulin and phytochrome B gene expressions were noted. This investigation was purposefully focused on subsequent molecular responses immediately following electromagnetic irradiation so that the possible effects of secondary stimulations could be avoided. Observed molecular responses strongly suggested that plants perceive 1837.50 MHz, 2.75 mW/m² electromagnetic irradiation similar to other injurious stimuli.

(NE) Lagroye I, Anane R, Wettring BA, Moros EG, Straube WL, Laregina M, Niehoff M, Pickard WF, Baty J, Roti JL. Measurement of DNA damage after acute exposure to pulsed-wave 2450 MHz microwaves in rat brain cells by two alkaline comet assay methods. Int J Radiat Biol. 80(1):11-20, 2004a. (VO, AE, GT)

Purpose: To investigate the effect of 2450 MHz pulsed-wave microwaves on the induction of DNA damage in brain cells of exposed rats and to discover whether proteinase K is needed to detect DNA damage in the brain cells of rats exposed to 2450 MHz microwaves. Materials and methods: Sprague-Dawley rats were exposed to 2450 MHz pulsed-wave microwaves and sacrificed 4 h after a 2-h exposure. Rats irradiated whole-body with 1 Gy (137)Cs were included as positive controls. DNA damage was assayed by two variants of the alkaline comet assay on separate aliquots of the same cell preparation. Results: Significant DNA damage was observed in the rat brain cells of rats exposed to gamma-rays using both versions of the alkaline comet assay independent of the presence or absence of proteinase K. However, neither version of the assay could detect any difference in comet length and/or normalized comet moment between sham- and 2450 MHz pulsed-wave microwave-exposed rats, regardless of the inclusion or omission of proteinase K in the comet assay. Conclusions: No DNA damage in brain cells was detected following exposure of rats to 2450 MHz microwaves pulsed-wave at a specific absorption rate of 1.2 W kg(-1) regardless of whether or not proteinase K was included in the assay. Thus, the results support the conclusion that low-level 2450 MHz pulsed-wave microwave exposures do not induce DNA damage detectable by the alkaline comet assay.

(NE) Lagroye I, Hook GJ, Wettring BA, Baty JD, Moros EG, Straube WL, Roti Roti JL. Measurements of alkali-labile DNA damage and protein-DNA crosslinks after 2450 MHz microwave and low-dose gamma irradiation In vitro. Radiat Res. 161(2): 201-214, 2004b. (VT, AE, GT)

In vitro experiments were performed to determine whether 2450 MHz microwave radiation induces alkali-labile DNA damage and/or DNA-protein or DNA-DNA crosslinks in C3H 10T(1/2) cells. After a 2-h exposure to either 2450 MHz continuous-wave (CW) microwaves at an SAR of 1.9 W/kg or 1 mM cisplatin (CDDP, a positive control for DNA crosslinks), C3H 10T(1/2) cells were irradiated with 4 Gy of gamma rays (¹³⁷Cs). Immediately after gamma irradiation, the single-cell gel electrophoresis assay was performed to detect DNA damage. For each exposure condition, one set of samples was treated with proteinase K (1 mg/ml) to remove any possible DNA-protein crosslinks. To measure DNA-protein crosslinks independent of DNA-DNA crosslinks, we quantified the proteins that were recovered with DNA after microwave exposure, using CDDP and gamma irradiation, positive controls for DNA-protein crosslinks. Ionizing radiation (4 Gy) induced significant DNA damage. However, no DNA damage could be detected after exposure to 2450 MHz CW microwaves alone. The crosslinking agent CDDP significantly reduced both the comet length and the normalized comet moment in C3H 10T(1/2) cells irradiated with 4 Gy gamma rays. In contrast, 2450 MHz microwaves did not impede the DNA migration induced by gamma rays. When control cells were treated with proteinase K, both parameters increased in the absence of any DNA damage. However, no additional effect of proteinase K was seen in samples exposed to 2450 MHz microwaves or in samples treated with the combination of microwaves and radiation. On the other hand, proteinase K treatment was ineffective in restoring any migration of the DNA in cells pretreated with CDDP and irradiated with gamma rays. When DNA-protein crosslinks were specifically measured, we found no evidence for the induction of DNA-protein crosslinks or changes in amount of the protein associated with DNA by 2450 MHz CW microwave exposure. Thus 2-h exposures to 1.9 W/ kg of 2450 MHz CW microwaves did not induce measurable alkali-labile DNA damage or DNA-DNA or DNA-protein crosslinks.

(E) Lai H, Singh NP, Acute low-intensity microwave exposure increases DNA single-strand breaks in rat brain cells. Bioelectromagnetics 16(3):207-210, 1995. (VO, AE, GT)

Levels of DNA single-strand break were assayed in brain cells from rats acutely exposed to low-intensity 2450 MHz microwaves using an alkaline microgel electrophoresis method. Immediately after 2 h of exposure to pulsed (2 microseconds width, 500 pulses/s) microwaves, no significant effect was observed, whereas a dose rate-dependent [0.6 and 1.2 W/kg whole body specific absorption rate (SAR)] increase in DNA single-strand breaks was found in brain cells of rats at 4 h postexposure. Furthermore, in rats exposed for 2 h to continuous-wave 2450 MHz microwaves (SAR 1.2 W/kg), increases in brain cell DNA single-strand breaks were observed immediately as well as at 4 h postexposure.

(E) Lai H, Singh NP, Single- and double-strand DNA breaks in rat brain cells after acute exposure to radiofrequency electromagnetic radiation. Int J Radiat Biol

69(4):513-521, 1996. (VO, AE, GT)

We investigated the effects of acute (2-h) exposure to pulsed (2-micros pulse width, 500 pulses s(-1)) and continuous wave 2450-MHz radiofrequency electromagnetic radiation on DNA strand breaks in brain cells of rat. The spatial averaged power density of the radiation was 2mW/cm², which produced a whole-body average-specific absorption rate of 1.2W/kg. Single- and double-strand DNA breaks in individual brain cells were measured at 4h post-exposure using a microgel electrophoresis assay. An increase in both types of DNA strand breaks was observed after exposure to either the pulsed or continuous-wave radiation, No significant difference was observed between the effects of the two forms of radiation. We speculate that these effects could result from a direct effect of radiofrequency electromagnetic energy on DNA molecules and/or impairment of DNA-damage repair mechanisms in brain cells. Our data further support the results of earlier in vitro and in vivo studies showing effects of radiofrequency electromagnetic radiation on DNA.

(E) Lai, H, Singh, NP, Melatonin and a spin-trap compound block radiofrequency electromagnetic radiation-induced DNA strand breaks in rat brain cells. Bioelectromagnetics 18(6):446-454, 1997. (VO, AE, GT, OX)

Effects of in vivo microwave exposure on DNA strand breaks, a form of DNA damage, were investigated in rat brain cells. In previous research, we have found that acute (2 hours) exposure to pulsed (2 microseconds pulses, 500 pps) 2450-MHz radiofrequency electromagnetic radiation (RFR) (power density 2 mW/cm², average whole body specific absorption rate 1.2 W/kg) caused an increase in DNA single- and double-strand breaks in brain cells of the rat when assayed 4 hours post exposure using a microgel electrophoresis assay. In the present study, we found that treatment of rats immediately before and after RFR exposure with either melatonin (1 mg/kg/injection, SC) or the spin-trap compound N-tert-butyl-alpha-phenylnitron (PBN) (100 mg/kg/injection, i.p.) blocks this effects of RFR. Since both melatonin and PBN are efficient free radical scavengers it is hypothesized that free radicals are involved in RFR-induced DNA damage in the brain cells of rats. Since cumulated DNA strand breaks in brain cells can lead to neurodegenerative diseases and cancer and an excess of free radicals in cells has been suggested to be the cause of various human diseases, data from this study could have important implications for the health effects of RFR exposure.

(E) Lai H, Singh NP, Interaction of microwaves and a temporally incoherent magnetic field on single and double DNA strand breaks in rat brain cells. Electromag Biol Med 24:23-29, 2005. (VO, AE, GT, IX)

The effect of a temporally incoherent magnetic field ('noise') on microwave-induced DNA single and double strand breaks in rat brain cells was investigated. Four treatment groups of rats were studied: microwave-exposure (continuous-wave 2450-MHz microwaves, power density 1 mW/cm², average whole body specific absorption rate of 0.6 W/kg), 'noise'-exposure (45 mG), 'microwave + noise'-exposure, and sham-exposure. Animals were exposed to these conditions for 2 hrs. DNA single and double strand breaks in brain cells of these animals were assayed 4 hrs later using a microgel electrophoresis assay. Results show that brain cells of microwave-

exposed rats had significantly higher levels of DNA single and double strand breaks when compared with sham-exposed animals. Exposure to 'noise' alone did not significantly affect the levels (i.e., they were similar to those of the sham-exposed rats). However, simultaneous 'noise' exposure blocked microwave-induced increases in DNA strand breaks. These data indicate that simultaneous exposure to a temporally incoherent magnetic field could block microwave-induced DNA damage in brain cells of the rat.

(E) Lai, H, Carino, MA, Singh, NP, Naltrexone blocks RFR-induced DNA double strand breaks in rat brain cells. Wireless Networks 3:471-476, 1997. (VO, AE, GT)

Previous research in our laboratory has shown that various effects of radiofrequency electromagnetic radiation (RFR) exposure on the nervous system are mediated by endogenous opioids in the brain. We have also found that acute exposure to RFR induced DNA strand breaks in brain cells of the rat. The present experiment was carried out to investigate whether endogenous opioids are also involved in RFR-induced DNA strand breaks. Rats were treated with the opioid antagonist naltrexone (1 mg/kg, IP) immediately before and after exposure to 2450-MHz pulsed (2 μ s pulses, 500 pps) RFR at a power density of 2 mW/cm² (average whole body specific absorption rate of 1.2 W/kg) for 2 hours. DNA double strand breaks were assayed in brain cells at 4 hours after exposure using a microgel electrophoresis assay. Results showed that the RFR exposure significantly increased DNA double strand breaks in brain cells of the rat, and the effect was partially blocked by treatment with naltrexone. Thus, these data indicate that endogenous opioids play a mediating role in RFR-induced DNA strand breaks in brain cells of the rat.

(E) Lakshmi NK, Tiwari R, Bhargava SC, Ahuja YR. Investigations on DNA damage and frequency of micronuclei in occupational exposure to electromagnetic fields (EMFs) emitted from video display terminals (VDTs). Gen Mol Biol 33, 154-158, 2010. (HU, LE, GT)

The potential effect of electromagnetic fields (EMFs) emitted from video display terminals (VDTs) to elicit biological response is a major concern for the public. The software professionals are subjected to cumulative EMFs in their occupational environments. This study was undertaken to evaluate DNA damage and incidences of micronuclei in such professionals. To the best of our knowledge, the present study is the first attempt to carry out cytogenetic investigations on assessing bioeffects in personal computer users. The study subjects (n = 138) included software professionals using VDTs for more than 2 years with age, gender, socioeconomic status matched controls (n = 151). DNA damage and frequency of micronuclei were evaluated using alkaline comet assay and cytochalasin blocked micronucleus assay respectively. Overall DNA damage and incidence of micronuclei showed no significant differences between the exposed and control subjects. With exposure characteristics, such as total duration (years) and frequency of use (minutes/day) sub-groups were assessed for such parameters. Although cumulative frequency of use showed no significant changes in the DNA integrity of the classified sub-groups, the long-term users (> 10 years) showed higher induction of DNA damage and increased frequency of micronuclei and micro nucleated cells.

(E) Lameth J, Arnaud-Cormos D, Lévêque P, Boillée S, Edeline JM, Mallat M. Effects of a single head exposure to GSM-1800 MHz signals on the transcriptome profile in the rat cerebral cortex: enhanced gene responses under proinflammatory conditions. Neurotox Res. 2020 Mar 21. doi: 10.1007/s12640-020-00191-3. [Epub ahead of print] (VO, AE, GE, IX)

Mobile communications are propagated by electromagnetic fields (EMFs), and since the 1990s, they operate with pulse-modulated signals such as the GSM-1800 MHz. The biological effects of GSM-EMF in humans affected by neuropathological processes remain seldom investigated. In this study, a 2-h head-only exposure to GSM-1800 MHz was applied to (i) rats undergoing an acute neuroinflammation triggered by a lipopolysaccharide (LPS) treatment, (ii) age-matched healthy rats, or (iii) transgenic hSOD1^{G93A} rats that modeled a presymptomatic phase of human amyotrophic lateral sclerosis (ALS). Gene responses were assessed 24 h after the GSM head-only exposure in a motor area of the cerebral cortex (mCx) where the mean specific absorption rate (SAR) was estimated to be 3.22 W/kg. In LPS-treated rats, a genome-wide mRNA profiling was performed by RNA-seq analysis and revealed significant (adjusted p value < 0.05) but moderate (fold changes < 2) upregulations or downregulations affecting 2.7% of the expressed genes, including genes expressed predominantly in neuronal or in glial cell types and groups of genes involved in protein ubiquitination or dephosphorylation. Reverse transcription-quantitative PCR analyses confirmed gene modulations uncovered by RNA-seq data and showed that in a set of 15 PCR-assessed genes, significant gene responses to GSM-1800 MHz depended upon the acute neuroinflammatory state triggered in LPS-treated rats, because they were not observed in healthy or in hSOD1^{G93A} rats. Together, our data specify the extent of cortical gene modulations triggered by GSM-EMF in the course of an acute neuroinflammation and indicate that GSM-induced gene responses can differ according to pathologies affecting the CNS.

(NE) Lamkowski A, Kreitlow M, Radunz J, Willenbockel M, Sabath F, Schuhn W, Stierner M, Fichte LO, Dudzinski M, Böhmelt S, Ullmann R, Majewski M, Franchini V, Eder S, Rump A, Port M, Abend M. Gene expression analysis in human peripheral blood cells after 900 MHz RF-EMF short-term exposure. Radiat Res. 189(5):529-540, 2018. (VT, AE, GE)

Radiofrequency electromagnetic fields (RF-EMF) are a basic requirement of modern wireless communication technology. Statutory thresholds of RF-EMF are established to limit relevant additional heat supply in human tissue. Nevertheless, to date, questions concerning nonthermal biological effects have yet to be fully addressed. New versions of microarrays (8 × 60K v2) provide a higher resolution of whole genome gene expression to display adaptive processes in cells after irradiation. In this ex vivo/ in vitro study, we irradiated peripheral blood cells from five donors with a continuous wave of 900 MHz RF-EMF for 0, 30, 60 and 90 min. Gene expression changes (P ≤ 0.05 and ≥twofold differences above or below the room temperature control exposed samples) were evaluated with microarray analysis. The results were compared with data from room temperature + 2°C samples. Verification of microarray results was performed using bioinformatic analyses and qRT-PCR. We registered a lack of an EMF-specific

gene expression response after applying the false discovery rate adjustment (FDR), using a high-stringency approach. Low-stringency analysis revealed 483 statistically significant deregulated transcripts in all RF-EMF groups relative to the room temperature exposed samples without an association with their corresponding room temperature + 2°C controls. Nevertheless, these transcripts must be regarded as statistical artefacts due to the absence of a targeted biological response, including enrichment and network analyses administered to microarray expressed gene subset profiles. Correspondingly, 14 most promising candidate transcripts examined by qRT-PCR displayed an absence of correlation with respect to the microarray results. In conclusion, these findings indicate that 900 MHz EMF exposure establishing an average specific absorption rate of 9.3 W/kg to whole blood cells is insufficient to induce nonthermal effects in gene expression during short-time exposure up to 90 min.

(NE) Lamkowski A, Kreitlow M, Radunz J, Willenbockel M, Stierner M, Fichte LO, Rädels CF, Majewski M, Ostheim P, Port M, Abend M. Analyzing the impact of 900 MHz EMF short-term exposure to the expression of 667 miRNAs in human peripheral blood cells. Sci Rep. 2021 Feb 24;11(1):4444. doi: 10.1038/s41598-021-82278-1. (VT, AE, GE)

More than ever before, people around the world are frequently exposed to different sections of the electromagnetic spectrum, mainly emitted from wireless modern communication technologies. Especially, the level of knowledge on non-thermal biological EMF effects remains controversial. New technologies allow for a more detailed detection of non-coding RNAs which affect the post-transcriptional control. Such method shall be applied in this work to investigate the response of human blood cells to electromagnetic irradiation. In this ex vivo in vitro study, we exposed peripheral blood cells from 5 male donors to a continuous wave of 900 MHz EMF for 0, 30, 60 and 90 min. Significant micro RNA (miRNA) expression changes ($p \leq 0.05$) above or below the SHAM exposed samples were evaluated using a quantitative real time PCR platform for simultaneous detection of 667 miRNAs called low density array. Only significant miRNA expression changes which were detectable in at least 60% of the samples per exposure group were analyzed. The results were compared with data from room temperature + 2 °C (RT + 2 °C) samples (here referred to as hyperthermia) to exclude miRNA expression altered by hyperthermia. The validation study by using the same donors and study design was performed after an interval of 2 years. When analyzing a total of 667 miRNAs during the screening study, 2 promising candidate miRNAs were identified, which were down regulated almost twice and showed a complete separation from the unexposed control group (miR-194 at 30 min and miR-939 at 60 min). The p-values even survived the Bonferroni correction for multiple comparisons ($p = 0.0007$ and $p = 0.004$, respectively). None of these miRNAs were expressed at a second time point after EMF exposure. Following an alternative analysis approach, we examined for miRNAs revealing an expected significant association of differential miRNA expression with the dose-time EMF exposure product, separately for each donor. Donors 2 and 3 revealed 11 and 10 miRNA species being significantly associated with EMF exposure which differed significantly from the other donors showing a minor number of differentially expressed miRNAs and could identify donors 2 and 3 as particularly EMF-responsive. The measurements were repeated after 2 years. The number of expressed/non-expressed miRNAs was almost similar (97.4%), but neither the number nor the previously differentially expressed miRNAs could be reproduced. Our data

neither support evidence of early changes at miRNA expression level in human whole blood cells after 900 MHz EMF exposure nor the identification of EMF-responsive individuals.

(E) Le Quément C, Nicolas Nicolaz C, Zhadobov M, Desmots F, Sauleau R, Aubry M, Michel D, Le Dréan Y. Whole-genome expression analysis in primary human keratinocyte cell cultures exposed to 60 GHz radiation. *Bioelectromagnetics* 33(2):147-158, 2012.(VT, AE, GE)

The main purpose of this study is to investigate potential responses of skin cells to millimeter wave (MMW) radiation increasingly used in the wireless technologies. Primary human skin cells were exposed for 1, 6, or 24 h to 60.4 GHz with an average incident power density of 1.8 mW/cm² and an average specific absorption rate of 42.4 W/kg. A large-scale analysis was performed to determine whether these exposures could affect the gene expression. Gene expression microarrays containing over 41,000 unique human transcript probe sets were used, and data obtained for sham and exposed cells were compared. No significant difference in gene expression was observed when gene expression values were subjected to a stringent statistical analysis such as the Benjamini-Hochberg procedure. However, when a t-test was employed to analyze microarray data, 130 transcripts were found to be potentially modulated after exposure. To further quantitatively analyze these preselected transcripts, real-time PCR was performed on 24 genes with the best combination of high fold change and low P-value. Five of them, namely CRIP2, PLXND1, PTX3, SERPINF1, and TRPV2, were confirmed as differentially expressed after 6 h of exposure. To the best of our knowledge, this is the first large-scale study reporting on potential gene expression modification associated with MMW radiation used in wireless communication applications.

(E) Lee K-S, Choi J-S, Hong S-Y, Son T-H, Yu K. Mobile phone electromagnetic radiation activates MAPK signaling and regulates viability in *Drosophila*. *Bioelectromagnetics* 29(5):371-379, 2008. (VO, AE, GE)

Mobile phones are widely used in the modern world. However, biological effects of electromagnetic radiation produced by mobile phones are largely unknown. In this report, we show biological effects of the mobile phone 835 MHz electromagnetic field (EMF) in the *Drosophila* model system. When flies were exposed to the specific absorption rate (SAR) 1.6 W/kg, which is the proposed exposure limit by the American National Standards Institute (ANSI), more than 90% of the flies were viable even after the 30 h exposure. However, in the SAR 4.0 W/kg strong EMF exposure, viability dropped from the 12 h exposure. These EMF exposures triggered stress response and increased the production of reactive oxygen species. The EMF exposures also activated extracellular signal regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) signaling, but not p38 kinase signaling. Interestingly, SAR 1.6 W/kg activated mainly ERK signaling and expression of an anti-apoptotic gene, whereas SAR 4.0 W/kg strongly activated JNK signaling and expression of apoptotic genes. In addition, SAR 4.0 W/kg amplified the number of apoptotic cells in the fly brain. These findings demonstrate that the exposure limit

on electromagnetic radiation proposed by ANSI triggered ERK-survival signaling but the strong electromagnetic radiation activated JNK-apoptotic signaling in *Drosophila*.

(E) Lee S, Johnson D, Dunbar K, Hui Dong, Xijin Ge, Yeong C Kim, Claudia Wing, Nimanthi Jayathilaka, Nimmi Emmanuel, Chenn Q Zhou, Howard L Gerber, Charles C Tseng, San Ming Wang. 2.45 GHz radiofrequency fields alter gene expression in cultured human cells. FEBS Lett 579(21):4829-4836, 2005. (VT, AE, GE)

The biological effect of radiofrequency (RF) fields remains controversial. We address this issue by examining whether RF fields can cause changes in gene expression. We used the pulsed RF fields at a frequency of 2.45 GHz that is commonly used in telecommunication to expose cultured human HL-60 cells. We used the serial analysis of gene expression (SAGE) method to measure the RF effect on gene expression at the genome level. We observed that 221 genes altered their expression after a 2-h exposure. The number of affected genes increased to 759 after a 6-h exposure. Functional classification of the affected genes reveals that apoptosis-related genes were among the upregulated ones and the cell cycle genes among the downregulated ones. We observed no significant increase in the expression of heat shock genes. These results indicate that the RF fields at 2.45 GHz can alter gene expression in cultured human cells through non-thermal mechanism.

(NE) Lerchl A, Klose M, Drees K. No Increased DNA Damage Observed in the Brain, Liver, and Lung of Fetal Mice Treated With Ethylnitrosourea and Exposed to UMTS Radiofrequency Electromagnetic Fields. Bioelectromagnetics 2020 Oct 8. doi: 10.1002/bem.22301. Online ahead of print. (VO, LE, GT, IX)

The widespread use of mobile phones and Wi-Fi-based communication devices makes exposure to radiofrequency electromagnetic fields (RF-EMF) unavoidable. Previous experiments have revealed the tumor-promoting effects of non-ionizing RF-EMF in adult carcinogen-treated mice in utero. To extend these investigations, we tested whether these effects are due to the co-carcinogenicity of RF-EMF which would manifest as elevated DNA damage. Similar to previous experiments, pregnant mice were exposed to RF-EMF (Universal Mobile Telecommunication System [UMTS] standard, approximately 1,960 MHz) from day 7 post-conception (p.c.) at 0 (sham), 0.04, and 0.4 W/kg SAR. At day 14 p.c., the mice were injected with the carcinogen ethylnitrosourea (ENU, 40 mg/kg). At three time-points specifically 24, 36, and 72 h later, the pregnant females were sacrificed and the fetuses (n = 24-57) were removed. A dye (cy3) specific for adenyl adducts was used to detect DNA damage by fluorescence microscopy in the brain, liver, and lung of each fetus. Compared to control (0 W/kg SAR), exposure to RF-EMF had no effect on the formation of DNA adducts in the inspected tissues. We conclude that increased adenyl formation of DNA by RF-EMF exposure is not a valid explanation for the previously reported tumor-promoting effects of RF-RMF. Our findings may help to gain a deeper insight into the biological effects of RF-EMF exposure in the context of malignancy.

(E) Li H, Yu G, Yong Z, Qiao S, Zhi W, Ma L, Xu X, Zhao X, Zhang J, Wang L, Hu X. Associations Between a Polymorphism in the Rat 5-HT1A Receptor Gene Promoter Region (rs198585630) and Cognitive Alterations Induced by Microwave Exposure. Front Pub

Health. 10. 2022. doi: 10.3389/fpubh.2022.802386. (VT, VO, LE, GE)

The nervous system is a sensitive target of electromagnetic radiation (EMR). Chronic microwave exposure can induce cognitive deficits, and 5-HT system is involved in this effect. Genetic polymorphisms lead to individual differences. In this study, we evaluated whether the single-nucleotide polymorphism (SNP) rs198585630 of 5-HT_{1A} receptor is associated with cognitive alterations in rats after microwave exposure with a frequency of 2.856 GHz and an average power density of 30 mW/cm². Rats were exposed to microwaves for 6 min three times a week for up to 6 weeks. PC12 cells and 293T cells were exposed to microwaves for 5 min up to 3 times at 2 intervals of 5 min. Transcriptional activity of 5-HT_{1A} receptor promoter containing rs198585630 C/T allele was determined in vitro. Electroencephalograms (EEGs), spatial learning and memory, and mRNA and protein expression of 5-HT_{1A} receptor were evaluated in vivo. We demonstrated that transcriptional activity of 5-HT_{1A} receptor promoter containing rs198585630 C allele was higher than that of 5-HT_{1A} receptor promoter containing T allele. The transcriptional activity of 5-HT_{1A} receptor promoter was stimulated by 30 mW/cm² microwave exposure, and rs198585630 C allele was more sensitive to microwave exposure, as it showed stronger transcriptional activation. Rats carrying rs198585630 C allele exhibited increased mRNA and protein expression of 5-HT_{1A} receptor and were more susceptible to 30 mW/cm² microwave exposure, showing cognitive deficits and inhibition of brain electrical activity. These findings suggest SNP rs198585630 of the 5-HT_{1A} receptor is an important target for further research exploring the mechanisms of hypersensitivity to microwave exposure.

(NE) Li L, Bisht KS, LaGroye I, Zhang P, Straube WL, Moros EG, Roti Roti JL. Measurement of DNA damage in mammalian cells exposed in vitro to radiofrequency fields at sars of 3-5 w/kg. Radiat Res 156:328-332, 2001. (VT, AE, GT)

In the present study, we determined whether exposure of mammalian cells to 3.2-5.1 W/kg specific absorption rate (SAR) radiofrequency fields could induce DNA damage in murine C3H 10T(1/2) fibroblasts. Cell cultures were exposed to 847.74 MHz code-division multiple access (CDMA) and 835.62 frequency-division multiple access (FDMA) modulated radiations in radial transmission line (RTL) irradiators in which the temperature was regulated to 37.0 +/- 0.3 degrees C. Using the alkaline comet assay to measure DNA damage, we found no statistically significant differences in either comet moment or comet length between sham-exposed cells and those exposed for 2, 4 or 24 h to CDMA or FDMA radiations in either exponentially growing or plateau-phase cells. Further, a 4-h incubation after the 2-h exposure resulted in no significant changes in comet moment or comet length. Our results show that exposure of cultured C3H 10T(1/2) cells at 37 degrees C CDMA or FDMA at SAR values of up to 5.1 W/kg did not induce measurable DNA damage.

(E) Li M, Hao B, Zhang M, Reiter RJ, Lin S, Zheng T, Chen X, Ren Y, Yue L, Abay B, Chen G, Xu X, Shi Y, Fan L. Melatonin enhances radiofrequency-induced NK antitumor immunity, causing cancer metabolism reprogramming and inhibition of multiple pulmonary tumor development. Signal Transduct Target Ther 2021 Sep 1;6(1):330. (VO, AE, GE, IX)

Surgery is the common treatment for early lung cancer with multiple pulmonary nodules, but it is often accompanied by the problem of significant malignancy of other nodules in non-therapeutic areas. In this study, we found that a combined treatment of local radiofrequency ablation (RFA) and melatonin (MLT) greatly improved clinical outcomes for early lung cancer patients with multiple pulmonary nodules by minimizing lung function injury and reducing the probability of malignant transformation or enlargement of nodules in non-ablated areas. Mechanically, as demonstrated in an associated mouse lung tumor model, RFA not only effectively remove treated tumors but also stimulate antitumor immunity, which could inhibit tumor growth in non-ablated areas. MLT enhanced RFA-stimulated NK activity and exerted synergistic antitumor effects with RFA. Transcriptomics and proteomics analyses of residual tumor tissues revealed enhanced oxidative phosphorylation and reduced acidification as well as hypoxia in the tumor microenvironment, which suggests reprogrammed tumor metabolism after combined treatment with RFA and MLT. Analysis of residual tumor further revealed the depressed activity of MAPK, NF-kappa B, Wnt, and Hedgehog pathways and upregulated P53 pathway in tumors, which was in line with the inhibited tumor growth. Combined RFA and MLT treatment also reversed the Warburg effect and decreased tumor malignancy. These findings thus demonstrated that combined treatment of RFA and MLT effectively inhibited the malignancy of non-ablated nodules and provided an innovative non-invasive strategy for treating early lung tumors with multiple pulmonary nodules.

(E) Li R, Ma M, Li L, Zhao L, Zhang T, Gao X, Zhang D, Zhu Y, Peng Q, Luo X, Wang M. The Protective Effect of Autophagy on DNA Damage in Mouse Spermatoocyte-Derived Cells Exposed to 1800 MHz Radiofrequency Electromagnetic Fields. Cell Physiol Biochem. 48(1):29-41, 2018. (VT, AE, GT, OX, RP)

Background/aims: The effects of exposure to radiofrequency electromagnetic fields (RF-EMFs) on the male reproductive system have raised public concern and studies have shown that exposure to RF-EMFs can induce DNA damage and autophagy. However, there are no related reports on the role of autophagy in DNA damage in spermatoocytes, especially after exposure to RF-EMFs. The aim of the present study was to determine the mechanism and role of autophagy induced by RF-EMFs in spermatozoa cells. **Methods:** Mouse spermatoocyte-derived cells (GC-2) were exposed to RF-EMFs 4 W/kg for 24 h. The level of reactive oxygen species (ROS) was determined by ROS assay kit. Comet assay was utilized to detect DNA damage. Autophagy was detected by three indicators: LC3II/LC3I, autophagic vacuoles, and GFP-LC3 dots, which were measured by western blot, transmission electron microscopy, and transfection with GFP-LC3, respectively. The expression of the molecular signaling pathway AMP-activated protein kinase (AMPK)/mTOR was determined by western blot. **Results:** The results showed that RF-EMFs induced autophagy and DNA damage in GC-2 cells via ROS generation, and the autophagy signaling pathway AMPK/mTOR was activated by ROS generation. Furthermore, following inhibition of autophagy by knockdown of AMPK α , increased DNA damage was observed in GC-2 cells following RF-EMFs exposure, and overexpression of AMPK α promoted autophagy and attenuated DNA damage. **Conclusions:** These findings demonstrated that the autophagy which was induced by RF-EMFs via the AMPK/mTOR signaling pathway could prevent DNA damage in spermatozoa cells.

(E) Li Y, Deng P, Chen C, Ma Q, Pi H, He M, Lu Y, Gao P, Zhou C, He Z, Zhang Y, Yu Z, Zhang L. 1,800 MHz Radiofrequency Electromagnetic Irradiation Impairs Neurite Outgrowth With a Decrease in Rap1-GTP in Primary Mouse Hippocampal Neurons and Neuro2a Cells. Front Public Health 2021 Nov 22;9:771508.(VT, AE, GE)

Background: With the global popularity of communication devices such as mobile phones, there are increasing concerns regarding the effect of radiofrequency electromagnetic radiation (RF-EMR) on the brain, one of the most important organs sensitive to RF-EMR exposure at 1,800 MHz. However, the effects of RF-EMR exposure on neuronal cells are unclear. Neurite outgrowth plays a critical role in brain development, therefore, determining the effects of 1,800 MHz RF-EMR exposure on neurite outgrowth is important for exploring its effects on brain development. **Objectives:** We aimed to investigate the effects of 1,800 MHz RF-EMR exposure for 48 h on neurite outgrowth in neuronal cells and to explore the associated role of the Rap1 signaling pathway. **Material and Methods:** Primary hippocampal neurons from C57BL/6 mice and Neuro2a cells were exposed to 1,800 MHz RF-EMR at a specific absorption rate (SAR) value of 4 W/kg for 48 h. CCK-8 assays were used to determine the cell viability after 24, 48, and 72 h of irradiation. Neurite outgrowth of primary hippocampal neurons (DIV 2) and Neuro2a cells was observed with a 20 × optical microscope and recognized by ImageJ software. Rap1a and Rap1b gene expressions were detected by real-time quantitative PCR. Rap1, Rap1a, Rap1b, Rap1GAP, and p-MEK1/2 protein expressions were detected by western blot. Rap1-GTP expression was detected by immunoprecipitation. The role of Rap1-GTP was assessed by transfecting a constitutively active mutant plasmid (Rap1-Gly_Val-GFP) into Neuro2a cells. **Results:** Exposure to 1,800 MHz RF-EMR for 24, 48, and 72 h at 4 W/kg did not influence cell viability. The neurite length, primary and secondary neurite numbers, and branch points of primary mouse hippocampal neurons were significantly impaired by 48-h RF-EMR exposure. The neurite-bearing cell percentage and neurite length of Neuro2a cells were also inhibited by 48-h RF-EMR exposure. Rap1 activity was inhibited by 48-h RF-EMR with no detectable alteration in either gene or protein expression of Rap1. The protein expression of Rap1GAP increased after 48-h RF-EMR exposure, while the expression of p-MEK1/2 protein decreased. Overexpression of constitutively active Rap1 reversed the decrease in Rap1-GTP and the neurite outgrowth impairment in Neuro2a cells induced by 1,800 MHz RF-EMR exposure for 48 h. **Conclusion:** Rap1 activity and related signaling pathways are involved in the disturbance of neurite outgrowth induced by 48-h 1,800 MHz RF-EMR exposure. The effects of RF-EMR exposure on neuronal development in infants and children deserve greater focus.

(E) Li ZQ, Zhang Y, Wan YM, Zhou Q, Liu C, Wu HX, Mu YZ, He YF, Rauniyar R, Wu XN. Testing of behavioral and cognitive development in rats after prenatal exposure to 1800 and 2400 MHz radiofrequency fields. J Radiat Res. 61:197-206, 2020. (VO, CE, GE, DE)

The objective of the study was to explore the effects of behavioral and cognitive development in rats after prenatal exposure to 1800 and 2400 MHz radiofrequency fields. Pregnant female rats were exposed to radiofrequency fields beginning on the 21st day of pregnancy. The indicators of physiological and behavioral development were observed and measured in the offspring rats: Y maze measured at 3-weeks postnatal, open field at 7-weeks postnatal, and the expression of N-methyl-D-aspartate receptors (NMDARs) measured by reverse transcription-PCR in the

hippocampus at 9-weeks postnatal. The body weight of the 1800 MHz group and the 1800 MHz + WiFi group showed a downward trend. The eye opening time of newborn rats was much earlier in the WiFi group than in the control group. Compared to the control group, the overall path length of the 1800 MHz + WiFi group was shortened and the stationary time was delayed. The path length of the WiFi group was shortened and the average velocity was increased in the error arm. The 1800 MHz + WiFi group displayed an increased trend in path length, duration, entry times and stationary time in the central area. In both the 1800 MHz + WiFi and WiFi groups, NR2A and NR2B expression was down-regulated, while NR2D, NR3A and NR3B were up-regulated. Moreover, NR1 and NR2C in the WiFi group were also up-regulated. Prenatal exposure to 1800 MHz and WiFi radiofrequency may affect the behavioral and cognitive development of offspring rats, which may be associated with altered mRNA expression of NMDARs in the hippocampus.

(E) Lian H-Y, Lin K-W, Yang C, Cai P. Generation and propagation of yeast prion [URE3] are elevated under electromagnetic field. Cell Stress Chaperones 23(4):581-594, 2018.(VO, AE, GE, OX)

In this study, we studied the effect of 2.0 GHz radio frequency electromagnetic field (RF-EMF) and 50 Hz extremely low frequency electromagnetic field (ELF-EMF) exposure on prion generation and propagation using two budding yeast strains, NT64C and SB34, as model organisms. Under exposure to RF-EMF or ELF-EMF, the de novo generation and propagation of yeast prions [URE3] were elevated in both strains. The elevation increased over time, and the effects of ELF-EMF occurred in a dose-dependent manner. The transcription and expression levels of the molecular chaperones Hsp104, Hsp70-Ssa1/2, and Hsp40-Ydj1 were not statistically significantly changed after exposure. Furthermore, the levels of ROS, as well as the activities of superoxide dismutase (SOD) and catalase (CAT), were significantly elevated after short-term, but not long-term exposure. This work demonstrated for the first time that EMF exposure could elevate the de novo generation and propagation of yeast prions and supports the hypothesis that ROS may play a role in the effects of EMF on protein misfolding. The effects of EMF on protein folding and ROS levels may mediate the broad effects of EMF on cell function.

(E) Lin KW, Yang CJ, Lian HY, Cai P. Exposure of ELF-EMF and RF-EMF increase the rate of glucose transport and TCA cycle in budding yeast. Front Microbiol. 7:1378, (2016). (VO, AE, GE)

In this study, we investigated the transcriptional response to 50 Hz extremely low frequency electromagnetic field (ELF-EMF) and 2.0 GHz radio frequency electromagnetic field (RF-EMF) exposure by Illumina sequencing technology using budding yeast as the model organism. The transcription levels of 28 genes were upregulated and those of four genes were downregulated under ELF-EMF exposure, while the transcription levels of 29 genes were upregulated and those of 24 genes were downregulated under RF-EMF exposure. After validation by reverse transcription quantitative polymerase chain reaction (RT-qPCR), a concordant direction of change both in differential gene expression (DGE) and RT-qPCR was demonstrated for nine genes under ELF-EMF exposure and for 10 genes under RF-EMF exposure. The RT-qPCR results revealed that ELF-EMF and RF-EMF exposure can upregulate the expression of genes

involved in glucose transportation and the tricarboxylic acid (TCA) cycle, but not the glycolysis pathway. Energy metabolism is closely related with the cell response to environmental stress including EMF exposure. Our findings may throw light on the mechanism underlying the biological effects of EMF.

(E) Liu C, Duan W, Xu S, Chen C, He M, Zhang L, Yu Z, Zhou Z. Exposure to 1800 MHz radiofrequency electromagnetic radiation induces oxidative DNA base damage in a mouse spermatocyte-derived cell line. Toxicol Lett 218(1): 2-9, 2013a. (VT, AE, GT, OX, RP)

Whether exposure to radiofrequency electromagnetic radiation (RF-EMR) emitted from mobile phones can induce DNA damage in male germ cells remains unclear. In this study, we conducted a 24 h intermittent exposure (5 min on and 10 min off) of a mouse spermatocyte-derived GC-2 cell line to 1800 MHz Global System for Mobile Communication (GSM) signals in GSM-Talk mode at specific absorption rates (SAR) of 1 W/kg, 2 W/kg or 4 W/kg. Subsequently, through the use of formamidopyrimidine DNA glycosylase (FPG) in a modified comet assay, we determined that the extent of DNA migration was significantly increased at a SAR of 4 W/kg. Flow cytometry analysis demonstrated that levels of the DNA adduct 8-oxoguanine (8-oxoG) were also increased at a SAR of 4 W/kg. These increases were concomitant with similar increases in the generation of reactive oxygen species (ROS); these phenomena were mitigated by co-treatment with the antioxidant α -tocopherol. However, no detectable DNA strand breakage was observed by the alkaline comet assay. Taking together, these findings may imply the novel possibility that RF-EMR with insufficient energy for the direct induction of DNA strand breaks may produce genotoxicity through oxidative DNA base damage in male germ cells.

(E) Liu C, Gao P, Xu SC, Wang Y, Chen CH, He MD, Yu ZP, Zhang L, Zhou Z. Mobile phone radiation induces mode-dependent DNA damage in a mouse spermatocyte-derived cell line: a protective role of melatonin. Int J Radiat Biol. 89(11):993-1001, 2013b. (VT, AE, GT, OX, RP)

Purpose: To evaluate whether exposure to mobile phone radiation (MPR) can induce DNA damage in male germ cells. Materials and methods: A mouse spermatocyte-derived GC-2 cell line was exposed to a commercial mobile phone handset once every 20 minutes in standby, listen, dialed or dialing modes for 24 h. DNA damage was determined using an alkaline comet assay. Results: The levels of DNA damage were significantly increased following exposure to MPR in the listen, dialed and dialing modes. Moreover, there were significantly higher increases in the dialed and dialing modes than in the listen mode. Interestingly, these results were consistent with the radiation intensities of these modes. However, the DNA damage effects of MPR in the dialing mode were efficiently attenuated by melatonin pretreatment. Conclusions: These results regarding mode-dependent DNA damage have important implications for the safety of inappropriate mobile phone use by males of reproductive age and also suggest a simple preventive measure, keeping our body from mobile phones as far away as possible, not only during conversations but during "dialed" and "dialing" operation modes as well. Since the "dialed" mode is actually part of the standby mode, mobile phones should be kept at a safe distance from our body even during standby operation. Furthermore, the protective role of melatonin suggests that it may be a promising pharmacological candidate for preventing mobile phone use-related reproductive impairments.

(E) Liu Y-x, Tai J-l, Li G-q, Zhang Z-w, Xue J-h, Liu H-s, Zhu H, Cheng J-d, Liu Y-L, Li A-m, Zhang Y. Exposure to 1950-MHz TD-SCDMA electromagnetic fields affects the apoptosis of astrocytes via caspase-3-dependent pathway. *PLoS One* 2012;7(8):e42332. **(VT, AE, GE)**

The usage of mobile phone increases globally. However, there is still a paucity of data about the impact of electromagnetic fields (EMF) on human health. This study investigated whether EMF radiation would alter the biology of glial cells and act as a tumor-promoting agent. We exposed rat astrocytes and C6 glioma cells to 1950-MHz TD-SCDMA for 12, 24 and 48 h respectively, and found that EMF exposure had differential effects on rat astrocytes and C6 glioma cells. A 48 h of exposure damaged the mitochondria and induced significant apoptosis of astrocytes. Moreover, caspase-3, a hallmark of apoptosis, was highlighted in astrocytes after 48 h of EMF exposure, accompanied by a significantly increased expression of bax and reduced level of bcl-2. The tumorigenicity assays demonstrated that astrocytes did not form tumors in both control and exposure groups. In contrast, the unexposed and exposed C6 glioma cells show no significant differences in both biological feature and tumor formation ability. Therefore, our results implied that exposure to the EMF of 1950-MHz TD-SCDMA may not promote the tumor formation, but continuous exposure damaged the mitochondria of astrocytes and induce apoptosis through a caspase-3-dependent pathway with the involvement of bax and bcl-2.

(E) Lixia S, Yao K, Kaijun W, Deqiang L, Huajun H, Xiangwei G, Baohong W, Wei Z, Jianling L, Wei W. Effects of 1.8GHz radiofrequency field on DNA damage and expression of heat shock protein 70 in human lens epithelial cells. *Mutat Res* 602(1-2):135-42, 2006. **(VT, AE, GT, GE)**

To investigate the DNA damage, expression of heat shock protein 70 (Hsp70) and cell proliferation of human lens epithelial cells (hLEC) after exposure to the 1.8GHz radiofrequency field (RF) of a global system for mobile communications (GSM). An Xc-1800 RF exposure system was used to employ a GSM signal at 1.8GHz (217Hz amplitude-modulated) with the output power in the specific absorption rate (SAR) of 1, 2 and 3W/kg. After 2h exposure to RF, the DNA damage of hLEC was accessed by comet assay at five different incubation times: 0, 30, 60, 120 and 240min, respectively. Western blot and RT-PCR were used to determine the expression of Hsp70 in hLECs after RF exposure. The proliferation rate of cells was evaluated by bromodeoxyuridine incorporation on days 0, 1 and 4 after exposure. The results show that the difference of DNA-breaks between the exposed and sham-exposed (control) groups induced by 1 and 2W/kg irradiation were not significant at any incubation time point ($P>0.05$). The DNA damage caused by 3W/kg irradiation was significantly increased at the times of 0 and 30min after exposure ($P<0.05$), a phenomenon that could not be seen at the time points of 60, 120 or 240min ($P>0.05$). Detectable mRNA as well as protein expression of Hsp70 was found in all groups. Exposure at SARs of 2 and 3W/kg for 2h exhibited significantly increased Hsp70 protein expression ($P<0.05$), while no change in Hsp70 mRNA expression could be found in any of the groups ($P>0.05$). No difference of the cell proliferation rate between the sham-exposed and exposed cells was found at any exposure dose tested ($P>0.05$). The results indicate that exposure to non-thermal dosages of RF for wireless communications can induce no or repairable DNA damage and the increased Hsp70 protein expression in hLECs occurred without change in the cell proliferation rate. The non-thermal stress response of Hsp70 protein increase to RF exposure

might be involved in protecting hLEC from DNA damage and maintaining the cellular capacity for proliferation.

(E) López-Furelos A, Leiro-Vidal JM, Salas-Sánchez AA, Ares-Pena FJ, López-Martín ME. Evidence of cellular stress and caspase-3 resulting from a combined two-frequency signal in the cerebrum and cerebellum of sprague-dawley rats. *Oncotarget* 7(40):64674-64689, 2016. (VO, AE, GE)

Multiple simultaneous exposures to electromagnetic signals induced adjustments in mammal nervous systems. In this study, we investigated the non-thermal SAR (Specific Absorption Rate) in the cerebral or cerebellar hemispheres of rats exposed in vivo to combined electromagnetic field (EMF) signals at 900 and 2450 MHz. Forty rats divided into four groups of 10 were individually exposed or not exposed to radiation in a GTEM chamber for one or two hours. After radiation, we used the Chemiluminescent Enzyme-Linked Immunosorbent Assay (ChELISA) technique to measure cellular stress levels, indicated by the presence of heat shock proteins (HSP) 90 and 70, as well as caspase-3-dependent pre-apoptotic activity in left and right cerebral and cerebellar hemispheres of Sprague Dawley rats. Twenty-four hours after exposure to combined or single radiation, significant differences were evident in HSP 90 and 70 but not in caspase 3 levels between the hemispheres of the cerebral cortex at high SAR levels. In the cerebellar hemispheres, groups exposed to a single radiofrequency (RF) and high SAR showed significant differences in HSP 90, 70 and caspase-3 levels compared to control animals. The absorbed energy and/or biological effects of combined signals were not additive, suggesting that multiple signals act on nervous tissue by a different mechanism.

(E) López-Martín E, Bregains J, Relova-Quinteiro JL, Cadarso-Suárez C, Jorge-Barreiro FJ, Ares-Pena FJ. The action of pulse-modulated GSM radiation increases regional changes in brain activity and c-Fos expression in cortical and subcortical areas in a rat model of picrotoxin-induced seizure proneness. *J Neurosci Res.* 87(6):1484-1499, 2009. (VO, AE, GE, WS)

The action of the pulse-modulated GSM radiofrequency of mobile phones has been suggested as a physical phenomenon that might have biological effects on the mammalian central nervous system. In the present study, GSM-exposed picrotoxin-pretreated rats showed differences in clinical and EEG signs, and in c-Fos expression in the brain, with respect to picrotoxin-treated rats exposed to an equivalent dose of unmodulated radiation. Neither radiation treatment caused tissue heating, so thermal effects can be ruled out. The most marked effects of GSM radiation on c-Fos expression in picrotoxin-treated rats were observed in limbic structures, olfactory cortex areas and subcortical areas, the dentate gyrus, and the central lateral nucleus of the thalamic intralaminar nucleus group. Nonpicrotoxin-treated animals exposed to unmodulated radiation showed the highest levels of neuronal c-Fos expression in cortical areas. These results suggest a specific effect of the pulse modulation of GSM radiation on brain activity of a picrotoxin-induced seizure-proneness rat model and indicate that this mobile-phone-type radiation might induce regional changes in previous preexcitability conditions of neuronal activation.

(E) López-Martín E, Jorge-Barreiro FJ, Relova-Quintero JL, Salas-Sánchez AA, Ares-Pena FJ. Exposure to 2.45 GHz radiofrequency modulates calcitonin-dependent

activity and HSP-90 protein in parafollicular cells of rat thyroid gland. Tissue and Cell 68: 101478, 2021.(VO, AE, GE)

In this study we analyzed the response of parafollicular cells in rat thyroid gland after exposure to radiofrequency at 2.45 GHz using a subthermal experimental diathermy model. Forty-two Sprague Dawley rats, divided into two groups of 21 rats each, were individually exposed at 0 (control), 3 or 12 W in a Gigahertz Transverse Electro-Magnetic (GTEM) chamber for 30 min. After radiation, we used simple or fluorescence immunohistochemistry to measure calcitonin cells or cellular stress levels, indicated by the presence hyperplasia of parafollicular cells, heat shock protein (HSP) 90. Immunomarking of calcitonin-positive cells was statistically significant higher in the thyroid tissue of rats exposed to 2.45 GHz radiofrequency and cell hyperplasia appeared 90 min after radiation at the SAR levels studied. At the same time, co-localized expression of HSP-90 and calcitonin in parafollicular cells was statistically significant attenuated 90 min after radiation and remained statistically significantly low 24 h after radiation, even though parafollicular cell levels normalized. These facts indicate that subthermal radiofrequency (RF) at 2.45 GHz constitutes a negative external stress stimulus that alters the activity and homeostasis of parafollicular cells in the rat thyroid gland. However, further research is needed to determine if there is toxic action in human C cells.

(E) Luukkonen J, Hakulinen P, Mäki-Paakkanen J, Juutilainen J, Naarala J. Enhancement of chemically induced reactive oxygen species production and DNA damage in human SH-SY5Y neuroblastoma cells by 872MHz radiofrequency radiation. Mutat Res 662:54-58, 2009. (VT, AE, GT, OX, WS)

The objective of the study was to investigate effects of 872 MHz radiofrequency (RF) radiation on intracellular reactive oxygen species (ROS) production and DNA damage at a relatively high SAR value (5W/kg). The experiments also involved combined exposure to RF radiation and menadione, a chemical inducing intracellular ROS production and DNA damage. The production of ROS was measured using the fluorescent probe dichlorofluorescein and DNA damage was evaluated by the Comet assay. Human SH-SY5Y neuroblastoma cells were exposed to RF radiation for 1h with or without menadione. Control cultures were sham exposed. Both continuous waves (CW) and a pulsed signal similar to that used in global system for mobile communications (GSM) mobile phones were used. Exposure to the CW RF radiation increased DNA breakage (p<0.01) in comparison to the cells exposed only to menadione. Comparison of the same groups also showed that ROS level was higher in cells exposed to CW RF radiation at 30 and 60 min after the end of exposure (p<0.05 and p<0.01, respectively). No effects of the GSM signal were seen on either ROS production or DNA damage. The results of the present study suggest that 872MHz CW RF radiation at 5W/kg might enhance chemically induced ROS production and thus cause secondary DNA damage. However, there is no known mechanism that would explain such effects from CW RF radiation but not from GSM modulated RF radiation at identical SAR.

(NE) Luukkonen J, Juutilainen J, Naarala J. Combined effects of 872 MHz radiofrequency radiation and ferrous chloride on reactive oxygen species production and DNA damage in human SH-SY5Y neuroblastoma cells. Bioelectromagnetics 31:417-424, 2010. (VT, AE, GT, OX)

The aim of the present study was to investigate possible cooperative effects of radiofrequency (RF) radiation and ferrous chloride (FeCl) on reactive oxygen species (ROS) production and DNA damage. In order to test intracellular ROS production as a possible underlying mechanism of DNA damage, we applied the fluorescent probe DCFH-DA. Integrity of DNA was quantified by alkaline comet assay. The exposures to 872 MHz RF radiation were conducted at a specific absorption rate (SAR) of 5 W/kg using continuous waves (CW) or a modulated signal similar to that used in Global System for Mobile Communications (GSM) phones. Four groups were included: Sham exposure (control), RF radiation, Chemical treatment, Chemical treatment, and RF radiation. In the ROS production experiments, human neuroblastoma (SH-SY5Y) cells were exposed to RF radiation and 10 microg/ml FeCl for 1 h. In the comet assay experiments, the exposure time was 3 h and an additional chemical (0.015% diethyl maleate) was used to make DNA damage level observable. The chemical treatments resulted in statistically significant responses, but no effects from either CW or modulated RF radiation were observed on ROS production, DNA damage or cell viability.

(E) Maes A, Verschaeve L, Arroyo A, De Wagter C, Vercruyssen L, In vitro cytogenetic effects of 2450 MHz waves on human peripheral blood lymphocytes. *Bioelectromagnetics* 14(6):495-501, 1993. (VT, AE, GT)

Cytogenetic analyses were performed on human peripheral blood lymphocytes exposed to 2450 MHz microwaves during 30 and 120 min at a constant temperature of 36.1 degrees C (body temperature). The temperature was kept constant by means of a temperature probe put in the blood sample which gives feedback to a microcomputer that controls the microwave supply. We found a marked increase in the frequency of chromosome aberrations (including dicentric chromosomes and acentric fragments) and micronuclei. On the other hand the microwave exposure did not influence the cell kinetics nor the sister chromatid exchange (SCE) frequency.

(E)Maes A, Collier M, Slaets D, Verschaeve L. Cytogenetic effects of microwaves from Mobile communication frequencies (954 MHz). *Electro Magneto Biol.* 1995; 14: 91-98. (VT, AE, GT)

Whole blood samples were exposed to a 954 MHz emitting antenna from a GSM (Global System for Mobile Communication) base station and cultivated for analysis of chromosome aberrations. A limited number of blood samples from maintenance workers being professionally exposed to microwaves of this and other frequencies was also investigated. Although some cytogenetic damage was obtained *in vitro* when blood samples were very close to the antenna, we may, according to our results, consider that microwaves emitted by a GSM base station are not able to induce defects in the general population.

(E) Maes A, Collier M, Slaets D, Verschaeve L, 954 MHz microwaves enhance the mutagenic properties of mitomycin C. *Environ Mol Mutagen* 28(1):26-30, 1996. (VT, AE, GT, IX)

This paper focuses on the combined effects of microwaves from mobile communication frequencies and a chemical DNA damaging agent mitomycin C (MMC). The investigation was performed *in vitro* by exposing whole blood samples to a 954 MHz emitting antenna from a

GSM (Global System for Mobile Communication) base station, followed by lymphocyte cultivation in the presence of MMC. A highly reproducible synergistic effect was observed as based on the frequencies of sister chromatid exchanges in metaphase figures.

(NE) Maes A, Collier M, Van Gorp U, Vandoninck S, Verschaeve L, Cytogenetic effects of 935.2-MHz (GSM) microwaves alone and in combination with mitomycin C. *Mutat Res* 393(1-2):151-156, 1997. (VT, AE, GT, IX)

This paper focuses on the genetic effects of microwaves from mobile communication frequencies (935.2 MHz) alone and in combination with a chemical DNA-damaging agent (mitomycin C). Three cytogenetic endpoints were investigated after in vitro exposure of human whole blood cells. These endpoints were the 'classical' chromosome aberration test, the sister chromatid exchange test and the alkaline comet assay. No direct cytogenetic effect was found. The combined exposure of the cells to the radiofrequency fields followed by their cultivation in the presence of mitomycin C revealed a very weak effect when compared to cells exposed to mitomycin C alone.

(NE) Maes A, Collier M, Verschaeve L Cytogenetic investigations on microwaves emitted by a 455.7 MHz car phone. *Folia Biol (Praha)* 46(5):175-180, 2000. (VT, AE, GT, IX)

The chromosome aberration or sister chromatid exchange frequency was determined in 455.7 MHz microwave-exposed human lymphocytes and in lymphocytes that were subsequently exposed to MMC or X-rays. The exposure was performed by placing the cells at 5 cm from the antenna of a car phone. In this way the specific absorption ratio was approximately 6.5 W/kg. The temperature and humidity was kept constant during the experiments. No statistically significant difference was found between microwave-exposed and unexposed control samples. When the microwave exposure was followed by exposure to MMC, some differences were found between the combined treatments and the MMC treatments alone. However, there was no consistency in the results. Combined treatments with X-rays did not provide any indication of a synergistic action between the RF fields and X-rays, either. Our data therefore do not support the hypothesis that RF fields act synergistically with chemical or physical mutagens.

(NE) Maes A, Collier M, Verschaeve L Cytogenetic effects of 900 MHz (GSM) microwaves on human lymphocytes. *Bioelectromagnetics* 22(2):91-96, 2001. (VT, AE, GT, IX)

The cytogenetic effects of 900 MHz radiofrequency fields were investigated with the chromosome aberration and sister chromatid exchange frequency methods. Three different modes of exposure (continuous, pseudo-random and dummy burst) were studied for different power outputs (0, 2, 8, 15, 25, 50 W). The specific absorption rates varied between 0 and 10 W/kg. We investigated the possible effects of the 900 MHz radiation alone as well as of combined exposure to the chemical or physical mutagens mitomycin C and X-rays. Overall, no indication was found of a mutagenic, and/or co-mutagenic/synergistic effect of this kind of nonionizing radiation.

(NE) Maes A, Van Gorp U, Verschaeve L. Cytogenetic investigation of subjects professionally exposed to radiofrequency radiation. *Mutagenesis* 21:139-142, 2006. (HU, VT, LE, GT, IX)

Nowadays, virtually everybody is exposed to radiofrequency radiation (RFR) from mobile phone base station antennas or other sources. At least according to some scientists, this exposure can have detrimental health effects. We investigated cytogenetic effects in peripheral blood lymphocytes from subjects who were professionally exposed to mobile phone electromagnetic fields in an attempt to demonstrate possible RFR-induced genetic effects. These subjects can be considered well suited for this purpose as their RFR exposure is 'normal' though rather high, and definitely higher than that of the 'general population'. The alkaline comet assay, sister chromatid exchange (SCE) and chromosome aberration tests revealed no evidence of RFR-induced genetic effects. Blood cells were also exposed to the well known chemical mutagen mitomycin C in order to investigate possible combined effects of RFR and the chemical. No cooperative action was found between the electromagnetic field exposure and the mutagen using either the comet assay or SCE test.

(NE) Malini V, Resolving the enigma of effect of mobile phone usage on spermatogenesis in humans in south Indian population. *Asian J Pharmaceut Clin Res.* 2017; 10: 233-237. (HU, LE, GT)

Objective: This study was aimed at to evaluate the possible risk of radiofrequency and electromagnetic waves of mobile phones on spermatogenic impairment and functional capacity of the spermatozoa along with oxidative stress, DNA damages, and hormone profile among mobile phone users. **Methods:** Mobile phone users were classified into three groups are 1-5, 6-10, and above 10 hrs/day, respectively, based on the exposure to electromagnetic radiation. Blood and semen samples are collected with informed consent letter. The semen samples used to carry out to the physical examination such as volume, liquefaction time, color, odor, pH, and viscosity, and functional status of the spermatozoa was carried out such as nuclear chromatin decondensation test, hypo-osmotic swelling test, and acrosomal intactness test. Seminal plasma was used for to evaluate the oxidative stress markers superoxide dismutase (SOD) activity, reactive oxygen species (ROS) levels, and total antioxidant capacity (TAC). Blood serum was used to estimate the level of follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone. DNA collected from blood used for DNA ladder assay. **Results:** In the present investigation, both physical and microscopic examinations were negatively correlated with mobile phone usage. No variation exists in functional status of spermatozoa. Oxidative stress markers such as the presence of ROS, enzymatic scavengers such as SOD and TAC showed no statistical variations between control group and mobile phone users and even no variations in hormone profile such as testosterone, FSH, and LH of users of mobile phone compared to normal reference values. **Conclusion:** In conclusion, though the literature has suggested that mobile phone use alters semen parameters, functional status of spermatozoa, increased oxidative stress, with subsequent sperm DNA damage in humans. The present study deviates from previous study stating nil impact of mobile phones on spermatogenic impairment in humans.

(NE) Malyapa RS, Ahern EW, Straube WL, Moros EG, Pickard WF, Roti Roti JL, Measurement of DNA damage after exposure to 2450 MHz electromagnetic radiation. *Radiat Res* 148(6):608-

617, 1997a. (VT, AE, GT)

Recent reports suggest that exposure to 2450 MHz electromagnetic radiation causes DNA single-strand breaks (SSBs) and double-strand breaks (DSBs) in cells of rat brain irradiated in vivo (Lai and Singh, Bioelectromagnetics 16, 207-210, 1995; Int. J. Radiat. Biol. 69, 513-521, 1996). Therefore, we endeavored to determine if exposure of cultured mammalian cells in vitro to 2450 MHz radiation causes DNA damage. The alkaline comet assay (single-cell gel electrophoresis), which is reportedly the most sensitive method to assay DNA damage in individual cells, was used to measure DNA damage after in vitro 2450 MHz irradiation. Exponentially growing U87MG and C3H 10T1/2 cells were exposed to 2450 MHz continuous-wave (CW) radiation in specially designed radial transmission lines (RTLs) that provided relatively uniform microwave exposure. Specific absorption rates (SARs) were calculated to be 0.7 and 1.9 W/kg. Temperatures in the RTLs were measured in real time and were maintained at 37 +/- 0.3 degrees C. Every experiment included sham exposure(s) in an RTL. Cells were irradiated for 2 h, 2 h followed by a 4-h incubation at 37 degrees C in an incubator, 4 h and 24 h. After these treatments samples were subjected to the alkaline comet assay as described by Olive et al. (Exp. Cell Res. 198, 259-267, 1992). Images of comets were digitized and analyzed using a PC-based image analysis system, and the "normalized comet moment" and "comet length" were determined. No significant differences were observed between the test group and the controls after exposure to 2450 MHz CW irradiation. Thus 2450 MHz irradiation does not appear to cause DNA damage in cultured mammalian cells under these exposure conditions as measured by this assay.

(NE) Malyapa RS, Ahern EW, Straube WL, Moros EG, Pickard WF, Roti Roti JL. Measurement of DNA damage after exposure to electromagnetic radiation in the cellular phone communication frequency band (835.62 and 847.74 MHz). Radiat Res 148(6):618-627, 1997b. (VT, AE, GT)

Mouse C3H 10T1/2 fibroblasts and human glioblastoma U87MG cells were exposed to cellular phone communication frequency radiations to investigate whether such exposure produces DNA damage in in vitro cultures. Two types of frequency modulations were studied: frequency-modulated continuous-wave (FMCW), with a carrier frequency of 835.62 MHz, and code-division multiple-access (CDMA) centered on 847.74 MHz. Exponentially growing (U87MG and C3H 10T1/2 cells) and plateau-phase (C3H 10T1/2 cells) cultures were exposed to either FMCW or CDMA radiation for varying periods up to 24 h in specially designed radial transmission lines (RTLs) that provided relatively uniform exposure with a specific absorption rate (SAR) of 0.6 W/kg. Temperatures in the RTLs were monitored continuously and maintained at 37 +/- 0.3 degrees C. Sham exposure of cultures in an RTL (negative control) and 137Cs gamma-irradiated samples (positive control) were included with every experiment. The alkaline comet assay as described by Olive et al. (Exp. Cell Res. 198, 259-269, 1992) was used to measure DNA damage. No significant differences were observed between the test group exposed to FMCW or CDMA radiation and the sham-treated negative controls. Our results indicate that exposure of cultured mammalian cells to cellular phone communication frequencies under these conditions at an SAR of 0.6 W/kg does not cause DNA damage as measured by the alkaline comet assay.

(NE) Malyapa RS, Ahern EW, Bi C, Straube WL, LaRegina M, Pickard WF, Roti RotiJL, DNA damage in rat brain cells after in vivo exposure to 2450 MHz electromagnetic radiation and various methods of euthanasia. Radiat Res 149(6):637-645, 1998. (VO, AE, GT)

The present study was done to confirm the reported observation that low-intensity acute exposure to 2450 MHz radiation causes DNA single-strand breaks (Lai and Singh, Bioelectromagnetics 16, 207-210, 1995). Male Sprague-Dawley rats weighing approximately 250 g were irradiated with 2450 MHz continuous-wave (CW) microwaves for 2 h at a specific absorption rate of 1.2 W/kg in a cylindrical waveguide system (Guy et al., Radio Sci. 14, 63-74, 1979). There was no associated rise in the core body temperature of the rats. After the irradiation or sham treatments, rats were euthanized by either CO₂ asphyxia or decapitation by guillotine (eight pairs of animals per euthanasia group). After euthanasia the brains were removed and immediately immersed in cold Ames medium and the cells of the cerebral cortex and the hippocampus were dissociated separately and subjected to the alkaline comet assay. Irrespective of whether the rats were euthanized by CO₂ asphyxia or decapitated by guillotine, no significant differences were observed between either the comet length or the normalized comet moment of cells from either the cerebral cortex or the hippocampus of sham-treated rats and those from the irradiated rats. However, the data for the rats asphyxiated with CO₂ showed more intrinsic DNA damage and more experiment-to-experiment variation than did the data for rats euthanized by guillotine. Therefore, the guillotine method of euthanasia is the most appropriate in studies relating to DNA damage. Furthermore, we did not confirm the observation that DNA damage is produced in cells of the rat cerebral cortex or the hippocampus after a 2-h exposure to 2450 MHz CW microwaves or at 4 h after the exposure.

(E) Manta AK, Papadopoulou D, Polyzos AP, Fragopoulou AF, Skouroliakou AS, Thanos D, Stravopodis DJ, Margaritis LH. Mobile-phone radiation-induced perturbation of gene-expression profiling, redox equilibrium and sporadic-apoptosis control in the ovary of *Drosophila melanogaster*. Fly (Austin). 11(2):75-95, 2017. (VO, AE, GE, OX)

The daily use by people of wireless communication devices has increased exponentially in the last decade, begetting concerns regarding its potential health hazards. *Drosophila melanogaster* four days-old adult female flies were exposed for 30 min to radiation emitted by a commercial mobile phone at a SAR of 0.15 W/kg and a SAE of 270 J/kg. ROS levels and apoptotic follicles were assayed in parallel with a genome-wide microarrays analysis. ROS cellular contents were found to increase by 1.6-fold (x), immediately after the end of exposure, in follicles of pre-choriogenic stages (germarium - stage 10), while sporadically generated apoptotic follicles (germarium 2b and stages 7-9) presented with an averaged 2x upregulation in their sub-population mass, 4 h after fly's irradiation with mobile device. Microarray analysis revealed 168 genes being differentially expressed, 2 h post-exposure, in response to radiofrequency (RF) electromagnetic field-radiation exposure ($\geq 1.25x$, $P < 0.05$) and associated with multiple and critical biological processes, such as basic metabolism and cellular subroutines related to stress response and apoptotic death. Exposure of adult flies to mobile-phone radiation for 30 min has an immediate impact on ROS production in animal's ovary, which seems to cause a global,

systemic and non-targeted transcriptional reprogramming of gene expression, 2 h post-exposure, being finally followed by induction of apoptosis 4 h after the end of exposure. Conclusively, this unique type of pulsed radiation, mainly being derived from daily used mobile phones, seems capable of mobilizing critical cytopathic mechanisms, and altering fundamental genetic programs and networks in D. melanogaster.

(E) Manti L, Braselmann H, Calabrese ML, Massa R, Pugliese M, Scampoli P, Sicignano G, Grossi G. Effects of modulated microwave radiation at cellular telephone frequency (1.95 GHz) on X-ray-induced chromosome aberrations in human lymphocytes in vitro. Radiat Res 169:575-583, 2008. (VT, AE, GT, IX)

The case for a DNA-damaging action produced by radiofrequency (RF) signals remains controversial despite extensive research. With the advent of the Universal Mobile Telecommunication System (UMTS) the number of RF-radiation-exposed individuals is likely to escalate. Since the epigenetic effects of RF radiation are poorly understood and since the potential modifications of repair efficiency after exposure to known cytotoxic agents such as ionizing radiation have been investigated infrequently thus far, we studied the influence of UMTS exposure on the yield of chromosome aberrations induced by X rays. Human peripheral blood lymphocytes were exposed in vitro to a UMTS signal (frequency carrier of 1.95 GHz) for 24 h at 0.5 and 2.0 W/kg specific absorption rate (SAR) using a previously characterized waveguide system. The frequency of chromosome aberrations was measured on metaphase spreads from cells given 4 Gy of X rays immediately before RF radiation or sham exposures by fluorescence in situ hybridization. Unirradiated controls were RF-radiation- or sham-exposed. No significant variations due to the UMTS exposure were found in the fraction of aberrant cells. However, the frequency of exchanges per cell was affected by the SAR, showing a small but statistically significant increase of 0.11 exchange per cell compared to 0 W/kg SAR. We conclude that, although the 1.95 GHz signal (UMTS modulated) does not exacerbate the yield of aberrant cells caused by ionizing radiation, the overall burden of X-ray-induced chromosomal damage per cell in first-mitosis lymphocytes may be enhanced at 2.0 W/kg SAR. Hence the SAR may either influence the repair of X-ray-induced DNA breaks or alter the cell death pathways of the damage response.

(E) Marinelli F, La Sala D, Ciccio G, Cattini L, Trimarchi C, Putti S, Zamparelli A, Giuliani L, Tomassetti G, Cinti C. Exposure to 900 MHz electromagnetic field induces an unbalance between pro-apoptotic and pro-survival signals in T-lymphoblastoid leukemia CCRF-CEM cells. J Cell Physiol. 198(2):324-332, 2004.(VT, AE, GT, GE)

It has been recently established that low-frequency electromagnetic field (EMFs) exposure induces biological changes and could be associated with increased incidence of cancer, while the issue remains unresolved as to whether high-frequency EMFs can have hazardous effect on health. Epidemiological studies on association between childhood cancers, particularly leukemia and brain cancer, and exposure to low- and high-frequency EMF suggested an etiological role of EMFs in inducing adverse health effects. To investigate whether exposure to high-frequency EMFs could affect in vitro cell survival, we cultured acute T-lymphoblastoid leukemia cells (CCRF-CEM) in the presence of unmodulated 900 MHz EMF, generated by a transverse electromagnetic (TEM) cell, at various exposure times. We evaluated the effects of high-

frequency EMF on cell growth rate and apoptosis induction, by cell viability (MTT) test, FACS analysis and DNA ladder, and we investigated pro-apoptotic and pro-survival signaling pathways possibly involved as a function of exposure time by Western blot analysis. At short exposure times (2-12 h), unmodulated 900 MHz EMF induced DNA breaks and early activation of both p53-dependent and -independent apoptotic pathways while longer continuous exposure (24-48 h) determined silencing of pro-apoptotic signals and activation of genes involved in both intracellular (Bcl-2) and extracellular (Ras and Akt1) pro-survival signaling. Overall our results indicate that exposure to 900 MHz continuous wave, after inducing an early self-defense response triggered by DNA damage, could confer to the survivor CCRF-CEM cells a further advantage to survive and proliferate.

(E) Markova E, Hillert L, Malmgren L, Persson BR, Belyaev IY. Microwaves from GSM mobile telephones affect 53BP1 and gamma-H2AX foci in human lymphocytes from hypersensitive and healthy persons. Environ Health Perspect. 113(9):1172-1177, 2005. (VT, AE, GT, WS)

The data on biologic effects of nonthermal microwaves (MWs) from mobile telephones are diverse, and these effects are presently ignored by safety standards of the International Commission for Non-Ionizing Radiation Protection (ICNIRP). In the present study, we investigated effects of MWs of Global System for Mobile Communication (GSM) at different carrier frequencies on human lymphocytes from healthy persons and from persons reporting hypersensitivity to electromagnetic fields (EMFs). We measured the changes in chromatin conformation, which are indicative of stress response and genotoxic effects, by the method of anomalous viscosity time dependence, and we analyzed tumor suppressor p53-binding protein 1 (53BP1) and phosphorylated histone H2AX (gamma-H2AX), which have been shown to colocalize in distinct foci with DNA double-strand breaks (DSBs), using immunofluorescence confocal laser microscopy. We found that MWs from GSM mobile telephones affect chromatin conformation and 53BP1/gamma-H2AX foci similar to heat shock. For the first time, we report here that effects of MWs from mobile telephones on human lymphocytes are dependent on carrier frequency. On average, the same response was observed in lymphocytes from hypersensitive and healthy subjects.

(E)Markovà E, Malmgren LO, Belyaev IY. Microwaves from mobile phones inhibit 53BP1 focus formation in human stem cells more strongly than in differentiated cells: possible mechanistic link to cancer risk. Environ Health Perspect. 118(3):394-399, 2010. (VT, AE, GT)

Background: It is widely accepted that DNA double-strand breaks (DSBs) and their misrepair in stem cells are critical events in the multistage origination of various leukemias and tumors, including gliomas. Objectives: We studied whether microwaves from mobile telephones of the Global System for Mobile Communication (GSM) and the Universal Global Telecommunications System (UMTS) induce DSBs or affect DSB repair in stem cells. Methods: We analyzed tumor suppressor TP53 binding protein 1 (53BP1) foci that are typically formed at the sites of DSB location (referred to as DNA repair foci) by laser confocal microscopy. Results:

Microwaves from mobile phones inhibited formation of 53BP1 foci in human primary fibroblasts and mesenchymal stem cells. These data parallel our previous findings for human lymphocytes. Importantly, the same GSM carrier frequency (915 MHz) and UMTS frequency band (1947.4 MHz) were effective for all cell types. Exposure at 905 MHz did not inhibit 53BP1 foci in differentiated cells, either fibroblasts or lymphocytes, whereas some effects were seen in stem cells at 905 MHz. Contrary to fibroblasts, stem cells did not adapt to chronic exposure during 2 weeks. Conclusions: The strongest microwave effects were always observed in stem cells. This result may suggest both significant imbalance in DSB repair and severe stress response. Our findings that stem cells are most sensitive to microwave exposure and react to more frequencies than do differentiated cells may be important for cancer risk assessment and indicate that stem cells are the most relevant cellular model for validating safe mobile communication signals.

(E) Martin C, Percevault F, Ryder K, Sani E, Le Cun JC, Zhadobov M, Sauleau R, Le Dréan Y, Habauzit D. Effects of radiofrequency radiation on gene expression: a study of gene expressions of human keratinocytes from different origins. *Bioelectromagnetics*. 2020 Oct;41(7):552-557. (VT, AE, GE)

(No abstract available.) Human keratinocytes from difference origins showed different patterns of gene expression after acute exposure to a 60-GHz RFR.

(E) Mashevich M, Folkman D, Kesar A, Barbul A, Korenstein R, Jerby E, Avivi L. Exposure of human peripheral blood lymphocytes to electromagnetic fields associated with cellular phones leads to chromosomal instability. *Bioelectromagnetics* 24:82-90, 2003. (VT, AE, GT)

Whether exposure to radiation emitted from cellular phones poses a health hazard is at the focus of current debate. We have examined whether in vitro exposure of human peripheral blood lymphocytes (PBL) to continuous 830 MHz electromagnetic fields causes losses and gains of chromosomes (aneuploidy), a major “somatic mutation” leading to genomic instability and thereby to cancer. PBL were irradiated at different average absorption rates (SAR) in the range of 1.6-8.8 W/kg for 72 hr in an exposure system based on a parallel plate resonator at temperatures ranging from 34.5-37.5 °C. The averaged SAR and its distribution in the exposed tissue culture flask were determined by combining measurements and numerical analysis based on a finite element simulation code. A linear increase in chromosome 17 aneuploidy was observed as a function of the SAR value, demonstrating that this radiation has a genotoxic effect. The SAR dependent aneuploidy was accompanied by an abnormal mode of replication of the chromosome 17 region engaged in segregation (repetitive DNA arrays associated with the centromere), suggesting that epigenetic alterations are involved in the SAR dependent genetic toxicity. Control experiments (i.e., without any RF radiation) carried out in the temperature range of 34.5-38.5 °C showed that elevated temperature is not associated with either the genetic or epigenetic alterations observed following RF radiation - the increased levels of aneuploidy and the modification in replication of the centromeric DNA arrays. These findings indicate that the genotoxic effect of the electromagnetic radiation is elicited via a non-thermal

pathway. Moreover, the fact that aneuploidy is a phenomenon known to increase the risk for cancer, should be taken into consideration in future evaluation of exposure guidelines.

(E) Mazor R, Korenstein-Ilan A, Barbul A, Eshet Y, Shahadi A, Jerby E, Korenstein R. Increased levels of numerical chromosome aberrations after in vitro exposure of human peripheral blood lymphocytes to radiofrequency electromagnetic fields for 72 hours. Radiat Res. 169(1):28-37, 2008. (VT, AE, GT)

We investigated the effects of 72 h in vitro exposure of 10 human lymphocyte samples to radiofrequency electromagnetic fields (800 MHz, continuous wave) on genomic instability. The lymphocytes were exposed in a specially designed waveguide resonator at specific absorption rates (SARs) of 2.9 and 4.1 W/kg in a temperature range of 36-37 degrees C. The induced aneuploidy of chromosomes 1, 10, 11 and 17 was determined by interphase FISH using semi-automated image analysis. We observed increased levels of aneuploidy depending on the chromosome studied as well as on the level of exposure. In chromosomes 1 and 10, there was increased aneuploidy at the higher SAR, while for chromosomes 11 and 17, the increases were observed only for the lower SAR. Multisomy (chromosomal gains) appeared to be the primary contributor to the increased aneuploidy. The effect of temperature on the level of aneuploidy was examined over the range of 33.5-40 degrees C for 72 h with no statistically significant difference in the level of aneuploidy compared to 37 degrees C. These findings suggest the possible existence of an athermal effect of RF radiation that causes increased levels of aneuploidy. These results contribute to the assessment of potential health risks after continuous chronic exposure to RF radiation at SARs close to the current levels set by ICNIRP guidelines.

(NE) McNamee JP, Bellier PV, Gajda GB, Miller SM, Lemay EP, Lavallee BF, Marro L, Thansandote A. DNA damage and micronucleus induction in human leukocytes after acute in vitro exposure to a 1.9 GHz continuous-wave radiofrequency field. Radiat Res 158(4):523-533, 2002a. (VT, AE, GT)

Human blood cultures were exposed to a 1.9 GHz continuous-wave (CW) radiofrequency (RF) field for 2 h using a series of six circularly polarized, cylindrical waveguides. Mean specific absorption rates (SARs) of 0.0, 0.1, 0.26, 0.92, 2.4 and 10 W/kg were achieved, and the temperature within the cultures during a 2-h exposure was maintained at 37.0 +/- 0.5 degrees C. Concurrent negative (incubator) and positive (1.5 Gy (137)Cs gamma radiation) control cultures were run for each experiment. DNA damage was quantified immediately after RF-field exposure using the alkaline comet assay, and four parameters (tail ratio, tail moment, comet length and tail length) were used to assess DNA damage for each comet. No evidence of increased primary DNA damage was detected by any parameter for RF-field-exposed cultures at any SAR tested. The formation of micronuclei in the RF-field-exposed blood cell cultures was assessed using the cytokinesis-block micronucleus assay. There was no significant difference in the binucleated cell frequency, incidence of micronucleated binucleated cells, or total incidence of micronuclei between any of the RF-field-exposed cultures and the sham-exposed controls at any SAR tested. These results do not support the hypothesis that acute, nonthermalizing 1.9 GHz CW RF-field exposure causes DNA damage in cultured human leukocytes.

(NE) McNamee JP, Bellier PV, Gajda GB, Lavallee BF, Lemay EP, Marro L, Thansandote A. DNA Damage in human leukocytes after acute in vitro exposure to a 1.9 GHz pulse-modulated radiofrequency field. Radiat Res 158(4):534-537, 2002b. (VT, AE, GT)

Blood cultures from human volunteers were exposed to an acute 1.9 GHz pulse-modulated radiofrequency (RF) field for 2 h using a series of six circularly polarized, cylindrical waveguides. Mean specific absorption rates (SARs) ranged from 0 to 10 W/kg, and the temperature within the cultures during the exposure was maintained at 37.0 +/- 0.5 degrees C. DNA damage was quantified in leukocytes by the alkaline comet assay and the cytokinesis-block micronucleus assay. When compared to the sham-treated controls, no evidence of increased primary DNA damage was detected by any parameter for any of the RF-field-exposed cultures when evaluated using the alkaline comet assay. Furthermore, no significant differences in the frequency of binucleated cells, incidence of micronucleated binucleated cells, or total incidence of micronuclei were detected between any of the RF-field-exposed cultures and the sham-treated control at any SAR tested. These results do not support the hypothesis that acute, nonthermalizing 1.9 GHz pulse-modulated RF-field exposure causes DNA damage in cultured human leukocytes.

(NE) McNamee, J. P., Bellier, P. V., Gajda, G. B., Lavallee, B. F., Marro, L., Lemay, E. and Thansandote, A. No evidence for genotoxic effects from 24 h exposure of human leukocytes to 1.9 GHz radiofrequency fields. Radiat Res 159:693-697, 2003. (VT, AE, GT)

The current study extends our previous investigations of 2-h radiofrequency (RF)-field exposures on genotoxicity in human blood cell cultures by examining the effect of 24-h continuous-wave (CW) and pulsed-wave (PW) 1.9 GHz RF-field exposures on both primary DNA damage and micronucleus induction in human leukocyte cultures. Mean specific absorption rates (SARs) ranged from 0 to 10 W/kg, and the temperature within the cultures was maintained at 37.0 +/- 1.0 degrees C for the duration of the 24-h exposure period. No significant differences in primary DNA damage were observed between the sham-treated controls and any of the CW or PW 1.9 GHz RF-field-exposed cultures when processed immediately after the exposure period by the alkaline comet assay. Similarly, no significant differences were observed in the incidence of micronuclei, incidence of micronucleated binucleated cells, frequency of binucleated cells, or proliferation index between the sham-treated controls and any of the CW or PW 1.9 GHz RF-field-exposed cultures. In conclusion, the current study found no evidence of 1.9 GHz RF-field-induced genotoxicity in human blood cell cultures after a 24-h exposure period.

(NE) McNamee JP, Bellier PV, Konkle AT, Thomas R, Wasoontarajaroen S, Lemay E, Gajda GB. Analysis of gene expression in mouse brain regions after exposure to 1.9 GHz radiofrequency fields. Int J Radiat Biol. 92(6):338-350, 2016. (VO, LE, GE)

PURPOSE: To assess 1.9 GHz radiofrequency (RF) field exposure on gene expression within a variety of discrete mouse brain regions using whole genome microarray analysis MATERIALS AND METHODS: Adult male C57BL/6 mice were exposed to 1.9 GHz pulse-modulated or continuous-wave RF fields for 4 h/day for 5 consecutive days at whole body average (WBA)

specific absorption rates of 0 (sham), ~0.2 W/kg and ~1.4 W/kg. Total RNA was isolated from the auditory cortex, amygdala, caudate, cerebellum, hippocampus, hypothalamus, and medial prefrontal cortex and differential gene expression was assessed using Illumina MouseWG-6 (v2) BeadChip arrays. Validation of potentially responding genes was conducted by RT-PCR. RESULTS: When analysis of gene expression was conducted within individual brain regions when controlling the false discovery rate (FDR), no differentially expressed genes were identified relative to the sham control. However, it must be noted that most fold changes among groups were observed to be less than 1.5-fold and this study had limited ability to detect such small changes. While some genes were differentially expressed without correction for multiple-comparisons testing, no consistent pattern of response was observed among different RF-exposure levels or among different RF-modulations. CONCLUSIONS: The current study provides the most comprehensive analysis of potential gene expression changes in the rodent brain in response to RF field exposure conducted to date. Within the exposure conditions and limitations of this study, no convincing evidence of consistent changes in gene expression was found in response to 1.9 GHz RF field exposure.

(NE) McNamee JP, Grybas VS, Qutob SS, Bellier PV **Effects of 1800 MHz radiofrequency fields on signal transduction and antioxidant proteins in human A172 glioblastoma cells.** Int J Radiat Biol 2021 May 28;1-34. doi: 10.1080/09553002.2021.1934751. Online ahead of print. (VT, AE, GE)

Purpose: To assess the effects of 1800 MHz radiofrequency electromagnetic field (RF-EMF) exposure on the expression of signal transduction and antioxidant proteins in a human-derived A172 glioblastoma cell line. **Materials and methods:** Adherent human-derived A172 glioblastoma cells (1.0×10^5 cells per 35 mm culture dish, containing 2 mL DMEM media) were exposed to 1800 MHz continuous-wave (CW) or GSM-modulated RF fields, in the presence or absence of serum for 5, 30 or 240 min at a specific absorption rate (SAR) of 0 (sham) or 2.0 W/kg. Concurrent negative (vehicle) and positive controls (1 μ g/mL anisomycin) were included in each experiment. Cell lysates were collected immediately after exposure, stabilized by protease and phosphatase inhibitors in lysis buffer, then frozen and maintained at -80 °C until analysis. The relative expression levels of phosphorylated- and total-signal transduction proteins (CREB, JNK, NF- κ B, ERK1/2, Akt, p70S6K, STAT3 and STAT5) and antioxidant proteins (SOD1, SOD2, CAT, TRX1, PRX2) were assessed using Milliplex magnetic bead array panels and a MagPix Multiplex imaging system. **Results:** In cells exposed to 1800 MHz continuous-wave RF-EMF with the presence of serum in the culture medium, CAT expression was statistically significantly decreased after a 30 min exposure, total JNK was decreased at both 30 and 240 min of exposure, STAT3 was decreased after 240 min of exposure and phosphorylated-CREB expression was decreased after 30 min of exposure. In cells exposed to 1800 MHz GSM-modulated RF-EMF in serum-free cultures, the expression level of total STAT5 was decreased after 30 and 240 min of exposure. These observed changes were detected sporadically across time-points, culture conditions and RF-EMF exposure conditions indicating the likelihood of false positive events. When cells were treated with anisomycin for 15 min as a positive control, dramatic increases in the expression of phosphorylated signalling proteins were observed in both serum-starved and serum-fed A172 cells, with larger fold change increases in the serum-free cultures. No statistically significant differences in the expression levels of SOD1, SOD2 or

TRX1 were observed under any tested conditions after exposure to RF-EMF. **Conclusions:** The current study found no consistent evidence of changes in the expression of antioxidant proteins (SOD1, SOD2, CAT or TRX2) or a variety of signal transduction proteins (CREB, JNK, NF- κ B, ERK1/2, Akt, p70S6K, STAT3, STAT5) in a human-derived glioblastoma A172 cell line in response to exposure to 1800 MHz continuous-wave or GSM-modulated RF-EMF for 5, 30 or 240 min in either serum-free or serum-containing cultures.

(E) Meena R, Kumari K, Kumar J, Rajamani P, Verma HN, Kesari KK. Therapeutic approaches of melatonin in microwave radiations-induced oxidative stress-mediated toxicity on male fertility pattern of Wistar rats. Electromag Biol Med. 2014; 33: 81-91. (VO, LE, GT, OX, RP)

Microwave (MW) radiation produced by wireless telecommunications and a number of electrical devices used in household or in healthcare institutions may adversely affect the reproductive pattern. Present study aimed to investigate the protective effects of melatonin (is well known antioxidant that protects DNA, lipids and proteins from free radical damage) against oxidative stress-mediated testicular impairment due to long-term exposure of MWs. For this, 70-day-old male Wistar rats were divided into four groups (n = 6/group): Sham exposed, Melatonin (Mel) treated (2 mg/kg), 2.45 GHz MWs exposed and MWs + Mel treated. Exposure took place in Plexiglas cages for 2 h a day for 45 days where, power density (0.21 mW/cm²) and specific absorption rate (SAR 0.14 W/Kg) were estimated. After the completion of exposure period, rats were sacrificed and various stress related parameters, that is LDH-X (lactate dehydrogenase isoenzyme) activity, xanthine oxidase (XO), ROS (reactive oxygen species), protein carbonyl content, DNA damage and MDA (malondialdehyde) were performed. Result shows that melatonin prevent oxidative damage biochemically by significant increase (p < 0.001) in the levels of testicular LDH-X, decreased (p < 0.001) levels of MDA and ROS in testis (p < 0.01). Meanwhile, it reversed the effects of MWs on XO, protein carbonyl content, sperm count, testosterone level and DNA fragmentation in testicular cells. These results concluded that the melatonin has strong antioxidative potential against MW induced oxidative stress mediated DNA damage in testicular cells.

(E) Megha K, Deshmukh PS, Ravi AK, Tripathi AK, Abegaonkar MP, Banerjee BD. Effect of Low-Intensity Microwave Radiation on Monoamine Neurotransmitters and Their Key Regulating Enzymes in Rat Brain. Cell Biochem Biophys. 73(1):93-100, 2015a. (VO, LE, GE, LI)

The increasing use of wireless communication devices has raised major concerns towards deleterious effects of microwave radiation on human health. The aim of the study was to demonstrate the effect of low-intensity microwave radiation on levels of monoamine neurotransmitters and gene expression of their key regulating enzymes in brain of Fischer rats. Animals were exposed to 900 MHz and 1800 MHz microwave radiation for 30 days (2 h/day, 5 days/week) with respective specific absorption rates as 5.953×10^{-4} and 5.835×10^{-4} W/kg. The levels of monoamine neurotransmitters viz. dopamine (DA), norepinephrine (NE), epinephrine (E) and serotonin (5-HT) were detected using LC-MS/MS in hippocampus of all

experimental animals. In addition, mRNA expression of key regulating enzymes for these neurotransmitters viz. tyrosine hydroxylase (TH) (for DA, NE and E) and tryptophan hydroxylase (TPH1 and TPH2) (for serotonin) was also estimated. Results showed significant reduction in levels of DA, NE, E and 5-HT in hippocampus of microwave-exposed animals in comparison with sham-exposed (control) animals. In addition, significant downregulation in mRNA expression of TH, TPH1 and TPH2 was also observed in microwave-exposed animals ($p < 0.05$). In conclusion, the results indicate that low-intensity microwave radiation may cause learning and memory disturbances by altering levels of brain monoamine neurotransmitters at mRNA and protein levels.

(E) Megha K, Deshmukh PS, Banerjee BD, Tripathi AK, Ahmed R, Abegaonkar MP. Low intensity microwave radiation induced oxidative stress, inflammatory response and DNA damage in rat brain. NeuroToxicol. 51: 158-165, 2015b. (VO, LE, GT, LI, OX)

Over the past decade people have been constantly exposed to microwave radiation mainly from wireless communication devices used in day to day life. Therefore, the concerns over potential adverse effects of microwave radiation on human health are increasing. Until now no study has been proposed to investigate the underlying causes of genotoxic effects induced by low intensity microwave exposure. Thus, the present study was undertaken to determine the influence of low intensity microwave radiation on oxidative stress, inflammatory response and DNA damage in rat brain. The study was carried out on 24 male Fischer 344 rats, randomly divided into four groups (n=6 in each group): group I consisted of sham exposed (control) rats, group II-IV consisted of rats exposed to microwave radiation at frequencies 900, 1800 and 2450 MHz, specific absorption rates (SARs) 0.59, 0.58 and 0.66 mW/kg, respectively in gigahertz transverse electromagnetic (GTEM) cell for 60 days (2h/day, 5 days/week). Rats were sacrificed and decapitated to isolate hippocampus at the end of the exposure duration. Low intensity microwave exposure resulted in a frequency dependent significant increase in oxidative stress markers viz. malondialdehyde (MDA), protein carbonyl (PCO) and catalase (CAT) in microwave exposed groups in comparison to sham exposed group ($p < 0.05$). Whereas, levels of reduced glutathione (GSH) and superoxide dismutase (SOD) were found significantly decreased in microwave exposed groups ($p < 0.05$). A significant increase in levels of pro-inflammatory cytokines (IL-2, IL-6, TNF- α , and IFN- γ) was observed in microwave exposed animal ($p < 0.05$). Furthermore, significant DNA damage was also observed in microwave exposed groups as compared to their corresponding values in sham exposed group ($p < 0.05$). In conclusion, the present study suggests that low intensity microwave radiation induces oxidative stress, inflammatory response and DNA damage in brain by exerting a frequency dependent effect. The study also indicates that increased oxidative stress and inflammatory response might be the factors involved in DNA damage following low intensity microwave exposure.

Meltz M. Radiofrequency exposure and mammalian cell toxicity, genotoxicity, and transformation. Bioelectromagnetics Suppl 6:S196-S213, 2003. (review)

The published in vitro literature relevant to the issue of the possible induction of toxicity, genotoxicity, and transformation of mammalian cells due to radiofrequency field (RF) exposure

is examined. In some instances, information about related in vivo studies is presented. The review is from the perspective of technical merit and also biological consistency, especially with regard to those publications reporting a positive effect. The weight of evidence available indicates that, for a variety of frequencies and modulations with both short and long exposure times, at exposure levels that do not (or in some instances do) heat the biological sample such that there is a measurable increase in temperature, RF exposure does not induce (a). DNA strand breaks, (b). chromosome aberrations, (c). sister chromatid exchanges (SCEs), (d). DNA repair synthesis, (e). phenotypic mutation, or (f). transformation (cancer-like changes). While there is limited experimental evidence that RF exposure induces micronuclei formation, there is abundant evidence that it does not. There is some evidence that RF exposure does not induce DNA excision repair, suggesting the absence of base damage. There is also evidence that RF exposure does not inhibit excision repair after the induction of thymine dimers by UV exposure, as well as evidence that indicates that RF is not a co-carcinogen or a tumor promoter. The article is in part a tutorial, so that the reader can consider similarities and discrepancies between reports of RF-induced effects relative to one another.

(NE) Meltz ML, Eagan P, Erwin DN, Proflavin and microwave radiation: absence of a mutagenic interaction. Bioelectromagnetics 11(2):149-157, 1990. (VT, AE, GT, IX)

The potential ability of radiofrequency electromagnetic radiation (RFR) in the microwave range to induce mutagenesis, chromosomal aberrations, and sister chromatid exchanges in mammalian cells is being explored in our laboratories. In addition, we have also been examining the ability of simultaneous exposure to RFR and chemical mutagens to alter the genotoxic damage induced by chemical mutagens acting alone. We have performed experiments to determine whether there is an interaction between 2.45-GHz, pulsed-wave, RFR and proflavin, a DNA-intercalating drug. The endpoint studied was forward mutation at the thymidine kinase locus in L5178Y mouse leukemic cells. Any effect on the size distribution of the resulting colonies of mutated cells was also examined. The exposures were performed at net forward powers of 500 or 600 W, resulting in a specific absorption rate (SAR) of approximately 40 W/kg. The culture-medium temperature reached a 3 degrees C maximal increase during the 4-h exposure; appropriate 37 degrees C and convection-heating temperature controls (TC) were performed. In no case was there any indication of a statistically significant increase in the induced mutant frequency due to the simultaneous exposure to RFR and proflavin, as compared with the proflavin exposures alone. There was also no indication of any change in the colony-size distribution of the resulting mutant colonies, neither, and there was no evidence in these experiments of any mutagenic action by the RFR exposure alone.

(E) Mildažienė V, Aleknavičiūtė V, Žūkienė R, Paužaitė G, Naučienė Z, Filatova I, Lyushkevich V, Haimi P, Tamošiūnė I, Baniulis D. Treatment of common sunflower (Helianthus annuus L.) seeds with radio-frequency electromagnetic field and cold plasma induces changes in seed phytohormone balance, seedling development and leaf protein expression. Sci Rep. 9(1):6437, 2019. (VT, AE, GE)

Treatment of plant seeds with electromagnetic fields or non-thermal plasmas aims to take advantage of plant functional plasticity towards stimulation of plant agricultural performance. In this study, the effects of pre-sowing seed treatment using 200 Pa vacuum (7 min), 5.28 MHz radio-frequency cold plasma (CP -2, 5, and 7 min) and electromagnetic field (EMF -5, 10, 15 min) on seed germination kinetics, content of phytohormones, morphometric parameters of seedlings and leaf proteome were assessed. CP 7 min and EMF 15 min treatments caused 19-24% faster germination in vitro; germination in the substrate was accelerated by vacuum (9%) and EMF 15 min (17%). The stressors did not change the seed germination percentage, with exception of EMF 5 min treatment that caused a decrease by 7.5%. Meanwhile both CP 7 min and EMF 15 min treatments stimulated germination, but the EMF treatment resulted in higher weight of leaves. Stressor-specific changes in phytohormone balance were detected in seeds: vacuum treatment decreased zeatin amount by 39%; CP treatments substantially increased gibberellin content, but other effects strongly varied with the treatment duration; the abscisic acid content was reduced by 55-60% after the EMF treatment. Analysis of the proteome showed that short exposure of seeds to the EMF or CP induced a similar long-term effect on gene expression in leaves, mostly stimulating expression of proteins involved in photosynthetic processes and their regulation.

(E) Millenbaugh NJ, Roth C, Sypniewska R, Chan V, Eggers JS, Kiel JL, Robert V Blystone RV, Mason PA. Gene expression changes in the skin of rats induced by prolonged 35 GHz millimeter-wave exposure. Radiat Res 169(3):288-300, 2008. (VO, AE, GE)

To better understand the cellular and molecular responses to overexposure to millimeter waves, alterations in the gene expression profile and histology of skin after exposure to 35 GHz radiofrequency radiation were investigated. Rats were subjected to sham exposure, to 42 degrees C environmental heat, or to 35 GHz millimeter waves at 75 mW/cm². Skin samples were collected at 6 and 24 h after exposure for Affymetrix GeneChip analysis. The skin was harvested from a separate group of rats at 3-6 h or 24-48 h after exposure for histopathology analysis. Microscopic findings observed in the dermis of rats exposed to 35 GHz millimeter waves included aggregation of neutrophils in vessels, degeneration of stromal cells, and breakdown of collagen. Changes were detected in 56 genes at 6 h and 58 genes at 24 h in the millimeter-wave-exposed rats. Genes associated with regulation of transcription, protein folding, oxidative stress, immune response, and tissue matrix turnover were affected at both times. At 24 h, more genes related to extracellular matrix structure and chemokine activity were altered. Up-regulation of Hspa1a, Timp1, S100a9, Ccl2 and Angptl4 at 24 h by 35 GHz millimeter-wave exposure was confirmed by real-time RT-PCR. These results obtained from histopathology, microarrays and RT-PCR indicate that prolonged exposure to 35 GHz millimeter waves causes thermally related stress and injury in skin while triggering repair processes involving inflammation and tissue matrix recovery.

(E) Misa Agustíño MJ, Leiro JM, Mora MTJ, Rodríguez-González JA, Barreiro FJJ, Ares-Pena FJ, López-Martín E. Electromagnetic fields at 2.45 GHz trigger changes in heat

shock proteins 90 and 70 without altering apoptotic activity in rat thyroid gland. Biol Open 1(9):831-838, 2012. (VO, AE, GE)

Non-ionizing radiation at 2.45 GHz may modify the expression of genes that codify heat shock proteins (HSP) in the thyroid gland. Using the enzyme-linked immunosorbent assay (ELISA) technique, we studied levels of HSP-90 and HSP-70. We also used hematoxylin eosin to look for evidence of lesions in the gland and applied the DAPI technique of fluorescence to search for evidence of chromatin condensation and nuclear fragmentation in the thyroid cells of adult female Sprague-Dawley rats. Fifty-four rats were individually exposed for 30 min to 2.45 GHz radiation in a Gigahertz transverse electromagnetic (GTEM) cell at different levels of non-thermal specific absorption rate (SAR), which was calculated using the finite difference time domain (FDTD) technique. Ninety minutes after radiation, HSP-90 and HSP-70 had decreased significantly ($P < 0.01$) after applying a SAR of 0.046 ± 1.10 W/Kg or $0.104 \pm 5.10(-3)$ W/Kg. Twenty-four hours after radiation, HSP-90 had partially recovered and HSP-70 had recovered completely. There were few indications of lesions in the glandular structure and signs of apoptosis were negative in all radiated animals. The results suggest that acute sub-thermal radiation at 2.45 GHz may alter levels of cellular stress in rat thyroid gland without initially altering their anti-apoptotic capacity.

(E) Misa-Agustiño MJ, Leiro-Vidal JM, Gomez-Amoza JL, Jorge-Mora MT, Jorge-Barreiro FJ, Salas-Sánchez AA, Ares-Pena FJ, López-Martín E. EMF radiation at 2450 MHz triggers changes in the morphology and expression of heat shock proteins and glucocorticoid receptors in rat thymus. Life Sci 127:1-11, 2015. (VO, AE, GE)

Aims: Electromagnetic fields (EMFs) can act as inducers or mediators of stress response through the production of heat shock proteins (HSPs) that modulate immune response and thymus functions. In this study, we analyzed cellular stress levels in rat thymus after exposure of the rats to a 2.45 GHz radio frequency (RF) using an experimental diathermic model in a Gigahertz Transverse Electromagnetic (GTEM) chamber. **Main methods:** In this experiment, we used H&E staining, the ELISA test and immunohistochemistry to examine Hsp70 and Hsp90 expression in the thymus and glucocorticoid receptors (GR) of 64 female Sprague-Dawley rats exposed individually to 2.45 GHz (at 0, 1.5, 3.0 or 12.0 W power). The 1 g averaged peak and mean SAR values in the thymus and whole body of each rat to ensure that sub-thermal levels of radiation were being reached. **Key findings:** The thymus tissue presented several morphological changes, including increased distribution of blood vessels along with the appearance of red blood cells and hemorrhagic reticuloepithelial cells. Levels of Hsp90 decreased in the thymus when animals were exposed to the highest power level (12 W), but only one group did not show recovery after 24 h. Hsp70 presented no significant modifications in any of the groups. The glucocorticoid receptors presented greater immunomarking on the thymic cortex in exposed animals. **Significance:** Our results indicate that non-ionizing sub-thermal radiation causes changes in the endothelial permeability and vascularization of the thymus, and is a tissue-modulating agent for Hsp90 and GR.

(NE) Miyakoshi, J., Yoshida, M., Tarusawa, Y., et al. (2002). Effects of high- frequency electromagnetic fields on DNA strand breaks using comet assay method. Electrical Eng. Japan. 141: 9–15. (VT, AS, GT)

We investigated whether exposure to high-frequency electromagnetic fields causes DNA damage in cells, using the alkaline comet assay. The exposure device made for this study used a TE01 circular waveguide operating at a frequency of 2.45 GHz. Cells of the human brain tumor-derived MO54 cell line were exposed to an electromagnetic field (input power: 7.8 W, average SAR in the middle well of an annular culture plate: CW 50 W/kg) for 2 hours and the tail moments of the cells in the inner, middle, and outer wells of the plate were compared with those of sham-exposed cells. There was no significant difference between the high-frequency electromagnetic field-exposed groups and the sham-exposed groups. Three studies performed under the same conditions gave similar results. Next, cells were exposed to a stronger electromagnetic field (input power: 13 W, average SAR in the middle well: CW 100 W/kg) for 2 hours and compared with sham-exposed cells. There was also no significant difference in the tail moments of cells in the inner, middle, and outer wells of the plate in the high-frequency electromagnetic field-exposed groups and the sham-exposed groups. These findings suggest that a high-frequency electromagnetic field does not cause direct DNA damage, and does not induce DNA strand breaks, even at a SAR of 100 W/kg.

(E) Miyakoshi J, Takemasa K, Takashima Y, Ding GR, Hirose H, Koyama S. Effects of exposure to a 1950 MHz radio frequency field on expression of Hsp70 and Hsp27 in human glioma cells. Bioelectromagnetics. 26(4):251-257, 2005. (VT, AE, GE)

Human glioma MO54 cells were used to investigate whether radio frequency (RF) field exposure could activate stress response genes. Cells were exposed to continuous wave 1950 MHz or sham conditions for up to 2 h. Specific absorption rates (SARs) were 1, 2, and 10 W/kg. For the cell growth experiment, cell numbers were counted at 0-4 days after exposure. Expression of Hsp27 and Hsp70, as well as the level of phosphorylated Hsp27 (78Ser) protein, was determined by Western blotting. It was found that sham exposed and RF exposed cells demonstrated a similar growth pattern up to 4 days after RFfield exposure. RFfield exposure at both 2 and 10 W/kg did not affect the growth of MO54 cells. In addition, there were no significant differences in protein expression of Hsp27 and Hsp70 between sham exposed and RF exposed cells at a SAR of 1, 2, or 10 W/kg for 1 and 2 h. However, exposure to RFfield at a SAR of 10 W/kg for 1 and 2 h decreased the protein level of phosphorylated Hsp27 (78Ser) significantly. Our results suggest that although exposure to a 1950 MHz RFfield has no effect on cell proliferation and expression of Hsp 27 and Hsp70, it may inhibit the phosphorylation of Hsp27 at Serine 78 in MO54 cells.

(NE) Miyakoshi J, Tonomura H, Koyama S, Narita E, Shinohara N. Effects of Exposure to 5.8 GHz Electromagnetic Field on Micronucleus Formation, DNA Strand Breaks, and Heat Shock Protein Expressions in Cells Derived From Human Eye. IEEE Trans Nanobioscience 18(2):257-260, 2019. (VT, AE, GT)

In the near future, electrification will be introduced to heavy-duty vehicles and passenger cars. However, the wireless power transfer (WPT) requires high energy levels, and the suitability of various types of WPT systems must be assessed. This paper describes a method for solving technical and safety issues associated with this technology. We exposed human corneal epithelial (HCE-T) cells derived from the human eye to 5.8-GHz electromagnetic fields for 24 h. We observed no statistically significant increase in micronucleus (MN) frequency in cells exposed to a 5.8-GHz field at 1 mW/cm² (the general public level in ICNIRP) relative to sham-exposed or incubator controls. Similarly, the DNA strand breaks, and the expression of heat shock protein (Hsp) Hsp27, Hsp70, and Hsp 90 α exhibited no statistically significant effects as a result of exposure. These results indicate that the exposure to 5.8-GHz electromagnetic fields at 1 mW/cm² for 24 h has little or no effect on micronucleus formation, DNA strand breaks, and Hsp expression in human eye cells.

(E) Moghadasi N, Alimohammadi I, Variani AS, Ashtarinezhad A. The Effect of Mobile Radiation on the Oxidative Stress Biomarkers in Pregnant Mice. J Family Reprod Health. 2021 Sep;15(3):172-178. doi: 10.18502/jfrh.v15i3.7134. (VO, LE, GT, OX)

Objective: Due to the growing use of communication instruments such as cell phones and wireless devices, there is growing public concern about possible harmful effects, especially in sensitive groups such as pregnant women. This study aimed to investigate the oxidative stress induced by exposure to 900 MHz mobile phone radiation and the effect of vitamin C intake on reducing possible changes in pregnant mice. Materials and methods: Twenty-one pregnant mice were divided into three groups (control, mobile radiation-exposed, and mobile radiation plus with vitamin C intake co-exposed (200 mg /kg)). The mice in exposure groups were exposed to 900 MHz, 2 watts, and a power density of 0.045 μ w /cm² mobile radiation for eight hours/day for ten consecutive days. After five days of rest, MDA (Malondialdehyde), 8-OHdG (8-hydroxy-2'-deoxyguanosine), and TAC (Total Antioxidant Capacity) levels were measured in the blood of animals. The results were analyzed by SPSS.22.0 software. Results: The results showed that exposure to mobile radiation increased MDA (P=0.002), and 8-OHdG (P=0.001) significantly and decreased Total Antioxidant Capacity in the exposed groups (P=0.001). Taking vitamin C inhibited the significant increase in MDA and 8-OHdG levels in exposed groups. Conclusion: Although exposure to mobile radiation can cause oxidative stress in the blood of pregnant mice, vitamin C as an antioxidant can prevent it.

(E) Mokarram P, Sheikhi M, Mortazavi SMJ, Saeb S, Shokrpour N. Effect of Exposure to 900 MHz GSM Mobile Phone Radiofrequency Radiation on Estrogen Receptor Methylation Status in Colon Cells of Male Sprague Dawley Rats. J Biomed Phys Eng 7(1):79-86, 2017. (VO, LE, GE, DE, RP, EP)

Background: Over the past several years, the rapidly increasing use of mobile phones has raised global concerns about the biological effects of exposure to radiofrequency (RF) radiation. Numerous studies have shown that exposure to electromagnetic fields (EMFs) can be associated with effects on the nervous, endocrine, immune, cardiovascular, hematopoietic and ocular

systems. In spite of genetic diversity, the onset and progression of cancer can be controlled by epigenetic mechanisms such as gene promoter methylation. There are extensive studies on the epigenetic changes of the tumor suppressor genes as well as the identification of methylation biomarkers in colorectal cancer. Some studies have revealed that genetic changes can be induced by exposure to RF radiation. However, whether or not RF radiation is capable of inducing epigenetic alteration has not been clarified yet. To date, no study has been conducted on the effect of radiation on epigenetic alterations in colorectal cancer (CRC). Several studies have also shown that methylation of estrogen receptor α (ER α), MYOD, MGMT, SFRP2 and P16 play an important role in CRC. It can be hypothesized that RF exposure can be a reason for the high incidence of CRC in Iran. This study aimed to investigate whether epigenetic pattern of ER α is susceptible to RF radiation and if RF radiation can induce radioadaptive response as epigenetic changes after receiving the challenge dose (γ -ray). **Material and method:** 40 male Sprague-Dawley rats were divided into 4 equal groups (Group I: exposure to RF radiation of a GSM cell phone for 4 hours and sacrificed after 24 hours; Group II: RF exposure for 4 hours, exposure to Co-60 gamma radiation (3 Gy) after 24 hours and sacrificed after 72 hrs; Group III: only 3Gy gamma radiation; Group 4: control group). DNA from colon tissues was extracted to evaluate the methylation status by methylation specific PCR. **Results:** Our finding showed that exposure to GSM cell phone RF radiation was capable of altering the pattern of ER α gene methylation compared to that of non-exposed controls. Furthermore, no adaptive response phenomenon was induced in the pattern of ER α gene methylation after exposure to the challenging dose of Co-60 γ -rays. **Conclusion:** It can be concluded that exposure to RF radiation emitted by GSM mobile phones can lead to epigenetic detrimental changes in ER α promoter methylation pattern.

(NE) Nakatani-Enomoto S, Okutsu M, Suzuki S, Suganuma R, Groiss SJ, Kadowaki S, et al. Effects of 1950MHz W-CDMA-Like signal on human spermatozoa. Bioelectromagnetics. 2016; 37: 373- 381. (VT, AE, GT)

There are growing concerns about how electromagnetic waves (EMW) emitted from mobile phones affect human spermatozoa. Several experiments have suggested harmful effects of EMW on human sperm quality, motility, velocity, or the deoxyribonucleic acid (DNA) of spermatozoa. In this study, we analyzed the effects on human spermatozoa (sperm motility and kinetic variables) induced by 1 h of exposure to 1950 MHz Wideband Code Division Multiple Access (W-CDMA)-like EMW with specific absorption rates of either 2.0 or 6.0 W/kg, using a computer-assisted sperm analyzer system. We also measured the percentage of 8-hydroxy-2'-deoxyguanosine (8-OHdG) positive spermatozoa with flow cytometry to evaluate damage to DNA. No significant differences were observed between the EMW exposure and the sham exposure in sperm motility, kinetic variables, or 8-OHdG levels. We conclude that W-CDMA-like exposure for 1 h under temperature-controlled conditions has no detectable effect on normal human spermatozoa. Differences in exposure conditions, humidity, temperature control, baseline sperm characteristics, and age of donors may explain inconsistency of our results with several previous studies.

(E) Narasimhan V, Huh WK, Altered restriction patterns of microwave irradiated lambda phage DNA. Biochem Int 25(2):363-370, 1991.(VT, AE, GT)

Samples of lambda phage DNA exposed to short pulses of microwave irradiation were subjected to restriction fragmentation by Eco RI and Bam HI. Eco RI digests of microwaved DNA samples yielded three additional fragments ranging in base pair lengths between 24,226 and 7,421 besides the six expected fragments. While Bam HI digests of the microwaved samples did not yield any additional fragments, mobilities of the Bam HI fragments from the microwaved DNA samples were slower and the bands were broader in comparison to those from native samples. We attribute these altered restriction patterns to the conformational anomalies in DNA resulting from single strand breaks and localized strand separations induced by microwave irradiation.

(E) Nikolova T, Czyz J, Rolletschek A, Blyszczuk P, Fuchs J, Jovtchev G, Schuderer J, Kuster N, Wobus AM. Electromagnetic fields affect transcript levels of apoptosis-related genes in embryonic stem cell-derived neural progenitor cells. FASEB J 19(12):1686-1688, 2005. (VT, AE, GT, GE, WS)

Mouse embryonic stem (ES) cells were used as an experimental model to study the effects of electromagnetic fields (EMF). ES-derived nestin-positive neural progenitor cells were exposed to extremely low frequency EMF simulating power line magnetic fields at 50 Hz (ELF-EMF) and to radiofrequency EMF simulating the Global System for Mobile Communication (GSM) signals at 1.71 GHz (RF-EMF). Following EMF exposure, cells were analyzed for transcript levels of cell cycle regulatory, apoptosis-related, and neural-specific genes and proteins; changes in proliferation; apoptosis; and cytogenetic effects. Quantitative RT-PCR analysis revealed that ELF-EMF exposure to ES-derived neural cells significantly affected transcript levels of the apoptosis-related *bcl-2*, *bax*, and cell cycle regulatory "growth arrest DNA damage inducible" *GADD45* genes, whereas mRNA levels of neural-specific genes were not affected. RF-EMF exposure of neural progenitor cells resulted in down-regulation of neural-specific *Nurr1* and in up-regulation of *bax* and *GADD45* mRNA levels. Short-term RF-EMF exposure for 6 h, but not for 48 h, resulted in a low and transient increase of DNA double-strand breaks. No effects of ELF- and RF-EMF on mitochondrial function, nuclear apoptosis, cell proliferation, and chromosomal alterations were observed. We may conclude that EMF exposure of ES-derived neural progenitor cells transiently affects the transcript level of genes related to apoptosis and cell cycle control. However, these responses are not associated with detectable changes of cell physiology, suggesting compensatory mechanisms at the translational and posttranslational level.

(E) Nittby H, Widegren B, Krogh M, Grafström G, Berlin H, Rehn G, Eberhardt JL, Malmgren L, Persson BRR, Salford L. Exposure to radiation from global system for mobile communications at 1,800 MHz significantly changes gene expression in rat hippocampus and cortex. Environmentalist 28(4), 458-465, 2008. (VO, AE, GE, LI)

We have earlier shown that radio frequency electromagnetic fields can cause significant leakage of albumin through the blood-brain barrier of exposed rats as compared to non-exposed rats, and also significant neuronal damage in rat brains several weeks after a 2 h exposure to a mobile phone, at 915 MHz with a global system for mobile communications (GSM) frequency modulation, at whole-body specific absorption rate values (SAR) of 200, 20, 2, and 0.2 mW/kg. We have now studied whether 6 h of exposure to the radiation from a GSM mobile test phone at

1,800 MHz (at a whole-body SAR-value of 13 mW/kg, corresponding to a brain SAR-value of 30 mW/kg) has an effect upon the gene expression pattern in rat brain cortex and hippocampus—areas where we have observed albumin leakage from capillaries into neurons and neuronal damage. Microarray analysis of 31,099 rat genes, including splicing variants, was performed in cortex and hippocampus of 8 Fischer 344 rats, 4 animals exposed to global system for mobile communications electromagnetic fields for 6 h in an anechoic chamber, one rat at a time, and 4 controls kept as long in the same anechoic chamber without exposure, also in this case one rat at a time. Gene ontology analysis (using the gene ontology categories biological processes, molecular functions, and cell components) of the differentially expressed genes of the exposed animals versus the control group revealed the following highly significant altered gene categories in both cortex and hippocampus: extracellular region, signal transducer activity, intrinsic to membrane, and integral to membrane. The fact that most of these categories are connected with membrane functions may have a relation to our earlier observation of albumin transport through brain capillaries.

(E) Nylund R, Leszczynski D. Mobile phone radiation causes changes in gene and protein expression in human endothelial cell lines and the response seems to be genome- and proteome-dependent. Proteomics 6:4769-4780, 2006. (VT, AE, GE, CS)

We have examined in vitro cell response to mobile phone radiation (900 MHz GSM signal) using two variants of human endothelial cell line: EA.hy926 and EA.hy926v1. Gene expression changes were examined in three experiments using cDNA Expression Arrays and protein expression changes were examined in ten experiments using 2-DE and PDQuest software. Obtained results show that gene and protein expression were altered, in both examined cell lines, in response to one hour mobile phone radiation exposure at an average specific absorption rate of 2.8 W/kg. However, the same genes and proteins were differently affected by the exposure in each of the cell lines. This suggests that the cell response to mobile phone radiation might be genome- and proteome-dependent. Therefore, it is likely that different types of cells and from different species might respond differently to mobile phone radiation or might have different sensitivity to this weak stimulus. Our findings might also explain, at least in part, the origin of discrepancies in replication studies between different laboratories.

(E) Odacı E, Hancı H, Yuluğ E, Türedi S, Aliyazıcıoğlu Y, Kaya H, Çolakoğlu S. Effects of prenatal exposure to a 900 MHz electromagnetic field on 60-day-old rat testis and epididymal sperm quality. Biotech Histochem. 91(1):9-19, 2016. (VO, CE, GT, OX, DE, RP)

We investigated the effects of exposure in utero to a 900 megahertz (MHz) electromagnetic field (EMF) on 60-day-old rat testis and epididymis. Pregnant rats were divided into control (CG; no treatment) and EMF (EMFG) groups. The EMFG was exposed to 900 MHz EMF for 1 h each day during days 13 - 21 of pregnancy. Newborn rats were either newborn CG (NCG) or newborn EMF groups (NEMFG). On postnatal day 60, a testis and epididymis were removed from each animal. Epididymal semen quality, and lipid and DNA oxidation levels, apoptotic index and histopathological damage to the testis were compared. We found a higher apoptotic index, greater DNA oxidation levels and lower sperm motility and vitality in the NEMFG compared to controls. Immature germ cells in the seminiferous tubule lumen, and altered seminiferous tubule

epithelium and seminiferous tubule structure also were observed in hematoxylin and eosin stained sections of NEMFG testis. Nuclear changes that indicated apoptosis were identified in TUNEL stained sections and large numbers of apoptotic cells were observed in most of the seminiferous tubule epithelium in the NEMFG. Sixty-day-old rat testes exposed to 900 MHz EMF exhibited altered sperm quality and biochemical characteristics.

(E) Ohtani S, Ushiyama A, Maeda M, Hattori K, Kunugita N, Wang J, Ishii K. Exposure time-dependent thermal effects of radiofrequency electromagnetic field exposure on the whole body of rats. J Toxicol Sci. 41(5):655-666, 2016. (VO, AE, GE) (thermal effect)

We investigated the thermal effects of radiofrequency electromagnetic fields (RF-EMFs) on the variation in core temperature and gene expression of some stress markers in rats. Sprague-Dawley rats were exposed to 2.14 GHz wideband code division multiple access (W-CDMA) RF signals at a whole-body averaged specific absorption rate (WBA-SAR) of 4 W/kg, which causes behavioral disruption in laboratory animals, and 0.4 W/kg, which is the limit for the occupational exposure set by the International Commission on Non-Ionizing Radiation Protection guideline. It is important to understand the possible in vivo effects derived from RF-EMF exposures at these intensities. Because of inadequate data on real-time core temperature analyses using free-moving animal and the association between stress and thermal effects of RF-EMF exposure, we analyzed the core body temperature under nonanesthetic condition during RF-EMF exposure. The results revealed that the core temperature increased by approximately 1.5°C compared with the baseline and reached a plateau till the end of RF-EMF exposure. Furthermore, we analyzed the gene expression of heat-shock proteins (Hsp) and heat-shock transcription factors (Hsf) family after RF-EMF exposure. At WBA-SAR of 4 W/kg, some Hsp and Hsf gene expression levels were significantly upregulated in the cerebral cortex and cerebellum following exposure for 6 hr/day but were not upregulated after exposure for 3 hr/day. On the other hand, there was no significant change in the core temperature and gene expression at WBA-SAR of 0.4 W/kg. Thus, 2.14-GHz RF-EMF exposure at WBA-SAR of 4 W/kg induced increases in the core temperature and upregulation of some stress markers, particularly in the cerebellum.

(NE) Ohtani S, Ushiyama A, Maeda M, Wada K, Suzuki Y, Hattori K, Kunugita N, Ishii K. Global Analysis of Transcriptional Expression in Mice Exposed to Intermediate Frequency Magnetic Fields Utilized for Wireless Power Transfer Systems. Int J Environ Res Public Health 2019 May 25;16(10):1851. (VO, C, GE)

Background: Intermediate frequency magnetic fields (IF-MFs) at around 85 kHz are a component of wireless power transfer systems used for charging electrical vehicles. However, limited data exist on the potential health effects of IF-MFs. We performed a comprehensive analysis of transcriptional expression in mice after IF-MF exposure. **Materials and methods:** We developed an IF-MF exposure system to generate a high magnetic flux density (25.3 mT). The system can expose the IF-MF for a mouse whole-body without considering thermal effects. After 10 days (1 h/day) of exposure, a comprehensive expression analysis was performed using microarray data from both the brain and liver. **Results:** No significant differences in transcriptional expression were detected in the 35,240 probe-sets when controlling the false discovery rate (FDR) under a fold change cutoff >1.5. However, several differential

expressions were detected without FDR-adjustment, but these were not confirmed by RT-PCR analysis. **Conclusions:** To our knowledge, this is the first in vivo study to evaluate the biological effects of IF-MF exposure with an intense magnetic flux density 253 times higher than the occupational restriction level defined by the International Commission on Non-Ionizing Radiation Protection guidelines. However, our findings indicate that transcriptional responses in the living body are not affected under these conditions.

(NE) Ohtani S, Ushiyama A, Wada K, Suzuki Y, Ishii K, Hattori K. No evidence for genotoxicity in mice due to exposure to intermediate-frequency magnetic fields used for wireless power-transfer systems. Mutat Res Mar-Apr 2021;863-864:503310. (VO, LE, GT)

Time varying magnetic fields (MFs) are used for the wireless power-transfer (WPT) technology. Especially, 85 kHz band MFs, which are included in the intermediate frequency (IF) band (300 Hz - 10 MHz), are commonly used WPT system for charging electric vehicles. Those applications of WPT technology have elicited public concern about health effects of IF-MF. However, existing data from health risk assessments are insufficient and additional data are needed. We assessed the genotoxic effects of IF-MF exposure on erythroid differentiation in mice. A high-intensity IF-MF mouse exposure system was constructed to induce an average whole-body electric field of 54.1 V/m. Blood samples were obtained from male mice before and after a 2-week IF-MF exposure (1 h/day, total: 10 h); X-irradiated mice were used as positive controls. We analyzed the blood samples with the micronucleus (MN) test and the Pig-a mutation assay. No significant differences were seen between IF-MF-exposed and sham-exposed mice in the frequencies of either MN or Pig-a mutations in mature erythrocytes and reticulocytes. IF-MF exposure did not induce genotoxicity in vivo under the study conditions (2.36× the basic restriction for occupational exposure, 22.9 V/m, in the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines). The absence of significant biological effects due to IF-MF exposure supports the practical application of this technology.

(NE) Ono T, Saito Y, Komura J, Ikehata H, Tarusawa Y, Nojima T, Goukon K, Ohba Y, Wang J, Fujiwara O, Sato R. Absence of mutagenic effects of 2.45 GHz radiofrequency exposure in spleen, liver, brain, and testis of lacZ-transgenic mouse exposed in utero. Tohoku J Exp Med. 202(2):93-103, 2004. (VO, LE, GT)

A possible mutagenic effect of 2.45 GHz radiofrequency exposure was examined using lacZ-transgenic Muta mice. Pregnant animals were exposed intermittently at a whole-body averaged specific absorption rate of 0.71 W/kg (10 seconds on, 50 seconds off which is 4.3 W/kg during the 10 seconds exposure). Offspring that were exposed in utero for 16 hours a day, from the embryonic age of 0 to 15 days, were examined at 10 weeks of age. To minimize thermal effects, the exposure was given in repeated bursts of 10 seconds of exposure followed by 50 seconds of no exposure. Mutation frequencies at the lacZ gene in spleen, liver, brain, and testis were similar to those observed in non-exposed mice. Quality of mutation assessed by sequencing the nucleotides of mutant DNAs revealed no appreciable difference between exposed and non-exposed samples. The data suggest that the level of radiofrequency exposure studied is not

mutagenic when administered in utero in short repeated bursts.

(E) Ozgur E, Guler G, Kismali G, Seyhan N. Mobile phone radiation alters proliferation of hepatocarcinoma cells. Cell Biochem Biophys. 70(2):983-991, 2014. (GT, WS) (not genetic effect paper)

This study investigated the effects of intermittent exposure (15 min on, 15 min off for 1, 2, 3, or 4 h, at a specific absorption rate of 2 W/kg) to enhanced data rates for global system for mobile communication evolution-modulated radiofrequency radiation (RFR) at 900- and 1,800-MHz frequencies on the viability of the Hepatocarcinoma cells (Hep G2). Hep G2 cell proliferation was measured by a colorimetric assay based on the cleavage of the tetrazolium salt WST-1 by mitochondrial dehydrogenases in viable cells. Cell injury was evaluated by analyzing the levels of lactate dehydrogenase (LDH) and glucose released from lysed cells into the culture medium. Morphological observation of the nuclei was carried out by 4',6-diamidino-2-phenylindole (DAPI) staining using fluorescence microscopy. In addition, TUNEL assay was performed to confirm apoptotic cell death. It was observed that cell viability, correlated with the LDH and glucose levels, changed according to the frequency and duration of RFR exposure. Four-hour exposure produced more pronounced effects than the other exposure durations. 1,800-MHz RFR had a larger impact on cell viability and Hep G2 injury than the RFR at 900 MHz. Morphological observations also supported the biochemical results indicating that most of the cells showed irregular nuclei pattern determined by using the DAPI staining, as well as TUNEL assay which shows DNA damage especially in the cells after 4 h of exposure to 1,800-MHz RFR. Our results indicate that the applications of 900- and 1,800-MHz (2 W/kg) RFR cause to decrease in the proliferation of the Hep G2 cells after 4 h of exposure. Further studies will be conducted on other frequency bands of RFR and longer duration of exposure.

(E) Pacini S, Ruggiero M, Sardi I, Aterini S, Gulisano F, Gulisano M. Exposure to global system for mobile communication (GSM) cellular phone radiofrequency alters gene expression, proliferation, and morphology of human skin fibroblasts. Oncol Res 13: 19-24, 2002. (VT, AE, GE)

Human skin fibroblasts were exposed to global system for mobile communication (GSM) cellular phone radiofrequency for 1 h. GSM exposure induced alterations in cell morphology and increased the expression of mitogenic signal transduction genes (e.g., MAP kinase kinase 3, G2/mitotic-specific cyclin G1), cell growth inhibitors (e.g., transforming growth factor-beta), and genes controlling apoptosis (e.g., bax). A significant increase in DNA synthesis and intracellular mitogenic second messenger formation matched the high expression of MAP kinase family genes. These findings show that these electromagnetic fields have significant biological effects on human skin fibroblasts.

(E) Panagopoulos DJ, Chavdoula ED, Nezis IP, Margaritis LH. Cell death induced by GSM 900-MHz and DCS 1800-MHz mobile telephony radiation. Mutat Res 626:69-78, 2007. (VO, LE, GT, RP)

In the present study, the TUNEL (Terminal deoxynucleotidyltransferase-UTP Nick End Labeling) assay - a well known technique widely used for detecting fragmented DNA in various

types of cells - was used to detect cell death (DNA fragmentation) in a biological model, the early and mid stages of oogenesis of the insect *Drosophila melanogaster*. The flies were exposed in vivo to either GSM 900-MHz (Global System for Mobile telecommunications) or DCS 1800-MHz (Digital Cellular System) radiation from a common digital mobile phone, for few minutes per day during the first 6 days of their adult life. The exposure conditions were similar to those to which a mobile phone user is exposed, and were determined according to previous studies of ours [D.J Panagopoulos, A. Karabarounis, L.H. Margaritis, Effect of GSM 900-MHz mobile phone radiation on the reproductive capacity of *D. melanogaster*, *Electromagn. Biol Med* 23 (2004) 29-43; D.J Panagopoulos, N. Messini, A. Karabarounis, A.L. Philippetis, L.H. Margaritis, Radio frequency electromagnetic radiation within "safety levels" alters the physiological function of insects, in: P. Kostarakis, P. Stavroulakis (Eds.), *Proceedings of the Millennium International Workshop on Biological Effects of Electromagnetic Fields*, Heraklion, Crete, Greece, October 17-20, 2000, pp. 169-175, ISBN: 960-86733-0-5; D.J Panagopoulos, L.H. Margaritis, Effects of electromagnetic fields on the reproductive capacity of *D. melanogaster*, in: P. Stavroulakis (Ed.), *Biological Effects of Electromagnetic Fields*, Springer, 2003, pp. 545-578], which had shown a large decrease in the oviposition of the same insect caused by GSM radiation. Our present results suggest that the decrease in oviposition previously reported, is due to degeneration of large numbers of egg chambers after DNA fragmentation of their constituent cells, induced by both types of mobile telephony radiation. Induced cell death is recorded for the first time, in all types of cells constituting an egg chamber (follicle cells, nurse cells and the oocyte) and in all stages of the early and mid-oogenesis, from germarium to stage 10, during which programmed cell death does not physiologically occur. Germarium and stages 7-8 were found to be the most sensitive developmental stages also in response to electromagnetic stress induced by the GSM and DCS fields and, moreover, germarium was found to be even more sensitive than stages 7-8.

(E) Panagopoulos DJ. Chromosome damage in human cells induced by UMTS mobile telephony radiation. *Gen Physiol Biophys.* 38(5):445-454, 2019. (VT, AE, GT)

Environmental exposure to modern microwave telecommunication electromagnetic fields (EMFs) has increased to unprecedented levels with consequent health complaints and concerns. Many studies have already reported genotoxic effects on a variety of organisms and cell/tissue types. Human peripheral blood lymphocytes from six healthy donors were stimulated for mitosis and exposed to microwave EMF of Universal Mobile Telecommunications System (UMTS) or third generation (3G) mobile telephony (MT) EMF/radiation emitted by a commercially available mobile phone handset. Lymphocytes exposed during the G2 phase of the cell division cycle and observed at metaphase, exhibited chromatid-type aberrations (gaps and breaks) at highly significant percentages - up to 275% - compared to the control (sham-exposed) samples. Each subject exhibited a different sensitivity to the microwave exposure. Moreover, the percentages of aberrations in the control samples among subjects were different due to genetic and environmental factors. The MT EMF exposure induced mainly achromatic lesions (gaps), and secondarily terminal deletions (breaks) in a smaller degree. In conclusion, the present study shows that microwave 3G MT EMF/radiation - within the current exposure limits - has significant genotoxic action on human cells, and human exposure to this EMF/radiation should be kept at levels as low as possible.

(E) Panagopoulos, D.J. (2020). Comparing chromosome damage induced by mobile telephony radiation and a high caffeine dose: Effect of combination and exposure duration. Gen. Physiol. Biophys. 39(6):531-544. (VT, AE, GT, IX)

I recently reported induction of chromatid-type aberrations in human peripheral blood lymphocytes after a single 15 min exposure to universal mobile telecommunications system (UMTS) mobile telephony (MT) electromagnetic field (EMF) from a mobile phone. Lymphocytes from six healthy subjects were stimulated for mitosis, and exposed during the G2/M phase at 1 cm distance from the handset during an active phone call in "talk" mode. The same type of cells from the same subjects treated with a high caffeine dose (~ 290 times above the permissible single dose for an adult human) exhibited the same type of aberrations in a little smaller but comparable degree. The combination of this caffeine dose and the 15 min MT EMF exposure increased dramatically the number of aberrations in all subjects. The combined effect increased almost linearly with increasing duration of exposure to the MT EMF. Thus, MT EMF exposure ~ 136 times below the official limit (ICNIRP 2020) exerts a genotoxic action even greater than that of a caffeine dose ~ 290 times above the corresponding limit. Therefore, with a reasonable approximation, the limit for MT EMFs should be lowered by at least $\sim 4 \times 10^4$ times (136×290) for short-term exposures, and $\sim 4 \times 10^6$ times for long-term exposures.

(E) Pandey N, Giri S, Das S, Upadhaya P. Radiofrequency radiation (900 MHz)-induced DNA damage and cell cycle arrest in testicular germ cells in swiss albino mice. Toxicol Ind Health. 33(4):33-384, 2017. (VO, LE, GT, OX, RP)

Even though there are contradictory reports regarding the cellular and molecular changes induced by mobile phone emitted radiofrequency radiation (RFR), the possibility of any biological effect cannot be ruled out. In view of a widespread and extensive use of mobile phones, this study evaluates alterations in male germ cell transformation kinetics following RFR exposure and after recovery. Swiss albino mice were exposed to RFR (900 MHz) for 4 h and 8 h duration per day for 35 days. One group of animals was terminated after the exposure period, while others were kept for an additional 35 days post-exposure. RFR exposure caused depolarization of mitochondrial membranes resulting in destabilized cellular redox homeostasis. Statistically significant increases in the damage index in germ cells and sperm head defects were noted in RFR-exposed animals. Flow cytometric estimation of germ cell subtypes in mice testis revealed 2.5-fold increases in spermatogonial populations with significant decreases in spermatids. Almost fourfold reduction in spermatogonia to spermatid turnover (1C:2C) and three times reduction in primary spermatocyte to spermatid turnover (1C:4C) was found indicating arrest in the premeiotic stage of spermatogenesis, which resulted in loss of post-meiotic germ cells apparent from testis histology and low sperm count in RFR-exposed animals. Histological alterations such as sloughing of immature germ cells into the seminiferous tubule lumen, epithelium depletion and maturation arrest were also observed. However, all these changes showed recovery to varied degrees following the post-exposure period indicating that the adverse effects of RFR on mice germ cells are detrimental but

reversible. To conclude, RFR exposure-induced oxidative stress causes DNA damage in germ cells, which alters cell cycle progression leading to low sperm count in mice.

(E) Pandey N, Giri S. Melatonin attenuates radiofrequency radiation (900 MHz)-induced oxidative stress, DNA damage and cell cycle arrest in germ cells of male Swiss albino mice. Toxicol Ind Health. 34(5):315-327, 2018. (VO, LE, GT, OX, RP)

Increasing male infertility of unknown aetiology can be associated with environmental factors. Extensive use of mobile phones has exposed the general population to unprecedented levels of radiofrequency radiations (RFRs) that may adversely affect male reproductive health. Therefore, the present study investigated the effect of RFR Global System for Mobile communication (GSM) type, 900 MHz and melatonin supplementation on germ cell development during spermatogenesis. Swiss albino mice were divided into four groups. One group received RFR exposure for 3 h twice/day for 35 days and the other group received the same exposure but with melatonin (N-acetyl-5-methoxytryptamine) (MEL; 5 mg/kg bw/day). Two other groups received only MEL or remain unexposed. Sperm head abnormality, total sperm count, biochemical assay for lipid peroxides, reduced glutathione, superoxide dismutase activity and testis histology were evaluated. Additionally, flow cytometric evaluation of germ cell subtypes and comet assay were performed in testis. Extensive DNA damage in germ cells of RFR-exposed animals along with arrest in pre-meiotic stages of spermatogenesis eventually leading to low sperm count and sperm head abnormalities were observed. Furthermore, biochemical assays revealed excess free radical generation resulting in histological and morphological changes in testis and germ cells morphology, respectively. However, these effects were either diminished or absent in RFR-exposed animals supplemented with melatonin. Hence, it can be concluded that melatonin inhibits pre-meiotic spermatogenesis arrest in male germ cells through its anti-oxidative potential and ability to improve DNA reparative pathways, leading to normal sperm count and sperm morphology in RFR-exposed animals.

(NE) Papparini A, Rossi P, Gianfranceschi G, Brugaletta V, Falsaperla R, De Luca P, Romano Spica V. No evidence of major transcriptional changes in the brain of mice exposed to 1800 MHz GSM signal. Bioelectromagnetics. 29(4):312-323, 2008. (VO, AE, GE)

To analyze possible effects of microwaves on gene expression, mice were exposed to global system for mobile communication (GSM) 1800 MHz signal for 1 h at a whole body SAR of 1.1 W/kg. Gene expression was studied in the whole brain, where the average SAR was 0.2 W/kg, by expression microarrays containing over 22,600 probe sets. Comparison of data from sham and exposed animals showed no significant difference in gene expression modulation. However, when less stringent constraints were adopted to analyze microarray results, 75 genes were found to be modulated following exposure. Forty-two probes showed fold changes ranging from 1.5 to 2.8, whereas 33 were down-regulated from 0.67- to 0.29-fold changes, but these differences in gene expression were not confirmed by real-time PCR. Under these specific limited conditions, no consistent indication of gene expression modulation in whole mouse brain was found associated to GSM 1800 MHz exposure.

(E) Paulraj R, Behari J. Single strand DNA breaks in rat brain cells exposed to microwave radiation. Mutat Res 596:76-80, 2006. (VO, LE, GT)

This investigation concerns with the effect of low intensity microwave (2.45 and 16.5GHz, SAR 1.0 and 2.01W/kg, respectively) radiation on developing rat brain. Wistar rats (35 days old, male, six rats in each group) were selected for this study. These animals were exposed for 35 days at the above mentioned frequencies separately in two different exposure systems. After the exposure period, the rats were sacrificed and the whole brain tissue was dissected and used for study of single strand DNA breaks by micro gel electrophoresis (comet assay). Single strand DNA breaks were measured as tail length of comet. Fifty cells from each slide and two slides per animal were observed. One-way ANOVA method was adopted for statistical analysis. This study shows that the chronic exposure to these radiations cause statistically significant (p<0.001) increase in DNA single strand breaks in brain cells of rat.

(E) Pesnya DS, Romanovsky AV. Comparison of cytotoxic and genotoxic effects of plutonium-239 alpha particles and mobile phone GSM 900 radiation in the Allium cepa test. Mutat Res. 750(1-2):27-33, 2013. (VO, AE, GT)

The goal of this study was to compare the cytotoxic and genotoxic effects of plutonium-239 alpha particles and GSM 900 modulated mobile phone radiation in the Allium cepa test. Three groups of bulbs were exposed to mobile phone radiation during 0 (sham), 3 and 9hours. A positive control group was treated during 20 min with plutonium-239 alpha-radiation. Mitotic abnormalities, chromosome aberrations, micronuclei and mitotic index were analyzed. Exposure to alpha-radiation from plutonium-239 and exposure to modulated radiation from mobile phone during 3 and 9h significantly increased the mitotic index. GSM 900 mobile phone radiation as well as alpha-radiation from plutonium-239 induced both clastogenic and aneugenic effects. However, the aneugenic activity of mobile phone radiation was more pronounced. After 9 hours of exposure to mobile phone radiation, polyploid cells, three-groups metaphases, amitoses and some unspecified abnormalities were detected, which were not registered in the other experimental groups. Importantly, GSM 900 mobile phone radiation increased the mitotic index, the frequency of mitotic and chromosome abnormalities, and the micronucleus frequency in a time-dependent manner. Due to its sensitivity, the Allium cepa test can be recommended as a useful cytogenetic assay to assess cytotoxic and genotoxic effects of radiofrequency electromagnetic fields.

(E) Phillips, J.L., Ivaschuk, O., Ishida-Jones, T., Jones, R.A., Campbell-Beachler, M. and Haggren, W. DNA damage in Molt-4 T- lymphoblastoid cells exposed to cellular telephone radiofrequency fields in vitro. Bioelectrochem. Bioenerg. 45:103-110, 1998. (VT, AE, LI, GT)

Molt-4 T-lymphoblastoid cells have been exposed to pulsed signals at cellular telephone frequencies of 813.5625 MHz (iDEN signal) and 836.55 MHz (TDMA signal). These studies were performed at low SAR (average = 2.4 and 24 microwatt/g for iDEN and 2.6 and 26 microwatt/g for TDMA) in studies designed to look for athermal RF effects. The alkaline comet, or single cell gel electrophoresis, assay was employed to measure DNA single-strand breaks in cell cultures exposed to the radiofrequency (RF) signal as compared to concurrent sham-exposed cultures.

Tail moment and comet extent were calculated as indicators of DNA damage. Statistical differences in the distribution of values for tail moment and comet extent between exposed and control cell cultures were evaluated with the SKolmogorov-Smirnoff distribution test. Data points for all experiments of each exposure condition were pooled and analyzed as single groups. It was found that: 1) exposure of cells to the iDEN signal at an SAR of 2.4 microwatt/g for 2 h or 21 h significantly decreased DNA damage; 2) exposure of cells to the TDMA signal at an SAR of 2.6 microwatt/g for 2 h and 21 h significantly decreased DNA damage; 3) exposure of cells to the iDEN signal at an SAR of 24 microwatt/g for 2 h and 21 h significantly increased DNA damage; 4) exposure of cells to the TDMA signal at an SAR of 26 microwatt/g for 2 h significantly decreased DNA damage. The data indicate a need to study the effects of exposure to RF signals on direct DNA damage and on the rate at which DNA damage is repaired.

(E) Pooam M, Jourdan N, Aguida B, Dahon C, Baouz S, Terry C, Raad H, Ahmad M. Exposure to 1.8 GHz radiofrequency field modulates ROS in human HEK293 cells as a function of signal amplitude. Commun Integr Biol 15(1):54-66, 2022.(VT, AE, OX, GE, WS)

The modern telecommunications industry is ubiquitous throughout the world, with a significant percentage of the population using cellular phones on a daily basis. The possible physiological consequences of wireless emissions in the GHz range are therefore of major interest, but remain poorly understood. Here, we show that exposure to a 1.8 GHz carrier frequency in the amplitude range of household telecommunications induces the formation of ROS (Reactive Oxygen Species) in human HEK293 cultured cells. The ROS concentrations detected by fluorescent imaging techniques increased significantly after 15 minutes of RF field exposure, and were localized to both nuclear and cytosolic cellular compartments. qPCR analysis showed altered gene expression of both anti-oxidative (SOD, GPX, GPX, and CAT) and oxidative (Nox-2) enzymes. In addition, multiple genes previously identified as responsive to static magnetic fields were found to also be regulated by RF, suggesting common features in response mechanisms. By contrast, many RF effects showed evidence of hormesis, whereby biological responsivity does not occur linearly as a function of signal amplitude. Instead, biphasic dose response curves occur with 'blind' spots at certain signal amplitudes where no measureable response occurs. We conclude that modulation of intracellular ROS can be a direct consequence of RF exposure dependent on signal frequency and amplitude. Since changes in intracellular ROS may have both harmful and beneficial effects, these could provide the basis for many reported physiological effects of RF exposure.

(NE) Port M, Abend M, Romer B, Van Beuningen D. Influence of high-frequency electromagnetic fields on different modes of cell death and gene expression. Int J Radiat Biol. 79(9):701-708, 2003.(VT, AE, GE)

PURPOSE: International thresholds for exposure to non-ionizing radiation leading to non-thermal effects were conservatively set by the International Commission on Non-Ionizing Radiation Protection (ICNIRP). The aim of this study was to examine whether biological effects such as different modes of cell death and gene expression modifications related to tumorigenesis are detectable above the threshold defined. MATERIALS AND METHODS: Human

leukaemia cells (HL-60) grown in vitro were exposed to electromagnetic fields (EMF; t 1/2(r) about 1 ns; field strength about 25 times higher than the ICNIRP reference levels for occupational exposure) leading to non-thermal effects using a high-voltage-improved GTEM cell 5302 (EMCO) connected to a pulse generator NP20 ($C = 1$ nF, $U(\text{Load}) = 20$ kV). HL-60 cells were harvested at 0, 24, 48 and 72 h after radiation exposure. Micronuclei, apoptosis and abnormal cells (e.g. necrosis) were determined using morphological criteria. In parallel, the expression of 1176 genes was measured using Atlas Human 1.2. Array. Based on high data reproducibility calculated from two independent experiments ($> 99\%$), array analysis was performed. RESULTS: No significant change in apoptosis, micronucleation, abnormal cells and differential gene expression was found. CONCLUSIONS: Exposure of HL-60 cells to EMFs 25 times higher than the ICNIRP reference levels for occupational exposure failed to induce any changes in apoptosis, micronucleation, abnormal morphologies and gene expression. Further experiments using EMFs above the conservatively defined reference level set by the ICNIRP may be desirable.

(E) Qin F, Cao H, Yuan H, Guo W, Pei H, Cao Y, Tong J. 1800 MHz radiofrequency fields inhibits testosterone production via CaMKI/ROR α pathway. Reprod Toxicol. 81:229-236, 2018. (VO, LE, GE, LI)

Exposure to radiofrequency fields (RF) has been reported to induce adverse effects on testosterone production and its daily rhythm. However, the mechanisms underneath this effect remain unknown. In this study, male mice were exposed to 1800 MHz radiofrequency fields (RF, $40 \mu\text{W}/\text{cm}^2$ power intensity and $0.0553 \text{ W}/\text{Kg}$ SAR) 2 h per day for 32 days. The data suggested that RF exposure: (i) significantly reduced testosterone levels, (ii) altered the expression of genes involved in its synthesis (Star, P450scc, P450c17 and 3β -Hsd) in testicular tissue, (iii) significantly reduced regulatory protein CaMKI/ROR α . Similar observations were also made in cultured primary Leydig cells exposed in vitro to RF. However, all of these observations were blocked by CaMK inhibitor, KN-93, and ionomycin reversed the down-regulation effects on intracellular $[\text{Ca}^{2+}]_i$ and CaMKI/ROR α expression induced by RF exposure. Thus, the data provided the evidence that RF-induced inhibition of testosterone synthesis might be mediated through CaMKI/ROR α signaling pathway. Capsule: CaMKI/ROR α signaling pathway was involved in the inhibition of testosterone synthesis induced by RF exposure.

(E) Qin F, Shen T, Cao H, Qian J, Zou D, Ye M, Pei H. CeO $_2$ NPs relieve radiofrequency radiation, improve testosterone synthesis, and clock gene expression in Leydig cells by enhancing antioxidation. Int J Nanomedicine. 2019 Jun 24;14:4601-4611. (VT, AE, GE, OX)

Introduction: The ratio of Ce $^{3+}$ /Ce $^{4+}$ in their structure confers unique functions on cerium oxide nanoparticles (CeO $_2$ NPs) containing rare earth elements in scavenging free radicals and protecting against oxidative damage. The potential of CeO $_2$ NPs to protect testosterone synthesis in primary mouse Leydig cells during exposure to 1,800 MHz radiofrequency (RF) radiation was examined in vitro. Methods: Leydig cells were treated with different concentrations of CeO $_2$ NPs

to identify the optimum concentration for cell proliferation. The cells were pretreated with the optimum dose of CeO₂NPs for 24 hrs and then exposed to 1,800 MHz RF at a power density of 200.27 $\mu\text{W}/\text{cm}^2$ (specific absorption rate (SAR), 0.116 W/kg) for 1 hr, 2 hrs, or 4 hrs. The medium was used to measure the testosterone concentration. The cells were collected to determine the antioxidant indices (catalase [CAT], malondialdehyde [MDA], and total antioxidant capacity [T-AOC]), and the mRNA expression of the testosterone synthase genes (*Star*, *Cyp11a1*, and *Hsd-3 β*) and clock genes (*Clock*, *Bmal1*, and *Rora*). Results: Our preliminary result showed that 128 $\mu\text{g}/\text{mL}$ CeO₂NPs was the optimum dose for cell proliferation. Cells exposed to RF alone showed reduced levels of testosterone, T-AOC, and CAT activities, increased MDA content, and the downregulated genes expression of *Star*, *Cyp11a1*, *Hsd-3 β* , *Clock*, *Bmal1*, and *Rora*. Pretreatment of the cells with 128 $\mu\text{g}/\text{mL}$ CeO₂NPs for 24 hrs followed by RF exposure significantly increased testosterone synthesis, upregulated the expression of the testosterone synthase and clock genes, and increased the resistance to oxidative damage in Leydig cells compared with those in cells exposed to RF alone. Conclusion: Exposure to 1,800 MHz RF had adverse effects on testosterone synthesis, antioxidant levels, and clock gene expression in primary Leydig cells. Pretreatment with CeO₂NPs prevented the adverse effects on testosterone synthesis induced by RF exposure by regulating their antioxidant capacity and clock gene expression in vitro. Further studies of the mechanism underlying the protective function of CeO₂NPs against RF in the male reproductive system are required.

(E) Qin F, Cao H, Feng C, Zhu T, Zhu B, Zhang J, Tong J, Pei H Microarray profiling of LncRNA expression in the testis of pubertal mice following morning and evening exposure to 1800 MHz radiofrequency fields. Chronobiol Int 2021 Aug 8;1-16. doi: 10.1080/07420528.2021.1962902. Online ahead of print. (VO, LE, GE, RP)

In this paper, the chronotoxicity of radiofrequency fields (RF) in the pubertal testis development and the involved molecular pathways were investigated by exposing four-week-old mice to RF (1800 MHz, SAR, 0.50 W/kg) in the morning and evening of each day for three weeks. Then, pathological changes and functional indices within the testis were determined. We also used a long non-coding RNA (lncRNA) microarray and GO/KEGG pathway analyses to determine lncRNA expression profiles and predict their potential functions. The cis and trans regulation of lncRNAs were investigated, and an interaction network was constructed using Cytoscape software. RF exposure led to a range of pathological changes in the testes of adolescent mice, as testicular weights and daily sperm productions decreased, and the testosterone secretion reduced. Furthermore, RF induced dysregulation in the expression of testicular lncRNAs. We identified 615 and 183 differentially expressed lncRNAs that were associated with morning and evening exposure to RF, respectively. From 15 differential expression lncRNAs both in morning RF group and evening RF group, we selected 6 lncRNAs to be validated by quantitative reverse transcription PCR (qRT-PCR). The differentially expressed lncRNAs induced by morning RF exposure were highly correlated with many different pathways, including Fanconi syndrome, metabolic processes, cell cycle, DNA damage, and DNA replication. Trans-regulation analyses further showed that differentially expressed lncRNAs were involved in multiple transcription factor-regulated pathways, such as TCFAP4, NF κ B, HINFP, TFDP2, FoxN1, and PAX5. These

transcription factors have all been shown to be involved in the modulation of testis development, cell cycle progression, and spermatogenesis. These findings suggest that the extent to which 1800 MHz RF induced toxicity in the testes and changed the expression of lncRNAs showed differences between morning exposure and evening exposure. These data indicate that differentially expressed lncRNAs play crucial roles in the RF exposure damage to the developing pubertal testis. Collectively, our findings provide a better understanding of the mechanisms underlying the toxic effects of RF exposure on testicular development.

(NE) Qutob SS, Chauhan V, Bellier PV, Yauk CL, Douglas GR, Berndt L, Williams A, Gajda GB, Lemay E, Thansandote A, McNamee JP. Microarray gene expression profiling of a human glioblastoma cell line exposed in vitro to a 1.9 GHz pulse-modulated radiofrequency field. Radiat Res 165:636-644, 2006. (VT, AE, GE)

The widespread use of mobile phones has led to public concerns about the health effects associated with exposure to radiofrequency (RF) fields. The paramount concern of most persons relates to the potential of these fields to cause cancer. Unlike ionizing radiation, RF fields used for mobile telecommunications (800-1900 MHz) do not possess sufficient energy to directly damage DNA. Most rodent bioassay and in vitro genotoxicity/mutation studies have reported that RF fields at non-thermal levels have no direct mutagenic, genotoxic or carcinogenic effects. However, some evidence has suggested that RF fields may cause detectable postexposure changes in gene expression. Therefore, the purpose of this study was to assess the ability of exposure to a 1.9 GHz pulse-modulated RF field for 4 h at specific absorption rates (SARs) of 0.1, 1.0 and 10.0 W/kg to affect global gene expression in U87MG glioblastoma cells. We found no evidence that non-thermal RF fields can affect gene expression in cultured U87MG cells relative to the nonirradiated control groups, whereas exposure to heat shock at 43 degrees C for 1 h up-regulated a number of typical stress-responsive genes in the positive control group. Future studies will assess the effect of RF fields on other cell lines and on gene expression in the mouse brain after in vivo exposure.

(E) Racuciu M, Effects of radiofrequency radiation on root tip cells of Zea mays. Roumanian Biotechnological Letters 14 (3) 4365-4369, 2009. (VT, AE, GT)

In this study, the mutagenic effects of low power radiofrequency radiation on Zea mays root tip were studied. Cells in different division phases and chromosomal aberration assay were used to determine the mitotic index and chromosomal aberration frequency of Zea mays root tip cells induced by 900MHz radiofrequency radiation. Zea mays seeds, having a uniform genophond, have been exposed to RF field of low power density, for different time intervals, between 1.0 and 36.0 hours. Exposure to RF field was applied to seeds before germination process. Continuous wave on 900 MHz was used for irradiation. Incident field distribution in the irradiation area was characterized, so as an as possible as uniform field to be applied in the volume of the sample. The results showed that the mitotic index and chromosomal aberration frequency showed linear increasing for radiofrequency radiation treatment of increased exposure time.

(E) Rago R, Salacone P, Caponecchia L, Sebastianelli A, Marcucci I, Calogero AE, Condorelli R, Vicari E, Morgia G, Favilla V, Cimino S, Arcoria AF, La Vignera S. The

semen quality of the mobile phone users. J Endocrinol Invest. 36(11):970-974, 2013. (HU, LE, GT, RP)

BACKGROUND: The increased use of mobile phones, the media's attention for general health, and the increase of idiopathic male infertility suggest to investigate the possible consequences of an excessive use of mobile phones on semen quality. **AIM:** To evaluate the conventional and some of the main biofunctional sperm parameters in healthy men according to the different use of the mobile phone. **SUBJECTS AND METHODS:** All the enrolled subjects in this study were divided into four groups according to their active cell phone use: group A= no use (no.=10 subjects); group B= <2 h/day (no.=16); group C= 2-4 h/day (no.=17); and group D= >4 h/day (no.=20). Among the subjects of the group D (>4 h/day), a further evaluation was made between the "trousers users"(no.=12) and "shirt users"(no.=8), and they underwent semen collection to evaluate conventional and biofunctional sperm parameters (density, total count, morphology, progressive motility, apoptosis, mitochondrial membrane potential, chromatin compaction, DNA fragmentation). **RESULTS:** None of the conventional sperm parameters examined were significantly altered. However, the group D and the trousers users showed a higher percentage of sperm DNA fragmentation compared to other groups. **CONCLUSION:** These results suggest that the sperm DNA fragmentation could represent the only parameter significantly altered in the subjects who use the mobile phone for more than 4 h/day and in particular for those who use the device in the pocket of the trousers.

(E) Rammal M, Jebai F, Rammal H, Joumaa WH. Effects of long-term exposure to RF/MW radiations on the expression of mRNA of stress proteins in *Lycopersicon esculentum*. WSEAS Transact Biol Biomed. 11:10-14, 2014. (VT, AE, GE, LI)

Low levels of RF radiation exposure can modify the protein's activity by stimulating or inhibiting their expression in cells. The proteinase inhibitor (Pin II) and *Lycopersicon esculentum basic leucine Zipper1* (lebZIP1) are two wound-plants genes. The aim of this work is to study the rate of accumulation of pin II and lebZIP1 at the level of messenger RNA after 10 days of electromagnetic waves exposure. Using RT-PCR and RT-qPCR, the results show that Pin II and lebZIP1 synthesis change at the level of cDNA. PinII and lebZIP1 surexpression influence the growth and the differentiation and evoke an increase of protein accumulation in the cell.

(NE) Regalbuto E, Anselmo A, De Sanctis S, Franchini V, Lista F, Benvenuto M, Bei R, Masuelli L, D'Inzeo G, Paffi A, Trodella E, Sgura A. Human fibroblasts in vitro exposed to 2.45 GHz continuous and pulsed wave signals: evaluation of biological effects with a multimethodological approach. Int J Mol Sci 21(19):E7069, 2020.(VT, AE, GT)

The increasing exposure to radiofrequency electromagnetic fields (RF-EMF), especially from wireless communication devices, raises questions about their possible adverse health effects. So far, several in vitro studies evaluating RF-EMF genotoxic and cytotoxic non-thermal effects have reported contradictory results that could be mainly due to inadequate experimental design and lack of well-characterized exposure systems and conditions. Moreover, a topic poorly investigated is related to signal modulation induced by electromagnetic fields. The aim of this study was to perform an analysis of the potential non-thermal biological effects induced by 2.45

GHz exposures through a characterized exposure system and a multimethodological approach. Human fibroblasts were exposed to continuous (CW) and pulsed (PW) signals for 2 h in a wire patch cell-based exposure system at the specific absorption rate (SAR) of 0.7 W/kg. The evaluation of the potential biological effects was carried out through a multimethodological approach, including classical biological markers (genotoxic, cell cycle, and ultrastructural) and the evaluation of gene expression profile through the powerful high-throughput next generation sequencing (NGS) RNA sequencing (RNA-seq) approach. Our results suggest that 2.45 GHz radiofrequency fields did not induce significant biological effects at a cellular or molecular level for the evaluated exposure parameters and conditions.

(E) Remondini D, Nylund R, Reivinen J, Poullietier de Gannes F, Veyret B, Lagroye I, Haro E, Trillo MA, Capri M, Franceschi C, Schlatterer K, Gminski R, Fitzner R, Tauber R, Schuderer J, Kuster N, Leszczynski D, Bersani F, Maercker C. Gene expression changes in human cells after exposure to mobile phone microwaves. *Proteomics* 6:4745-4754, 2006. (VT, AE, GE, CS)

Possible biological effects of mobile phone microwaves were investigated in vitro. In this study, which was part of the 5FP EU project REFLEX (Risk Evaluation of Potential Environmental Hazards From Low-Energy Electromagnetic Field Exposure Using Sensitive in vitro Methods), six human cell types, immortalized cell lines and primary cells, were exposed to 900 and 1800 MHz. RNA was isolated from exposed and sham-exposed cells and labeled for transcriptome analysis on whole-genome cDNA arrays. The results were evaluated statistically using bioinformatics techniques and examined for biological relevance with the help of different databases. NB69 neuroblastoma cells, T lymphocytes, and CHME5 microglial cells did not show significant changes in gene expression. In EA.hy926 endothelial cells, U937 lymphoblastoma cells, and HL-60 leukemia cells we found between 12 and 34 up- or down-regulated genes. Analysis of the affected gene families does not point towards a stress response. However, following microwave exposure, some but not all human cells might react with an increase in expression of genes encoding ribosomal proteins and therefore up-regulating the cellular metabolism.

(E) Romano-Spica V, Mucci N, Ursini CL, Ianni A, Bhat NK(2000). Ets1 oncogene induction by ELF-modulated 50 MHz radiofrequency electromagnetic field. *Bioelectromagnetics* 21(1):8-18, 2000. (VT, AE, GE)

We have analyzed gene expression in hemopoietic and testicular cell types after their exposure to 50 MHz radiofrequency (RF) non-ionizing radiation modulated (80%) with a 16 Hz frequency. The exposure system generates a 0.2 microT magnetic field parallel to the ground and a 60 V/m electric field orthogonal to the earth's magnetic field. Exposure conditions were selected so as to interfere with the calcium ion flow. Under these electromagnetic field (EMF) conditions, we observed an overexpression of the ets1 mRNA in Jurkat T-lymphoblastoid and Leydig TM3 cell lines. This effect was observed only in the presence of the 16 Hz modulation, corresponding to the resonance frequency for calcium

ion with a DC magnetic field of 45.7 microT. We have also identified a putative candidate gene repressed after EMF exposure. The experimental model described in this paper may contribute to the understanding of the biological mechanisms involved in EMF effects.

Romeo S, Zeni O, Sannino A, Lagorio S, Biffoni M, Scarfi MR. Genotoxicity of radiofrequency electromagnetic fields: Protocol for a systematic review of in vitro studies. Environ Int. 2021 Jan 21;148:106386. doi: 10.1016/j.envint.2021.106386. (Opinion)

Background: Exposure to radiofrequency electromagnetic fields (RF-EMF, 100 kHz - 300 GHz) emitted by wireless communication technologies is pervasive and ubiquitous. Concern has been raised about possible adverse effects to human health. In 2011 the International Agency for Research on Cancer has classified RF-EMF as possibly carcinogenic to humans, highlighting that the evidence is weak and far from conclusive. Updated systematic reviews of the scientific literature on this topic are lacking, especially for mechanistic studies. Objectives: To develop a protocol for a systematic review of experimental studies investigating genotoxic effects induced by RF-EMF in in vitro cellular models. Genotoxicity is one of the key-biological indicators of carcinogenicity, and the most common characteristics of established carcinogens. The predefined procedures for conducting the systematic review are outlined below. Methods: We will follow the guidelines developed by the National Toxicology Program-Office of Health Assessment and Translation (NTP-OHAT), adapted to the evaluation of in vitro studies. Eligibility criteria: We will include experimental in vitro studies addressing the relationship between controlled exposures to RF-EMF and genotoxicity in mammalian cells only. Eligibility for inclusion will be further restricted to peer reviewed articles reporting findings from primary studies. Information sources: We will search the scientific literature databases NCBI PubMed, Web of Science, and EMF-Portal. No filter on publication date will be applied. Only studies published in English will be considered. The reference lists of the included papers and available reviews will be screened for unidentified relevant papers. References will be managed through Endnote X9 software. Data extraction and synthesis of results: Data from included papers will be extracted according to predefined forms. Heterogeneity within the available evidence will determine the type of evidence synthesis that is appropriate. Findings will be summarized in tables, graphical displays and in a narrative synthesis of the available evidences. A meta-analysis will be carried out if subgroups of studies homogeneous in terms of exposure characteristics, endpoint, and cell types will be identified. Risk of bias: The internal validity of included studies will be assessed using the NTP-OHAT Risk of Bias Rating Tool for animal studies, adapted to in vitro studies. This stage of the process will be managed through the Health Assessment Workspace Collaborative (HAWC). Evidence appraisal: To rate confidence in the body of evidence, we will use the OHAT GRADE-based approach for animal studies.

(NE) Ros-Llor I, Sanchez-Siles M, Camacho-Alonso F, Lopez-Jornet P. Effect of mobile phones on micronucleus frequency in human exfoliated oral mucosal cells. Oral Dis. 18:786-792, 2012. (HU, LE, GT)

Objective: In the last two decades, the use of mobile phones has increased enormously all over the world. The controversy regarding whether radiofrequency (RF) fields exert effects upon

biological systems is a concern for the general population. An evaluation is made of DNA damage and cytogenetic defects, proliferative potential, and cell death because of RF radiation emitted by mobile phones in healthy young users. Study design: This cohort study was carried out in 50 Caucasian mobile phone users. We collected two cell samples from each subject (a total of 100 cell samples), corresponding to the right and left cheek mucosa, respectively. Case histories and personal information were assessed, including age, gender, body height and weight, history of cancer, smoking and alcohol consumption, exposure to chemical carcinogens or radiation, and dietary habits. Sampling comprised cell collection from both cheeks with a cytobrush, centrifugation, slide preparation, fixation, and staining, followed by fluorescent microscopic analysis. A total of 2000 exfoliated cells were screened for nuclear abnormalities, especially micronucleus. Results: No statistically significant changes were recorded in relation to age, gender, body mass index, or smoking status. A comparison of the results vs the control area according to the side of the face on which the mobile phone was placed, and in relation to the duration of exposure (years) to mobile phone radiation in the total 100 samples, yielded no significant differences. Conclusions: No genotoxic effects because of RF exposure were observed in relation to any of the study parameters.

(NE) Roti Roti JL , Malyapa RS, Bisht KS, Ahern EW, Moros EG, Pickard WF, Straube WL, Neoplastic Transformation in C3H 10T(1/2) cells after exposure to 835.62 MHz FDMA and 847.74 MHz CDMA radiations. Radiat Res 155(1):239-247, 2001.(VT, LE, GT)

The effect of radiofrequency (RF) radiation in the cellular phone communication range (835.62 MHz frequency division multiple access, FDMA; 847.74 MHz code division multiple access, CDMA) on neoplastic transformation frequency was measured using the in vitro C3H 10T(1/2) cell transformation assay system. To determine if 835.62 MHz FDMA or 847.74 MHz CDMA radiations have any genotoxic effects that induce neoplastic transformation, C3H 10T(1/2) cells were exposed at 37 degrees C to either of the above radiations [each at a specific absorption rate (SAR) of 0.6 W/kg] or sham-exposed at the same time for 7 days. After the culture medium was changed, the cultures were transferred to incubators and refed with fresh growth medium every 7 days. After 42 days, the cells were fixed and stained with Giemsa, and transformed foci were scored. To determine if exposure to 835.62 MHz FDMA or 847.74 MHz CDMA radiation has any epigenetic effects that can promote neoplastic transformation, cells were first exposed to 4.5 Gy of X rays to induce the transformation process and then exposed to the above radiations (SAR = 0.6 W/kg) in temperature-controlled irradiators with weekly refeeding for 42 days. After both the 7-day RF exposure and the 42-day RF exposure after X irradiation, no statistically significant differences in the transformation frequencies were observed between incubator controls, the sham-exposed (maintained in irradiators without power to the antenna), and the 835.62 MHz FDMA or 847.74 MHz CDMA-exposed groups.

(E) Roux D, Vian A, Girard S, Bonnet P, Paladian F, Davies E, Ledoigt G. Electromagnetic fields (900 MHz) evoke consistent molecular responses in tomato plants. *Physiologia Plantarum* 128: 283–288, 2006. (VO, AE, GE, LI)

Although the effects of high-frequency electromagnetic fields on biological systems have been studied frequently, unequivocal results have rarely been obtained, primarily because suitably controlled experiments could not be performed. In the present work, tomato plants were exposed to a homogeneous and isotropic field (900 MHz) using a mode stirred reverberation chamber, and the stress-related transcripts (calmodulin, protease inhibitor and chloroplast mRNA-binding protein) were assayed by real-time quantitative PCR. Exposure to an electromagnetic field induced a biphasic response, in which the levels of all three transcripts increased four- to six-fold 15 min after the end of electromagnetic stimulation, dropped to close to initial levels by 30 min, and then increased again at 60 min. We deliberately focused on the very early molecular responses to high-frequency electromagnetic fields in order to minimize secondary effects.

(E) Roux D, Vian A, Girard S, Bonnet P, Paladian F, Davies E, Ledoigt G. High frequency (900 MHz) low amplitude (5 V m⁻¹) electromagnetic field: a genuine environmental stimulus that affects transcription, translation, calcium and energy charge in tomato. *Planta*. 227(4):883-891, 2008. (VO, AE, GE)

Using an especially-designed facility, the Mode Stirred Reverberation Chamber, we exposed tomato plants (*Lycopersicon esculentum* Mill. VFN8) to low level (900 MHz, 5 V m⁻¹) electromagnetic fields for a short period (10 min) and measured changes in abundance of three specific mRNA soon after exposure. Within minutes of electromagnetic stimulation, stress-related mRNA (calmodulin, calcium-dependent protein kinase and proteinase inhibitor) accumulated in a rapid, large and 3-phase manner typical of an environmental stress response. Accumulation of these transcripts into the polysomal RNA also took place (indicating that the encoded proteins were translated) but was delayed (indicating that newly-synthesized mRNA was not immediately recruited into polysomes). Transcript accumulation was maximal at normal Ca(2+) levels and was depressed at higher Ca(2+), especially for those encoding calcium-binding proteins. Removal of Ca(2+) (by addition of chelating agents or Ca(2+) channel blocker) led to total suppression of mRNA accumulation. Finally, 30 min after the electromagnetic treatment, ATP concentration and adenylate energy charge were transiently decreased, while transcript accumulation was totally prevented by application of the uncoupling reagent, CCCP. These responses occur very soon after exposure, strongly suggesting that they are the direct consequence of application of radio-frequency fields and their similarities to wound responses strongly suggests that this radiation is perceived by plants as an injurious stimulus.

(E) Sagripanti JL, Swicord ML. DNA structural changes caused by microwave radiation. *Int J Radiat Biol Relat Stud Phys Chem Med*. 50(1):47-50, 1986. (VT, AE, GT)

No Abstract available. "A 28 µl solution containing 10 µg purified DNA was exposed to 2.55GHz

microwave radiation at 20°C for 20min". "SAR_{min} and SAR_{max} ranges: 0, 2-8-5 and 21-85 mW/g". "These results suggest that exposure to microwave radiation can cause single as well as double-strand breaks in DNA in solution."

(E) Sagripanti JL, Swicord ML, Davis CC. Microwave effects on plasmid DNA. Radiat Res. 110(2):219-221, 1987. (VT, AE, GT)

The exposure of purified plasmid DNA to microwave radiation at nonthermal levels in the frequency range from 2.00 to 8.75 GHz produces single- and double-strand breaks that are detected by agarose gel electrophoresis. Microwave-induced damage to DNA depends on the presence of small amounts of copper. This effect is dependent upon both the microwave power and the duration of the exposure. Cuprous, but not cupric, ions were able to mimic the effects produced by microwaves on DNA.

(E) Sahin D, Ozgur E, Guler G, Tomruk A, Unlu I, Sepici-Dincel A, et al. The 2100 MHz radiofrequency radiation of a 3G-mobile phone and the DNA oxidative damage in brain. J Chem Neuroanatmy. 2016; 75: 94-98. (VO, LE, GT, OX)

We aimed to evaluate the effect of 2100MHz radiofrequency radiation emitted by a generator, simulating a 3G-mobile phone on the brain of rats during 10 and 40 days of exposure. The female rats were randomly divided into four groups. Group I; exposed to 3G modulated 2100MHz RFR signal for 6h/day, 5 consecutive days/wk for 2 weeks, group II; control 10 days, were kept in an inactive exposure set-up for 6h/day, 5 consecutive days/wk for 2 weeks, group III; exposed to 3G modulated 2100MHz RFR signal for 6h/day, 5 consecutive days/wk for 8 weeks and group IV; control 40 days, were kept in an inactive exposure set-up for 6h/day, 5 consecutive days/wk for 8 weeks. After the genomic DNA content of brain was extracted, oxidative DNA damage (8-hydroxy-2'deoxyguanosine, pg/mL) and malondialdehyde (MDA, nmoL/g tissue) levels were determined. Our main finding was the increased oxidative DNA damage to brain after 10 days of exposure with the decreased oxidative DNA damage following 40 days of exposure compared to their control groups. Besides decreased lipid peroxidation end product, MDA, was observed after 40 days of exposure. The measured decreased quantities of damage during the 40 days of exposure could be the means of adapted and increased DNA repair mechanisms.

(E) Said-Salman IH, Jebaii FA, Yusef HH, Moustafa ME. Global gene expression analysis of Escherichia coli K-12 DH5α after exposure to 2.4 GHz wireless fidelity radiation. Sci Rep. 9(1):14425, 2019. (VT, AE, GE)

This study investigated the non-thermal effects of Wi-Fi radiofrequency radiation of 2.4 GHz on global gene expression in Escherichia coli K-12 DH5α. High-throughput RNA-sequencing of 2.4 GHz exposed and non-exposed bacteria revealed that 101 genes were differentially expressed (DEGs) at $P \leq 0.05$. The up-regulated genes were 52 while the down-regulated ones were 49. QRT-PCR analysis of pgaD, fliC, cheY, malP, malZ, motB, alsC, alsK, appB and appX confirmed the RNA-seq results. About 7% of DEGs are involved in cellular component organization, 6% in response to stress stimulus, 6% in biological regulation, 6% in localization, 5% in locomotion and 3% in cell adhesion. Database for annotation, visualization and integrated

discovery (DAVID) functional clustering revealed that DEGs with high enrichment score included genes for localization of cell, locomotion, chemotaxis, response to external stimulus and cell adhesion. Kyoto encyclopedia of genes and genomes (KEGG) pathways analysis showed that the pathways for flagellar assembly, chemotaxis and two-component system were affected. Go enrichment analysis indicated that the up-regulated DEGs are involved in metabolic pathways, transposition, response to stimuli, motility, chemotaxis and cell adhesion. The down-regulated DEGs are associated with metabolic pathways and localization of ions and organic molecules. Therefore, the exposure of E. coli DH5 α to Wi-Fi radiofrequency radiation for 5 hours influenced several bacterial cellular and metabolic processes.

(NE) Sakuma N, Komatsubara Y, Takeda H, Hirose H, Sekijima M, Nojima T, Miyakoshi J. DNA strand breaks are not induced in human cells exposed to 2.1425 GHz band CW and W-CDMA modulated radiofrequency fields allocated to mobile radio base stations. Bioelectromagnetics 27:51-57, 2006. (VT, AE, CT)

We conducted a large-scale in vitro study focused on the effects of low level radiofrequency (RF) fields from mobile radio base stations employing the International Mobile Telecommunication 2000 (IMT-2000) cellular system in order to test the hypothesis that modulated RF fields may act as a DNA damaging agent. First, we evaluated the responses of human cells to microwave exposure at a specific absorption rate (SAR) of 80 mW/kg, which corresponds to the limit of the average whole body SAR for general public exposure defined as a basic restriction in the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines. Second, we investigated whether continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) modulated signal RF fields at 2.1425 GHz induced different levels of DNA damage. Human glioblastoma A172 cells and normal human IMR-90 fibroblasts from fetal lungs were exposed to mobile communication frequency radiation to investigate whether such exposure produced DNA strand breaks in cell culture. A172 cells were exposed to W-CDMA radiation at SARs of 80, 250, and 800 mW/kg and CW radiation at 80 mW/kg for 2 and 24 h, while IMR-90 cells were exposed to both W-CDMA and CW radiations at a SAR of 80 mW/kg for the same time periods. Under the same RF field exposure conditions, no significant differences in the DNA strand breaks were observed between the test groups exposed to W-CDMA or CW radiation and the sham exposed negative controls, as evaluated immediately after the exposure periods by alkaline comet assays. Our results confirm that low level exposures do not act as a genotoxicant up to a SAR of 800 mW/kg.

(NE) Sakurai T, Kiyokawa T, Narita E, Suzuki Y, Taki M, Miyakoshi J. Analysis of gene expression in a human-derived glial cell line exposed to 2.45 GHz continuous radiofrequency electromagnetic fields. J Radiat Res. 52(2):185-192, 2011. (VT, AE, GE)

The increasing use of mobile phones has aroused public concern regarding the potential health risks of radiofrequency (RF) fields. We investigated the effects of exposure to RF fields (2.45 GHz, continuous wave) at specific absorption rate (SAR) of 1, 5, and 10 W/kg for 1, 4, and 24 h on gene expression in a normal human glial cell line, SVGp12, using DNA microarray. Microarray analysis revealed 23 assigned gene spots and 5 non-assigned gene spots as prospective altered gene spots. Twenty-two genes out of the 23 assigned gene spots were further

analyzed by reverse transcription-polymerase chain reaction to validate the results of microarray, and no significant alterations in gene expression were observed. Under the experimental conditions used in this study, we found no evidence that exposure to RF fields affected gene expression in SVGp12 cells.

(NE) Salmen SH, Alharbi SA, Faden AA, Wainwright M. Evaluation of effect of high frequency electromagnetic field on growth and antibiotic sensitivity of bacteria. Saudi J Biol Sci. 25(1):105-110, 2018. (VO, AE, GT)

This study was aimed to evaluate the impact of high frequency electromagnetic fields (HF-EMF at 900 and 1800 MHz) on DNA, growth rate and antibiotic susceptibility of *S. aureus*, *S. epidermidis*, and *P. aeruginosa*. In this study, bacteria were exposed to 900 and 1800 MHz for 2 h and then inoculated to new medium when their growth rate and antibiotic susceptibility were evaluated. Results for the study of bacterial DNA unsuccessful to appearance any difference exposed and non-exposed *S. aureus* and *S. epidermidis*. Exposure of *S. epidermidis* and *S. aureus* to electromagnetic fields mostly produced no statistically significant decrease in bacterial growth, except for *S. aureus* when exposure to 900 MHz at 12 h. Exposure of *P. aeruginosa* to electromagnetic fields at 900 MHz however, lead to a significant reduction in growth rate, while 1800 MHz had insignificant effect. With the exception of *S. aureus*, treated with amoxicillin (30 µg) and exposed to electromagnetic fields, radiation treatment had no significant effect on bacterial sensitivity to antibiotics.

(NE) Sannino A, Calabrese ML, d'Ambrosio G, Massa R, Petraglia G, Mita P, et al. Evaluation of cytotoxic and genotoxic effects in human peripheral blood leukocytes following exposure to 1950- MHz modulated signal. IEEE Trans Plasma Sci. 34: 1441-1448, 2006. (VT, AE, GT)

Human peripheral blood leukocytes from six volunteers were exposed to a Universal Mobile Telecommunication System (UMTS) signal (frequency carrier of 1950 MHz) for 24 h. The exposures were carried out in a waveguide system at specific absorption rates (SAR) of 0.5 and 2.0 W/kg, and for each blood donor, sham-exposed samples were also set up. The alkaline comet assay was used to quantify DNA damage, while cytotoxicity was determined by the Trypan blue exclusion method. The results obtained indicate the absence of genotoxic and cytotoxic effects at both SAR levels investigated, as assessed by comparing sham-exposed and exposed samples. Therefore, the findings indicate that, in the experimental conditions adopted, 24-h in vitro exposure to 1950-MHz radio-frequency radiation (UMTS signal) does not induce DNA damage in human leukocytes

(NE) Sannino A, Di Costanzo G, Brescia F, Sarti M, Zeni O, Juutilainen J, Scarfi MR. Human fibroblasts and 900 MHz radiofrequency radiation: evaluation of DNA damage after exposure and co-exposure to 3-Chloro-4-(dichloromethyl)-5-Hydroxy-2(5h)-furanone (MX). Radiat Res 171:743-751, 2009a. (VT, AE, NT, IX)

The aim of this study was to investigate DNA damage in human dermal fibroblasts from a healthy subject and from a subject affected by Turner's syndrome that were exposed for 24 h to radiofrequency (RF) radiation at 900 MHz. The RF-radiation exposure was carried out alone or in combination with 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), a well-

known environmental mutagen and carcinogen produced during the chlorination of drinking water. Turner's syndrome fibroblasts were also exposed for a shorter time (1 h). A signal similar to that emitted by Global System for Mobile Communications (GSM) mobile phones was used at a specific absorption rate of 1 W/kg under strictly controlled conditions of temperature and dosimetry. To evaluate DNA damage after RF-radiation exposure alone, the alkaline comet assay and the cytokinesis-block micronucleus assay were used. In the combined-exposure experiments, MX was given at a concentration of 25 microM for 1 h immediately after the RF-radiation exposure, and the effects were evaluated by the alkaline comet assay. The results revealed no genotoxic and cytotoxic effects from RF radiation alone in either cell line. As expected, MX treatment induced an increase in DNA migration in the comet assay, but no enhancement of the MX-induced DNA damage was observed in the cells exposed to RF radiation.

(NE) Sannino A, Costanzo GD, Brescia F, Sarti M, Zeni O, Jautilainen J, et al. Human fibroblasts and 900 MHz radiofrequency radiation: Evaluation of DNA damage after exposure and co-exposure to 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone. Radiat Res. 2009b; 171: 743-751. (VT, AE, GT)

The aim of this study was to investigate DNA damage in human dermal fibroblasts from a healthy subject and from a subject affected by Turner's syndrome that were exposed for 24 h to radiofrequency (RF) radiation at 900 MHz. The RF-radiation exposure was carried out alone or in combination with 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), a well-known environmental mutagen and carcinogen produced during the chlorination of drinking water. Turner's syndrome fibroblasts were also exposed for a shorter time (1 h). A signal similar to that emitted by Global System for Mobile Communications (GSM) mobile phones was used at a specific absorption rate of 1 W/kg under strictly controlled conditions of temperature and dosimetry. To evaluate DNA damage after RF-radiation exposure alone, the alkaline comet assay and the cytokinesis-block micronucleus assay were used. In the combined-exposure experiments, MX was given at a concentration of 25 microM for 1 h immediately after the RF-radiation exposure, and the effects were evaluated by the alkaline comet assay. The results revealed no genotoxic and cytotoxic effects from RF radiation alone in either cell line. As expected, MX treatment induced an increase in DNA migration in the comet assay, but no enhancement of the MX-induced DNA damage was observed in the cells exposed to RF radiation.

(E) Sannino A, Zeni O, Sarti M, Romeo S, Reddy SB, Belisario MA, et al. (2011) Induction of adaptive response in human blood lymphocytes exposed to 900 MHz radiofrequency fields: influence of cell cycle. Int J Radiat Biol. 2011; 87: 993-999. (VT, AE, GT, IX)

Purpose: To investigate the influence of cell cycle on the adaptive response (AR) induced by the exposure of human blood lymphocytes to radiofrequency fields (RF). **Materials and methods:** Human peripheral blood lymphocytes in G(0)-, G(1)- or S-phase of the cell cycle were exposed for 20 hours to an adaptive dose (AD) of 900 MHz RF at an average specific absorption rate of 1.25 W/kg and then treated with a challenge dose (CD) of 100 ng/ml mitomycin C (MMC). Un-exposed and sham-exposed controls as well as cells treated with MMC alone were included in the study. The incidence of micronuclei (MN) was evaluated to determine the induction of AR. **Results:** The results indicated that the cells which were exposed to AD of RF in G(0)- and G(1)-phase of the cell cycle did not exhibit AR while such a response was observed

when the cells were exposed to AD of RF in S-phase of the cell cycle. **Conclusions:** These results confirmed the observations reported in our previous investigation where AR was observed in human blood lymphocytes exposed to AD of RF in S-phase of the cell cycle and further suggested that the timing of AD exposure of RF is important to elicit AR.

(E) Sannino A, Zeni O, Romeo S, Massa R, Gialanella G, Grossi G, et al. Adaptive response in human blood lymphocytes exposed to non-ionizing radiofrequency fields: resistance to ionizing radiation-induced damage. J Radiat Res. 2014; 55: 210-217. (VT, AE, GT, IX)

The aim of this preliminary investigation was to assess whether human peripheral blood lymphocytes which have been pre-exposed to non-ionizing radiofrequency fields exhibit an adaptive response (AR) by resisting the induction of genetic damage from subsequent exposure to ionizing radiation. Peripheral blood lymphocytes from four healthy donors were stimulated with phytohemagglutinin for 24 h and then exposed for 20 h to 1950 MHz radiofrequency fields (RF, adaptive dose, AD) at an average specific absorption rate of 0.3 W/kg. At 48 h, the cells were subjected to a challenge dose (CD) of 1.0 or 1.5 Gy X-irradiation (XR, challenge dose, CD). After a 72 h total culture period, cells were collected to examine the incidence of micronuclei (MN). There was a significant decrease in the number of MN in lymphocytes exposed to RF + XR (AD + CD) as compared with those subjected to XR alone (CD). These observations thus suggested a RF-induced AR and induction of resistance to subsequent damage from XR. There was variability between the donors in RF-induced AR. The data reported in our earlier investigations also indicated a similar induction of AR in human blood lymphocytes that had been pre-exposed to RF (AD) and subsequently treated with a chemical mutagen, mitomycin C (CD). Since XR and mitomycin-C induce different kinds of lesions in cellular DNA, further studies are required to understand the mechanism(s) involved in the RF-induced adaptive response.

(E) Sannino A, Zeni O, Romeo S, Massa R, Scarfi MR. Adverse and beneficial effect in Chinese hamster lung fibroblast cells following radiofrequency exposure. Bioelectromagnetics. 2017; 38: 245-254. (VT, AE, GT)

In this study, the effect of radiofrequency (RF) exposure to 1950 MHz, Universal Mobile Telecommunication System signal, was investigated in Chinese hamster lung fibroblast cell line (V79). Genotoxic and cytotoxic effects of 20-h exposure at specific absorption rate (SAR) values from 0.15 W/kg to 1.25 W/kg were measured by means of cytokinesis-block micronucleus (MN) assay. Exposure was carried out blinded under strictly controlled conditions of dosimetry and temperature. The effect of RF exposure alone at four SAR values was tested, that is, 0.15, 0.3, 0.6, and 1.25 W/kg. A statistically significant increase in MN frequency was found in cultures exposed to 0.15 and 0.3 W/kg ($P < 0.05$) compared to sham-exposed ones, in the absence of cytotoxicity. SAR values of 0.6 and 1.25 W/kg did not exert any effect. Moreover, to evaluate the ability of RF to exert protective effects with respect to a chemical mutagen, cell cultures were also pre-exposed for 20 h at 0.3 or 1.25 W/kg, and then treated with 500 ng/ml of mitomycin-C (MMC). A significant reduction in the frequency of MN was detected in cultures pre-exposed to 1.25 W/kg compared to cultures treated with MMC alone ($P < 0.05$), indicating induction of adaptive response. Such a decrease was not induced by pre-exposure at 0.3 W/kg SAR. Taken

together, our results indicated that V79 is a sensitive cell model to evidence either adverse or beneficial effects of RF exposure, depending on experimental conditions applied.

(E) Sarimov R, Malmgren L.O.G., Markova, E., Persson, B.R.R., Belyaev, I.Y. Nonthermal GSM microwaves affect chromatin conformation in human lymphocytes similar to heat shock. IEEE Trans Plasma Sci 32:1600-1608, 2004. (VT, AE, GT, LI, WS)

Here we investigated whether microwaves (MWs) of Global System for Mobile Communication (GSM) induce changes in chromatin conformation in human lymphocytes. Effects of MWs were studied at different frequencies in the range of 895-915 MHz in experiments with lymphocytes from seven healthy persons. Exposure was performed in transverse electromagnetic transmission line cell (TEM-cell) using a GSM test-mobile phone. All standard modulations included 2 W output power in the pulses, specific absorbed rate (SAR) being 5.4 mW/kg. Changes in chromatin conformation, which are indicative of stress response and genotoxic effects, were measured by the method of anomalous viscosity time dependencies (AVTD). Heat shock and treatment with the genotoxic agent camptothecin, were used as positive controls. 30-min exposure to MWs at 900 and 905 MHz resulted in statistically significant condensation of chromatin in lymphocytes from 1 of 3 tested donors. This condensation was similar to effects of heat shock within the temperature window of 40/spl deg/C-44/spl deg/C. Analysis of pooled data from all donors showed statistically significant effect of 30-min exposure to MWs. Stronger effects of MWs was found following 1-h exposure. In replicated experiments, cells from four out of five donors responded to 905 MHz. Responses to 915 MHz were observed in cells from 1 out of 5 donors, $p < 0.002$. Dependent on donor, condensation, 3 donors, or decondensation, 1 donor, of chromatin was found in response to 1-h exposure. Analysis of pooled data from all donors showed statistically significant effect of 1-h exposure to MWs. In cells from one donor, this effect was frequency-dependent ($p < 0.01$). Effects of MWs correlated statistically significantly with effects of heat shock and initial state of chromatin before exposure. MWs at 895 and 915 MHz affected chromatin conformation in transformed lymphocytes. The conclusion-GSM microwaves under specific conditions of exposure affected human lymphocytes similar to stress response. The data suggested that the MW effects differ at various GSM frequencies and vary between donors.

(E) Sarkar S, Ali S, Behari J, Effect of low power microwave on the mouse genome: a direct DNA analysis. Mutat Res 320(1-2):141-147, 1994. (VO, LE, GT)

The potential mutagenic effect of low power microwave at the DNA sequence level in the mouse genome was evaluated by direct DNA analysis. Animals were exposed to microwave at a power density of 1 mW/cm² for 2 h/day at a frequency of 2.45 GHz over a period of 120, 150 and 200 days. HinfI digested DNA samples from testis and brain of control and exposed animals were hybridized with a synthetic oligo probe (OAT 36) comprising nine repeats of 5'-GACA-3'. As compared to control animals, band patterns in exposed animals were found to be distinctly altered in the range of 7-8 kb which was also substantiated by densitometric analysis. Though

the mechanism of this rearrangement is not yet clear, the results obtained at the present dose are of significance. This dose, which has been set as the safe limit for general public exposure by the Non-Ionizing Radiation Committee of the International Radiation Protection Association, may imply a need for (re)evaluation of the mutagenic potential of microwaves at the prescribed safe limit for the personnel and people who are being exposed.

(E) Saxena AK, Singh A, Chilkoti GT, Sharma T, Banerjee BD, Das S, Singhal A. Modulation of signal transduction gene expression following pulsed radiofrequency in dorsal root ganglia and pregabalin therapy. Pain Manag 2021 Nov 17. doi: 10.2217/pmt-2021-0057. Online ahead of print. (HU, LE, GE)

Introduction: A randomized controlled study was conducted to assess modulation of signal transduction genes (*PKA*, *PKC* and *ERK*) following integrated multimodal approach encompassing pulsed radiofrequency treatment (PRF) of dorsal root ganglion and pregabalin in thoracic postherpetic neuralgia (PHN). Clinical variables such as pain intensity and quality of life were also explored. **Material & methods:** A total of 40 Patients of PHN were recruited. 20 patients randomly assigned to each of the two groups, group PP administered PRF with pregabalin and group SP administered pregabalin alone. **Results:** Significant downregulation of *PKA* and *ERK* observed in group PP at end of 10th week ($p < 0.05$). A significantly positive correlation demonstrated between Visual analog scale scores and signal transduction genes expression in PHN patients. **Conclusion:** Downregulation of all three signal transduction genes was observed following the integrated multimodal approach; however, significant downregulation was observed with *PKA* and *ERK* only. A positive correlation observed between signal transduction gene expression and visual analog scale scores signify their role in the pathogenesis of PHN.

(E) Scarfi MR, Lioi MB, d'Ambrosio G, Massa R, Zeni O, Pietro RD, et al. Genotoxic effects of mitomycin-C and microwave radiation on bovine lymphocytes. Electro Magneto Biol. 1996; 15, 99-107. (VT, AE, GT)

The aim of this work was to ascertain whether microwave radiation (frequency 9 GHz; specific absorption rate 70 mW/gr, exposure duration 10 min) may produce genotoxic effects, assessed by cytokinesis-block micro-nucleus (MN) assay, in bovine (*Bos taurus* L.) peripheral blood lymphocyte cultures. As positive control for the MN induction, mitomycin-C (MMC) was used: it is known that this alkylating agent has a potent genotoxic (radiomimetic) action. To evaluate possible cooperative effects, some cultures from microwave-exposed samples were treated with MMC. The results obtained indicate that the optimal dose of MMC to induce MN frequency increase in this species is 0.044 $\mu\text{g/ml}$ and that microwave radiation induces a statistically significant increase of MN frequency both with and without MMC.

(NE) Scarfi MR, Romano M, Di Pietro R, Zeni O, Doria A, Gallerano GP, et al. THz exposure of whole blood for the study of biological effects on human lymphocytes. J Biol Phy. 2003; 29: 171-177. (VT, AE, GT)

The aim of the present study is to investigate the genotoxic effect of THz radiation in human peripheral blood lymphocytes following 20 minutes exposure to 1 mW average power Free

Electron Laser radiation in the frequency range 120-140GHz. For this purpose 9 healthy donors were employed and cytokinesis block technique was applied to study micronucleus frequency and cell proliferation. The results obtained indicate that all the electromagnetic conditions adopted so far do not alter the investigated parameters, suggesting absence of direct chromosomal damage and alteration of cell cycle kinetics (two tailed paired Student's test: $p > 0.05$ in all cases).

(NE) Scarfi MR, Fresegna AM, Villani P, Pinto R, Marino C, Sarti M, Altavista P, Sannino A, Lovisolo GA. Exposure to radiofrequency radiation (900 MHz, GSM signal) does not affect micronucleus frequency and cell proliferation in human peripheral blood lymphocytes: an interlaboratory study. Radiat Res. 165(6):655-663, 2006. (VT, AE, GT)

The objective of this study was to investigate whether 24 h exposure to radiofrequency electromagnetic fields similar to those emitted by mobile phones induces genotoxic effects and/or effects on cell cycle kinetics in cultured human peripheral blood lymphocytes. The effect of 900 MHz exposure (GSM signal) was evaluated at four specific absorption rates (SARs, 0, 1, 5 and 10 W/kg peak values). The exposures were carried out in wire patch cells under strictly controlled conditions of both temperature and dosimetry, and the induction of genotoxic effects was evaluated in lymphocyte cultures from 10 healthy donors by applying the cytokinesis-block micronucleus assay. Positive controls were provided by using mitomycin C. Two research groups were involved in the study, one at ENEA, Rome, and the other at CNR-IREA, Naples. Each laboratory tested five donors, and the resulting slides were scored by both laboratories. Following this experimental scheme, it was also possible to compare the results obtained by cross-scoring of slides. The results obtained provided no evidence for the existence of genotoxic or cytotoxic effects in the range of SARs investigated. These findings were confirmed in the two groups of five donors examined in the two laboratories and when the same slides were scored by two operators.

(NE) Schuermann D, Ziemann C, Barekati Z, Capstick M, Oertel A, Focke F, Murbach M, Kuster N, Dasenbrock C, Schär P. Assessment of Genotoxicity in Human Cells Exposed to Modulated Electromagnetic Fields of Wireless Communication Devices. Genes (Basel). 2020 Mar 25;11(4). pii: E347. doi: 10.3390/genes11040347. (VT, AE, GT)

Modulated electromagnetic fields (wEMFs), as generated by modern communication technologies, have raised concerns about adverse health effects. The International Agency for Research on Cancer (IARC) classifies them as "possibly carcinogenic to humans" (Group 2B), yet, the underlying molecular mechanisms initiating and promoting tumorigenesis remain elusive. Here, we comprehensively assess the impact of technologically relevant wEMF modulations on the genome integrity of cultured human cells, investigating cell type-specificities as well as time- and dose-dependencies. Classical and advanced methodologies of genetic toxicology and DNA repair were applied, and key experiments were performed in two separate laboratories. Overall, we found no conclusive evidence for an induction of DNA damage nor for alterations of the DNA repair capacity in cells exposed to several wEMF modulations (i.e., GSM, UMTS, WiFi, and RFID). Previously reported observations of increased DNA damage after

exposure of cells to GSM-modulated signals could not be reproduced. Experimental variables, presumably underlying the discrepant observations, were investigated and are discussed. On the basis of our data, we conclude that the possible carcinogenicity of wEMF modulations cannot be explained by an effect on genome integrity through direct DNA damage. However, we cannot exclude non-genotoxic, indirect, or secondary effects of wEMF exposure that may promote tumorigenesis in other ways.

(E) Schwarz C, Kratochvil E, Pilger A, Kuster N, Adlkofer F, Rüdiger HW. Radiofrequency electromagnetic fields (UMTS, 1,950 MHz) induce genotoxic effects in vitro in human fibroblasts but not in lymphocytes. Int Arch Occup Environ Health 81:755-767, 2008. (VT, AE, GT, CS)

OBJECTIVE: Universal Mobile Telecommunication System (UMTS) was recently introduced as the third generation mobile communication standard in Europe. This was done without any information on biological effects and genotoxic properties of these particular high-frequency electromagnetic fields. This is disconcerting, because genotoxic effects of the second generation standard Global System for Mobile Communication have been reported after exposure of human cells in vitro. METHODS: Human cultured fibroblasts of three different donors and three different short-term human lymphocyte cultures were exposed to 1,950 MHz UMTS below the specific absorption rate (SAR) safety limit of 2 W/kg. The alkaline comet assay and the micronucleus assay were used to ascertain dose and time-dependent genotoxic effects. Five hundred cells per slide were visually evaluated in the comet assay and comet tail factor (CTF) was calculated. In the micronucleus assay 1,000 binucleated cells were evaluated per assay. The origin of the micronuclei was determined by fluorescence labeled anticentromere antibodies. All evaluations were performed under blinded conditions. RESULTS: UMTS exposure increased the CTF and induced centromere-negative micronuclei (MN) in human cultured fibroblasts in a dose and time-dependent way. Incubation for 24 h at a SAR of 0.05 W/kg generated a statistically significant rise in both CTF and MN ($P = 0.02$). At a SAR of 0.1 W/kg the CTF was significantly increased after 8 h of incubation ($P = 0.02$), the number of MN after 12 h ($P = 0.02$). No UMTS effect was obtained with lymphocytes, either unstimulated or stimulated with Phytohemagglutinin. CONCLUSION: UMTS exposure may cause genetic alterations in some but not in all human cells in vitro.

(E) Sekeroğlu V, Akar A, Sekeroğlu ZA. Cytotoxic and genotoxic effects of high-frequency electromagnetic fields (GSM 1800 MHz) on immature and mature rats. Ecotoxicol Environ Saf. 80:140-144, 2012. (VO, LE, GT, DE)

We investigated the cytogenotoxic effects of high frequency electromagnetic fields (HF-EMF) for 45 day and the effect of a recovery period of 15 day after exposure to EMF on bone marrow cells of immature and mature rats. The animals in treatment groups were exposed to 1800 MHz EMF at SAR of 0.37 W/kg and 0.49 W/kg for 2h/day for 45 day. Two recovery groups were kept for a recovery period of 15 day without EMF after exposure to HF-EMF. Two control groups for both immature and mature rats were also included. Significant differences were also observed in chromosome aberrations (CA), micronucleus (MN) frequency, mitotic index (MI) and ratio of polychromatic erythrocytes (PCEs) in all treatment groups. The cytogenotoxic damage was more

remarkable in immature rats and, the recovery period did not improve this damage in immature rats. Because much higher and irreversible cytogenotoxic damage was observed in immature rats than in mature rats, further studies are needed to understand effects of EMF on DNA damage and DNA repair, and to determine safe limits for environment and human, especially for children.

(E) Sekeroglu ZA, Akar A, Sekeroglu V. Evaluation of the cytogenetic damage in immature and mature rats exposed to 900 MHz radiofrequency electromagnetic fields. Int J Radiat Biol. 2013; 89:985-892. (VO, RE, GT, DE)

Purpose: One of the most important issues regarding radiofrequency electromagnetic fields (RF-EMF) is their effect on genetic material. Therefore, we investigated the cytogenotoxic effects of 900 MHz radiofrequency electromagnetic fields (RF-EMF) and the effect of a recovery period after exposure to RF-EMF on bone marrow cells of immature and mature rats. **Materials and methods:** The immature and mature rats in treatment groups were exposed to RF-EMF for 2 h/day for 45 days. Average electrical field values for immature and mature rats were 28.1 ± 4.8 V/m and 20.0 ± 3.2 V/m, respectively. Whole-body specific absorption rate (SAR) values for immature and mature rats were in the range of 0.38-0.78 W/kg, and 0.31-0.52 W/kg during the 45 days, respectively. Two recovery groups were kept for 15 days after RF-EMF exposure. **Results:** Significant differences were observed in chromosome aberrations (CA), micronucleus (MN) frequency, mitotic index (MI) and ratio of polychromatic erythrocytes (PCE) in all treatment and recovery groups. The cytogenotoxic damage in immature rats was statistically higher than the mature rats. The recovery period did not reduce the damage to the same extent as the corresponding control groups. **Conclusions:** The exposure of RF-EMF leads to cytotoxic and genotoxic damage in immature and mature rats. More sensitive studies are required to elucidate the possible carcinogenic risk of EMF exposure in humans, especially children.

(NE) Sekijima M, Takeda H, Yasunaga K, Sakuma N, Hirose H, Nojima T, Miyakoshi J. 2-GHz band CW and W-CDMA modulated radiofrequency fields have no significant effect on cell proliferation and gene expression profile in human cells. J Radiat Res. 51(3):277-284, 2010. (VT, AE, GE)

We investigated the mechanisms by which radiofrequency (RF) fields exert their activity, and the changes in both cell proliferation and the gene expression profile in the human cell lines, A172 (glioblastoma), H4 (neuroglioma), and IMR-90 (fibroblasts from normal fetal lung) following exposure to 2.1425 GHz continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) RF fields at three field levels. During the incubation phase, cells were exposed at the specific absorption rates (SARs) of 80, 250, or 800 mW/kg with both CW and W-CDMA RF fields for up to 96 h. Heat shock treatment was used as the positive control. No significant differences in cell growth or viability were observed between any test group exposed to W-CDMA or CW radiation and the sham-exposed negative controls. Using the Affymetrix Human Genome Array, only a very small (< 1%) number of available genes (ca. 16,000 to 19,000) exhibited altered expression in each experiment. The results confirm that low-level exposure to 2.1425 GHz CW and W-CDMA RF fields for up to 96 h did not act as an acute cytotoxicant in either cell proliferation or the gene expression profile. These results suggest that RF exposure up

to the limit of whole-body average SAR levels as specified in the ICNIRP guidelines is unlikely to elicit a general stress response in the tested cell lines under these conditions.

(E) Semin IuA, Shvartsburg LK, Dubovik BV. [Changes in the secondary structure of DNA under the influence of external low-intensity electromagnetic field] Radiats Biol Radioecol 35(1):36-41, 1995. (VT, AE, GT, WS)

The effect of weak RF on the stability of DNA secondary structure was studied in vitro. DNA was exposed in the presence of glycine and formaldehyde. Aminomethynol compounds, which form in this medium, react with DNA bases at single-strand sites, which prevents recovery from damage to the DNA secondary structure. The damage accumulates during the incubation, and its amount can be estimated from the dynamics of thermal DNA denaturalization after RF or sham exposure. Samples were exposed in an anechoic chamber at 18°C at 10 different microwave frequencies simultaneously (4- to 8 GHz, 25 ms pulses, 0.4 to 0.7 mW/cm² peak power, 1- to 6-Hz repetition rate, no heating). Parallel control samples were sham exposed in a shielded area in the same chamber. The experiments established that irradiation at 3 or 4 Hz and 0.6 mW/cm² peak power clearly increased the accumulated damage to the DNA secondary structure ($P < .00001$). However, changing the pulse repetition rate to 1, 5, 6 Hz, as well as changing the peak power to 0.4 or 0.7 mW/cm², eliminated the effect entirely. Thus, the effect occurred only within narrow 'windows' of the peak intensities and modulation frequencies.

(NE) Senturk E, Tugrul S, Doğan R, Eren SB, Ozturan O, Koçyiğit A, Kesgin S. Effect of radiofrequency on DNA damage and oxidative status in patients with turbinate hypertrophy. Indian J Otolaryngol Head Neck Surg. 71(Suppl 3):1810-1815, 2019. (HU, LE, GT, OX)

The radiofrequency devices that are used generate radiofrequency in the frequency range of 1.5 and 2.5 MHz. This study aims to demonstrate whether systematic oxidative status and DNA are influenced in this frequency range. In study, 27 patients who received radiofrequency treatment on inferior turbinate as they were diagnosed with inferior turbinate hypertrophy. DNA damage was assessed by alkaline comet assay in peripheral lymphocyte cells. Plasma levels of total antioxidant status (TAS), total oxidative status (TOS) were determined by using an automated measurement method and oxidative stress index (OSI) was calculated (OSI was calculated as: $OSI = (TOS/TAS) \times 100$). There were increased in the OSI and TOS values on days 1 and 15 as compared to the samples taken before the radiofrequency administration. Significant decreases were seen in TAS values on days 1 and 15. As for the DNA damage, no significant differences were found on day 15 compared to the preoperative values even though there was a statistically insignificant increase on day 1. Administration of radiofrequency radiation on inferior turbinates results in increased oxidative stress in the acute period and a decrease in the anti-oxidative system. Although this effect causes a slight increase in the DNA damage in the early post-operative period, the damage is restored to the pre-operative levels on day 15. Therefore, we believe that a more conservative approach should be selected for radiofrequency treatment instead of using it routinely.

(E) Shah C, Nair A, Naik M2, Bakshi S. Cell phone radiation and genomic damage: In vitro exposure and assessment. Int J Innov Res Sci Eng Technol. 2015; 4: 401-405. (VT, AE, GT)

The health concerns have been raised following the enormous increase in the use of wireless mobile telephones throughout the world. According to the International Agency for Research in Cancer (IARC), a part of World Health Organization (WHO) has designated cell phone radiation i.e. non-ionizing radiofrequency radiation as „Possible Human Carcinogen“ [Class 2B] in May, 2011. It is believed that the effect is caused because of the electromagnetic frequency generated by the radio frequency which couples with the human tissues which results in induced electric and magnetic fields that cause field distribution in the body. Thus, human body acts as an antenna that receives electromagnetic waves externally. Therefore, effect of radiofrequency radiation needs to be studied by examining the target tissues that are directly exposed to electromagnetic waves i.e. brain tissue, circulating blood, and facial muscles. In this study, circulating blood was taken as target tissue and subjected to cell phone radiation in vitro and following short term cultures metaphase chromosomes were analyzed for frequency of breakage. The results indicated significant increase in chromosomal damage at higher power level and longer exposure times.

(E) Shahin S, Singh VP, Shukla RK, Dhawan A, Gangwar RK, Singh SP, Chaturvedi CM. 2.45 GHz microwave irradiation-induced oxidative stress affects implantation or pregnancy in mice, *Mus musculus*. Appl Biochem Biotechnol. 169(5):1727-1751, 2013. (VO, LE, GT, LI, OX)

The present experiment was designed to study the 2.45 GHz low-level microwave (MW) irradiation-induced stress response and its effect on implantation or pregnancy in female mice. Twelve-week-old mice were exposed to MW radiation (continuous wave for 2 h/day for 45 days, frequency 2.45 GHz, power density=0.033549 mW/cm²), and specific absorption rate=0.023023 W/kg). At the end of a total of 45 days of exposure, mice were sacrificed, implantation sites were monitored, blood was processed to study stress parameters (hemoglobin, RBC and WBC count, and neutrophil/lymphocyte (N/L) ratio), the brain was processed for comet assay, and plasma was used for nitric oxide (NO), progesterone and estradiol estimation. Reactive oxygen species (ROS) and the activities of ROS-scavenging enzymes- superoxide dismutase, catalase, and glutathione peroxidase-were determined in the liver, kidney and ovary. We observed that implantation sites were affected significantly in MW-irradiated mice as compared to control. Further, in addition to a significant increase in ROS, hemoglobin (p<0.001), RBC and WBC counts (p<0.001), N/L ratio (p<0.01), DNA damage (p<0.001) in brain cells, and plasma estradiol concentration (p<0.05), a significant decrease was observed in NO level (p<0.05) and antioxidant enzyme activities of MW-exposed mice. Our findings led us to conclude that a low level of MW irradiation-induced oxidative stress not only suppresses implantation, but it may also lead to deformity of the embryo in case pregnancy continues. We also suggest that MW radiation-induced oxidative stress by increasing ROS production in the body may lead to DNA strand breakage in the brain cells and implantation failure/resorption or abnormal pregnancy in mice.

(E) Shahin NN, El-Nabarawy NA, Gouda AS, Mégarbane B. The protective role of spermine against male reproductive aberrations induced by exposure to electromagnetic field - An experimental investigation in the rat. Toxicol Appl Pharmacol. 370:117-130, 2019. (VO, LE, GT, OX, RP)

The exponentially increasing use of electromagnetic field (EMF)-emitting devices imposes substantial health burden on modern societies with particular concerns of male infertility. Limited studies have addressed the modulation of this risk by protective agents. We investigated the hazardous effects of rat exposure to EMF (900 MHz, 2 h/day for 8 weeks) on male fertility and evaluated the possible protective effect of the polyamine, spermine, against EMF-induced alterations. Exposure to EMF significantly decreased sperm count, viability and motility, and increased sperm deformities. EMF-exposed rats exhibited significant reductions in serum inhibin B and testosterone along with elevated activin A, follicle-stimulating hormone, luteinizing hormone and estradiol concentrations. Testicular steroidogenic acute regulatory protein (StAR), c-kit mRNA expression and testicular activities of the key androgenic enzymes 3 β - and 17 β -hydroxysteroid dehydrogenases were significantly attenuated following exposure to EMF. Exposure led to testicular lipid peroxidation, decreased catalase and glutathione peroxidase activities and triggered nuclear factor-kappa B p65, inducible nitric oxide synthase, cyclooxygenase-2 and caspase-3 overexpression. EMF-exposed rats showed testicular DNA damage as indicated by elevated comet parameters. Spermine administration (2.5 mg/Kg/day intraperitoneally for 8 weeks) prevented EMF-induced alterations in the sperm and hormone profiles, StAR and c-kit expression and androgenic enzyme activities. Spermine hampered EMF-induced oxidative, inflammatory, apoptotic and DNA perturbations. Histological and histomorphometric analysis of the testes supported all biochemical findings. In conclusion, rat exposure to EMF disrupts sperm and hormone profiles with underlying impairment of steroidogenesis and spermatogenesis. Spermine confers protection against EMF-associated testicular and reproductive aberrations, at least in part, via antioxidant, anti-inflammatory and anti-apoptotic mechanisms.

(E) Sharma A, Shrivastava S, Shukla S. Exposure of Radiofrequency Electromagnetic Radiation on Biochemical and Pathological Alterations. Neurol India Sep-Oct 2020;68(5):1092-1100. (VO, LE, GT)

Introduction: In the era of globalization, too much dependency on mobile phones is a cause of concern. **Objective:** The present study was designed to evaluate the risk assessment of microwave radiation (MWR) at 1800 MHz frequency and specific absorption rate 0.433 (W/kg) on male Wistar rats. **Methodology:** Animals were divided into two groups: the first group is the control group, and the second group was exposed to 1800 MHz radiation for 90 days at 4 h/5 days/week in a month. **Results:** Chronic exposure of MWR may alter GSH homeostasis due to alteration in various GSH cycle regulating enzymes such as GR, GPx, GST, and G6PDH which showed an imbalance in GSH content and causes an increase in the oxidative stress and release of inflammatory cytokines. A remarkable increase in the DNA damage was seen due to disorganization and pyknosis of neurons in exposed animal's brain when compared with the control group ($P \leq 0.05$). There was also a significant decline in AChE level. **Conclusion:** The study concludes that MWR may cause neurochemical and pathophysiological damage by initiating the inflammatory process in various brain regions, especially in hippocampus and

cerebral cortex. These effects are further associated with a remarkable elevation in the genotoxicity of neurons with reference to the control group.

(E) Sharma S, Shukla S. Effect of electromagnetic radiation on redox status, acetylcholine esterase activity and cellular damage contributing to the diminution of the brain working memory in rats. J Chem Neuroanat. 2020 Mar 20:101784. (VO, LE, GT, OX)

Behavioral impairments are the most pragmatic outcome of long-term mobile uses but the underlying causes are still poorly understood. Therefore, the Aim of the present study to determine the possible mechanism of mobile induced behavioral alterations by observing redox status, cholinesterase activity, cellular, genotoxic damage and cognitive alterations in rat hippocampus. This study was carried out on 24 male Wistar rats, randomly divided into four groups (n = 6 in each group): group I consisted of sham-exposed (control) rats, group II-IV consisted of rats exposed to microwave radiation (900 MHz) at different time duration 1 h, 2 h, and 4 h respectively for 90 days. After 90 days of exposure, rats were assessing learning ability by using T-Maze. A significantly increased level of malondialdehyde (MDA) with concomitantly depleted levels of superoxide dismutase (SOD), catalase (CAT) and redox enzymes (GSH, GPX, GR, GST, G-6PDH) indicated an exposure of mobile emitted EMR induced oxidative stress by the depleted redox status of brain cells. The depletion in the acetylcholinesterase (AChE) level reveals altered neurotransmission in brain cells. Resultant cellular degeneration was also observed in the radiation-exposed hippocampus. Conclusively, the present study revealed that microwave radiation induces oxidative stress, depleted redox status, and causes DNA damage with the subsequent reduction in working memory in a time-dependent manner. This study provides insight over the associative reciprocity between redox status, cellular degeneration and reduced cholinergic activity, which presumably leads to the behavioral alterations following mobile emitted electromagnetic radiation.

(E) Sharma S, Bahel S, Katnoria JK. Evaluation of oxidative stress and genotoxicity of 900 MHz electromagnetic radiations using Trigonella foenum-graecum test system. Protoplasma 2022 May 11. doi: 10.1007/s00709-022-01768-9. Online ahead of print. (VO, LE, GT)

Unprecedented growth in the communication sector and expanded usage of the number of wireless devices in the past few decades have resulted in a tremendous increase in emissions of non-ionizing electromagnetic radiations (EMRs) in the environment. The widespread EMRs have induced many significant changes in biological systems leading to oxidative stress as well as DNA damage. Considering this, the present study was planned to study the effects of EMRs at 900 MHz frequency with the power density of 10.0 dBm (0.01 W) at variable exposure periods (0.5 h, 1 h, 2 h, 4 h, and 8 h per day for 7 days) on percentage germination, morphological characteristics, protein content, lipid peroxidation in terms of malondialdehyde content (MDA), and antioxidant defense system of Trigonella foenum-graecum test system. The genotoxicity was also evaluated using similar conditions. It was observed that EMRs significantly decreased the germination percentage at an exposure time of 4 h and 8 h. Fresh weight and dry weight of root

and shoot did not show significant variations, while the root and shoot length have shown significant variations for 4 h and 8 h exposure period. Further, EMRs enhanced MDA indicating lipid peroxidation. In response to exposure of EMRs, there was a significant up-regulation in the activities of enzymes such as ascorbate peroxidase (APX), superoxide dismutase (SOD), glutathione-S-transferase (GST), guaiacol peroxidase (POD), and glutathione reductase (GR) in the roots and shoots of *Trigonella-foenum graecum*. The genotoxicity study showed the induction of chromosomal aberrations in root tip cells of the *Trigonella foenum-graecum* test system. The present study revealed the induction of oxidative stress and genotoxicity of EMRs exposure in the test system.

(E) Sharma S, Bahel S, Katnoria JK. Evaluation of oxidative stress and genotoxicity of 900 MHz electromagnetic radiations using *Trigonella foenum-graecum* test system. *Protoplasma* 2022 May 11. doi: 10.1007/s00709-022-01768-9. Online ahead of print. (VO, LE, GT)

Unprecedented growth in the communication sector and expanded usage of the number of wireless devices in the past few decades have resulted in a tremendous increase in emissions of non-ionizing electromagnetic radiations (EMRs) in the environment. The widespread EMRs have induced many significant changes in biological systems leading to oxidative stress as well as DNA damage. Considering this, the present study was planned to study the effects of EMRs at 900 MHz frequency with the power density of 10.0 dBm (0.01 W) at variable exposure periods (0.5 h, 1 h, 2 h, 4 h, and 8 h per day for 7 days) on percentage germination, morphological characteristics, protein content, lipid peroxidation in terms of malondialdehyde content (MDA), and antioxidant defense system of *Trigonella foenum-graecum* test system. The genotoxicity was also evaluated using similar conditions. It was observed that EMRs significantly decreased the germination percentage at an exposure time of 4 h and 8 h. Fresh weight and dry weight of root and shoot did not show significant variations, while the root and shoot length have shown significant variations for 4 h and 8 h exposure period. Further, EMRs enhanced MDA indicating lipid peroxidation. In response to exposure of EMRs, there was a significant up-regulation in the activities of enzymes such as ascorbate peroxidase (APX), superoxide dismutase (SOD), glutathione-S-transferase (GST), guaiacol peroxidase (POD), and glutathione reductase (GR) in the roots and shoots of *Trigonella-foenum graecum*. The genotoxicity study showed the induction of chromosomal aberrations in root tip cells of the *Trigonella foenum-graecum* test system. The present study revealed the induction of oxidative stress and genotoxicity of EMRs exposure in the test system

(E) Shckorbatov YG, Pasiuga VN, Kolchigin NN, Grabina VA, Batrakov DO, Kalashnikov VV, et al. The influence of differently polarized microwave radiation on chromatin in human cells. *Int J Radiat Biol.* 2009; 85: 322-329. (VT, AE, GT, WS)

Purpose: To determine the possible biological effects of differently polarised microwave radiation on the chromatin state in human cells. **Materials and methods:** Isolated human buccal epithelium cells were irradiated by microwaves of frequency $f = 35$ GHz and surface power density $E = 30$ microW/cm². The state of chromatin in human cells was determined by methods of light and electron microscopy. The state of cell membranes was evaluated by the

method of vital indigo carmine staining. **Results:** The microwave-induced condensation of chromatin in human cells is revealed. Degree of microwave-induced condensation depends on the state of polarisation of electromagnetic wave: In some cases left circularly polarised waves induce less effect than linearly polarised radiation. The linearly polarised electromagnetic waves induce cell membrane damage revealed by increase of cell staining. The data obtained are discussed in connection with mechanisms of biological effects of electromagnetic fields. **Conclusion:** The data obtained in this work demonstrate important biological effects of monochromatic microwave irradiation at 35 GHz. Low-level microwave irradiation induces chromatin condensation in human cells and damages of cell membranes.

(E) Shckorbatov YG, Pasiuga VN, Goncharuk EI, Petrenko TP, Grabina VA, Kolchigin NN, et al. Effects of differently polarized microwave radiation on the microscopic structure of the nuclei in human fibroblasts. J Zhejiang Univ Sci B. 2010; 11: 801-805. (VT, AE, GT)

To investigate the influence of microwave radiation on the human fibroblast nuclei, the effects of three variants of electromagnetic wave polarization, linear and left-handed and right-handed elliptically polarized, were examined. Experimental conditions were: frequency (f) 36.65 GHz, power density (P) at the surface of exposed object 1, 10, 30, and 100 $\mu\text{W}/\text{cm}^2$, exposure time 10 s. Human fibroblasts growing in a monolayer on a cover slide were exposed to microwave electromagnetic radiation. The layer of medium that covered cells during microwave exposure was about 1 mm thick. Cells were stained immediately after irradiation by 2% (w/v) orcein solution in 45% (w/v) acetic acid. Experiments were made at room temperature (25 °C), and control cell samples were processed in the same conditions. We assessed heterochromatin granule quantity (HGQ) at 600 \times magnification. Microwave irradiation at the intensity of 1 $\mu\text{W}/\text{cm}^2$ produced no effect, and irradiation at the intensities of 10 and 100 $\mu\text{W}/\text{cm}^2$ induced an increase in HGQ. More intense irradiation induced more chromatin condensation. The right-handed elliptically polarized radiation revealed more biological activity than the left-handed polarized one.

(NE) Shi D, Zhu C, Lu R, Mao S, Qi Y. Intermediate frequency magnetic field generated by a wireless power transmission device does not cause genotoxicity in vitro. Bioelectromagnetics. 35(7):512-518, 2014. (VT, AE, GT)

The aim of this study was to evaluate effects of intermediate frequency magnetic fields (IFMF) generated by a wireless power transmission (WPT) based on magnetic resonance from the perspective of cellular genotoxicity on cultured human lens epithelial cells (HLECs). We evaluated the effects of exposure to 90 kHz magnetic fields at 93.36 μT on cellular genotoxicity in vitro for 2 and 4 h. The magnetic flux density is approximately 3.5 times higher than the reference level recommended by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines. For assessment of genotoxicity, we studied cellular proliferation, apoptosis and DNA damage by Cell Counting Kit-8 (CCK-8) assay, flow cytometry analysis, alkaline comet assay and phosphorylated histone H2AX (γH2AX) foci formation test. We did not detect any effect of a 90 kHz IFMF generated by WPT based on

magnetic resonance on cell proliferation, apoptosis, comet assay, and γ H2AX foci formation test. Our results indicated that exposure to 90 kHz IFMF generated by WPT based on magnetic resonance at 93.36 μ T for 2 and 4 h does not cause detectable cellular genotoxicity.

(NE) Silva V, Hilly O, Strenov Y, Tzabari C, Hauptman Y, Feinmesser R. Effect of cell phone-like electromagnetic radiation on primary human thyroid cells. Int J Radiat Biol. 2016; 92: 107-115. (VT, AE, GE)

Purpose: To evaluate the potential carcinogenic effects of radiofrequency energy (RFE) emitted by cell phones on human thyroid primary cells. **Materials and methods:** Primary thyroid cell culture was prepared from normal thyroid tissue obtained from patients who underwent surgery at our department. Subconfluent thyroid cells were irradiated under different conditions inside a cell incubator using a device that simulates cell phone-RFE. Proliferation of control and irradiated cells was assessed by the immunohistochemical staining of antigen Kiel clone-67 (Ki-67) and tumor suppressor p53 (p53) expression. DNA ploidy and the stress biomarkers heat shock protein 70 (HSP70) and reactive oxygen species (ROS) was evaluated by fluorescence-activated cell sorting (FACS). **Results:** Our cells highly expressed thyroglobulin (Tg) and sodium-iodide symporter (NIS) confirming the origin of the tissue. None of the irradiation conditions evaluated here had an effect neither on the proliferation marker Ki-67 nor on p53 expression. DNA ploidy was also not affected by RFE, as well as the expression of the biomarkers HSP70 and ROS. **Conclusion:** Our conditions of RFE exposure seem to have no potential carcinogenic effect on human thyroid cells. Moreover, common biomarkers usually associated to environmental stress also remained unchanged. We failed to find an association between cell phone-RFE and thyroid cancer. Additional studies are recommended.

Sisakht M, Darabian M, Mahmoodzadeh A, Bazi A, Shafiee SM, Mokarram P, Khoshdel Z. The role of radiation induced oxidative stress as a regulator of radio-adaptive responses. Int J Radiat Biol. 2020 Feb 7:1-16. (review)

Purpose: Various sources of radiation including radiofrequency, electromagnetic radiation (EMR), low- dose X-radiation, low-level microwave radiation and ionizing radiation (IR) are indispensable parts of modern life. In the current review, we discussed the adaptive responses of biological systems to radiation with a focus on the impacts of radiation-induced oxidative stress (RIOS) and its molecular downstream signaling pathways. **Materials and methods:** A comprehensive search was conducted in Web of Sciences, PubMed, Scopus, Google Scholar, Embase, and Cochrane Library. Keywords included Mesh terms of "radiation," "electromagnetic radiation," "adaptive immunity," "oxidative stress," and "immune checkpoints." Manuscripts published up until December 2019 were included. **Results:** RIOS induces various molecular adaptors connected with adaptive responses in radiation exposed cells. One of these adaptors includes p53 which promotes various cellular signaling pathways. RIOS also activates the intrinsic apoptotic pathway by depolarization of the mitochondrial membrane potential and activating the caspase apoptotic cascade. RIOS is also involved in radiation-induced proliferative responses through interaction with mitogen-activated protein kinases (MAPks) including p38 MAPK, ERK, and c-Jun N-terminal kinase (JNK). Protein kinase B (Akt)/phosphoinositide 3-kinase (PI3K) signaling pathway has also

been reported to be involved in RIOS-induced proliferative responses. Furthermore, RIOS promotes genetic instability by introducing DNA structural and epigenetic alterations, as well as attenuating DNA repair mechanisms. Inflammatory transcription factors including macrophage migration inhibitory factor (MIF), nuclear factor κ B (NF- κ B), and signal transducer and activator of transcription-3 (STAT-3) play major role in RIOS-induced inflammation. **Conclusion:** In conclusion, RIOS considerably contributes to radiation induced adaptive responses. Other possible molecular adaptors modulating RIOS-induced responses are yet to be divulged in future studies.

(E)Smith-Roe SL, Wyde ME, Stout MD, Winters JW, Hobbs CA, Shepard KG, Green AS, Kissling GE, Shockley KR, Tice RR, Bucher JR, Witt KL. Evaluation of the genotoxicity of cell phone radiofrequency radiation in male and female rats and mice following subchronic exposure. Environ Mol Mutagen. 61(2):276-290, 2020. (VO, LE, GT)

The National Toxicology Program tested two common radiofrequency radiation (RFR) modulations emitted by cellular telephones in a 2-year rodent cancer bioassay that included interim assessments of additional animals for genotoxicity endpoints. Male and female Hsd:Sprague Dawley SD rats and B6C3F1/N mice were exposed from Gestation day 5 or Postnatal day 35, respectively, to code division multiple access (CDMA) or global system for mobile modulations over 18 hr/day, at 10-min intervals, in reverberation chambers at specific absorption rates of 1.5, 3, or 6 W/kg (rats, 900 MHz) or 2.5, 5, or 10 W/kg (mice, 1,900 MHz). After 19 (rats) or 14 (mice) weeks of exposure, animals were examined for evidence of RFR-associated genotoxicity using two different measures. Using the alkaline (pH > 13) comet assay, DNA damage was assessed in cells from three brain regions, liver cells, and peripheral blood leukocytes; using the micronucleus assay, chromosomal damage was assessed in immature and mature peripheral blood erythrocytes. Results of the comet assay showed significant increases in DNA damage in the frontal cortex of male mice (both modulations), leukocytes of female mice (CDMA only), and hippocampus of male rats (CDMA only). Increases in DNA damage judged to be equivocal were observed in several other tissues of rats and mice. No significant increases in micronucleated red blood cells were observed in rats or mice. In conclusion, these results suggest that exposure to RFR is associated with an increase in DNA damage.

(E) Sokolovic D, Djordjevic B, Kocic G, Stoimenov TJ, Stanojkovic Z, Sokolovic DM, et al. The effects of melatonin on oxidative stress parameters and DNA fragmentation in testicular tissue of rats exposed to microwave radiation. Adv Clin Exp Med. 24(3):429-436, 2015. (VO, LE, GT, OX, RP)

BACKGROUND: Microwaves from mobile phones are one of the environmental toxicants that are capable of compromising male fertility by inducing oxidative stress and apoptosis in the testes. Melatonin is a lipophilic tryptophan indole amine and a potent antioxidant.

OBJECTIVES: The aim of the study was to evaluate the effect of melatonin treatment on oxidative stress parameters and DNA fragmentation in the testicular tissue of rats exposed to microwave radiation (4 h/day). **MATERIAL AND METHODS:** Adult Wistar rats were divided in 4 groups: I - treated with saline; II - treated with melatonin; III - exposed to microwaves; IV - exposed to microwaves and treated with melatonin. The melatonin (2 mg/kg ip) was administered daily. The animals were sacrificed after 20, 40 and 60 days. **RESULTS:** Melatonin

treatment prevented previously registered increases in malondialdehyde after only 20 days. Furthermore, it reversed the effects of microwave exposure on xanthine oxidase (after 40 days) and acid-DNase activity (after 20 days). However, neither protein carbonyl content nor catalase and alkaline Dnase activity were changed due to melatonin treatment. **CONCLUSIONS:** Melatonin exerts potent antioxidant effects in the testes of rats exposed to microwaves by decreasing the intensity of oxidative stress; it also reduces DNA fragmentation.

(E) Soubere Mahamoud Y, Aite M, Martin C, Zhadobov M, Sauleau R, Le Dréan Y, Habauzit D. Additive Effects of Millimeter Waves and 2-Deoxyglucose Co-Exposure on the Human Keratinocyte Transcriptome. PLoS One.;11(8):e0160810, 2016. (VT, AE, GE, IX)

Millimeter Waves (MMW) will be used in the next-generation of high-speed wireless technologies, especially in future Ultra-Broadband small cells in 5G cellular networks. Therefore, their biocompatibilities must be evaluated prior to their massive deployment. Using a microarray-based approach, we analyzed modifications to the whole genome of a human keratinocyte model that was exposed at 60.4 GHz-MMW at an incident power density (IPD) of 20 mW/cm² for 3 hours in athermic conditions. No keratinocyte transcriptome modifications were observed. We tested the effects of MMWs on cell metabolism by co-treating MMW-exposed cells with a glycolysis inhibitor, 2-deoxyglucose (2dG, 20 mM for 3 hours), and whole genome expression was evaluated along with the ATP content. We found that the 2dG treatment decreased the cellular ATP content and induced a high modification in the transcriptome (632 coding genes). The affected genes were associated with transcriptional repression, cellular communication and endoplasmic reticulum homeostasis. The MMW/2dG co-treatment did not alter the keratinocyte ATP content, but it did slightly alter the transcriptome, which reflected the capacity of MMW to interfere with the bioenergetic stress response. The RT-PCR-based validation confirmed 6 MMW-sensitive genes (SOCS3, SPRY2, TRIB1, FAM46A, CSRNPI and PPP1R15A) during the 2dG treatment. These 6 genes encoded transcription factors or inhibitors of cytokine pathways, which raised questions regarding the potential impact of long-term or chronic MMW exposure on metabolically stressed cells.

(E) Souza LD, Cerqueira ED, Meireles JR. Assessment of nuclear abnormalities in exfoliated cells from the oral epithelium of mobile phone users. Electromagn Biol Med. 33(2):98-102, 2014. (HU, LE, GE)

Transmission and reception of mobile telephony signals take place through electromagnetic wave radiation, or electromagnetic radiofrequency fields, between the mobile terminal and the radio base station. Based on reports in the literature on adverse effects from exposure to this type of radiation, the objective of this study was to evaluate the genotoxic and cytotoxic potential of such exposure, by means of the micronucleus test on exfoliated cells from the oral epithelium. The sample included 45 individuals distributed in 3 groups according to the amount of time in

hours per week (t) spent using mobile phones: group I, $t > 5$ h; group II, $t > 1$ h and ≤ 5 h; and group III, $t \leq 1$ h. Cells from the oral mucosa were analyzed to assess the numbers of micronuclei, broken egg structures and degenerative nuclear abnormalities indicative of apoptosis (condensed chromatin, karyorrhexis and pyknosis) or necrosis (karyolysis in addition to these changes). The occurrences of micronuclei and degenerative nuclear abnormalities did not differ between the groups, but the number of broken egg (structures that may be associated with gene amplification) was significantly greater in the individuals in group I ($p < 0.05$).

(NE) Speit G, Schütz P, Hoffmann H. Genotoxic effects of exposure to radiofrequency electromagnetic fields (RF-EMF) in cultured mammalian cells are not independently reproducible. Mutat Res. 626(1-2):42-47, 2007. (VT, AE, GT)

Conflicting results have been published regarding the induction of genotoxic effects by exposure to radiofrequency electromagnetic fields (RF-EMF). Using the comet assay, the micronucleus test and the chromosome aberration test with human fibroblasts (ES1 cells), the EU-funded "REFLEX" project (Risk Evaluation of Potential Environmental Hazards From Low Energy Electromagnetic Field Exposure Using Sensitive in vitro Methods) reported clearly positive effects for various exposure conditions. Because of the ongoing discussion on the biological significance of the effects observed, it was the aim of the present study to independently repeat the results using the same cells, the same equipment and the same exposure conditions. We therefore exposed ES1 cells to RF-EMF (1800 MHz; SAR 2 W/kg, continuous wave with intermittent exposure) for different time periods and then performed the alkaline ($\text{pH} > 13$) comet assay and the micronucleus test (MNT). For both tests, clearly negative results were obtained in independently repeated experiments. We also performed these experiments with V79 cells, a sensitive Chinese hamster cell line that is frequently used in genotoxicity testing, and also did not measure any genotoxic effect in the comet assay and the MNT. Appropriate measures of quality control were considered to exclude variations in the test performance, failure of the RF-EMF exposure or an evaluation bias. The reasons for the difference between the results reported by the REFLEX project and our experiments remain unclear.

(NE) Speit G, Richard Gminski, Tauber R. Genotoxic effects of exposure to radiofrequency electromagnetic fields (RF-EMF) in HL-60 cells are not reproducible. Mutat Res 755(2):163-166, 2013. (VT, AE, GT)

Conflicting results have been published regarding the induction of genotoxic effects by exposure to radiofrequency electromagnetic fields (RF-EMF). Various results indicating a genotoxic potential of RF-EMF were reported by the collaborative EU-funded REFLEX (Risk Evaluation of Potential Environmental Hazards From Low Energy Electromagnetic Field Exposure Using Sensitive in vitro Methods) project. There has been a long-lasting scientific debate about the reliability of the reported results and an attempt to reproduce parts of the results obtained with human fibroblasts failed. Another part of the REFLEX study was performed in Berlin with the human lymphoblastoid cell line HL-60; genotoxic effects of RF-EMF were measured by means of the comet assay and the micronucleus test. The plausibility and reliability of these results were also questioned. In order to contribute to a clarification of the biological significance of the reported findings, a repeat study was performed, involving scientists of the original study.

Comet-assay experiments and micronucleus tests were performed under the same experimental conditions that had led to genotoxic effects in the REFLEX study. Here we report that the attempts to reproduce the induction of genotoxic effects by RF-EMF in HL-60 cells failed. No genotoxic effects of RF-EMF were measured in the repeat experiments. We could not find an explanation for the conflicting results. However, the negative repeat experiments suggest that the biological significance of genotoxic effects of RF-EMF reported by the REFLEX study should be re-assessed.

(E) Srujana Aravinda VS, Kandregula CR, Muppa R, Krishna MM, Nikitha BS, Yenni M. A cross-sectional and histological analysis to understand the cytological effects of cell phone radiation on buccal mucosa of children. J Indian Soc Pedod Prev Dent 2022;40(1):74-80. (HU, LE, GT)

Context: The ongoing pandemic has affected all the spheres of life and one of the severely affected avenues is the education of a child. The online education has seen an upward curve since the start of COVID-19 pandemic. Schools globally have adopted online class tutorials as the main method to impart education and directly increasing the screen time for a child.

Aim: The aim of the present study was to evaluate the cytological effects of prolonged mobile phone usage on the buccal mucosa of children.

Settings and design: Stratified sampling was used for the selection of subjects for the study. After a questionnaire regarding the usage of a mobile phone was distributed among the parents of children. Among them, 90 children were selected on the basis of pattern and frequency of mobile phone usage in the child. **Materials and methodology:** The children were divided into three groups based on the per day hours of viewing of mobile phone, i.e., Group 1: Usage of 1-2 h a day, Group 2: Usage of 3-6 h a day, and Group 3: Usage of >6 h a day. The time frame taken into consideration was 1 year after the pandemic started. This was specifically to understand the impact of the online education. Swab was obtained by using the conventional ice-cream stick method from the buccal mucosa.

Statistical analysis: The samples were subjected to histological and microscopical analysis to observe for cytological changes. One-way ANOVA was used to determine the statistical significance if any. **Results:** The results obtained clearly showed that Group 3 (>6 h usage per day) showed the highest number of cellular and chromosomal aberrations which was significant.

Conclusion: The results indicated that impact due to the prolonged screen time on the buccal mucosa is significant. A direct proportionality was seen between the apoptotic changes and chromosomal aberrations and the number of daily hour usage.

(NE) Stronati L, Testa A, Moquet J, Edwards A, Cordelli E, Villani P, Marino C, Fresegna AM, Appolloni M, Lloyd D. 935 MHz cellular phone radiation. An in vitro study of genotoxicity in human lymphocytes. Int J Radiat Biol 82:339-346, 2006. (VT, AE, GT, IX)

Purpose: The possibility of genotoxicity of radiofrequency radiation (RFR) applied alone or in combination with x-rays was investigated in vitro using several assays on human lymphocytes. The chosen specific absorption rate (SAR) values are near the upper limit of actual energy absorption in localized tissue when persons use some cellular telephones. The purpose of the combined exposures was to examine whether RFR might act epigenetically by reducing the fidelity of repair of DNA damage caused by a well-characterized and established mutagen. **Methods:** Blood specimens from 14 donors were exposed continuously for 24 h to a

Global System for Mobile Communications (GSM) basic 935 MHz signal. The signal was applied at two SAR; 1 and 2 W/Kg, alone or combined with a 1-min exposure to 1.0 Gy of 250 kVp x-rays given immediately before or after the RFR. The assays employed were the alkaline comet technique to detect DNA strand breakage, metaphase analyses to detect unstable chromosomal aberrations and sister chromatid exchanges, micronuclei in cytokinesis-blocked binucleate lymphocytes and the nuclear division index to detect alterations in the speed of in vitro cell cycling. Results: By comparison with appropriate sham-exposed and control samples, no effect of RFR alone could be found for any of the assay endpoints. In addition RFR did not modify any measured effects of the x-radiation. Conclusions: This study has used several standard in vitro tests for chromosomal and DNA damage in Go human lymphocytes exposed in vitro to a combination of x-rays and RFR. It has comprehensively examined whether a 24-h continuous exposure to a 935 MHz GSM basic signal delivering SAR of 1 or 2 W/Kg is genotoxic per se or whether, it can influence the genotoxicity of the well-established clastogenic agent; x-radiation. Within the experimental parameters of the study in all instances no effect from the RFR signal was observed.

(NE) Su L, Wei X, Xu Z, Chen G. RF-EMF exposure at 1800 MHz did not elicit DNA damage or abnormal cellular behaviors in different neurogenic cells. Bioelectromagnetics 38(3):175-185, 2017. (VT, AE, GT)

Despite many years of studies, the debate on genotoxic effects of radiofrequency electromagnetic fields (RF-EMF) continues. To systematically evaluate genotoxicity of RF-EMF, this study examined effects of RF-EMF on DNA damage and cellular behavior in different neurogenic cells. Neurogenic A172, U251, and SH-SY5Y cells were intermittently (5 min on/10 min off) exposed to 1800 MHz RF-EMF at an average specific absorption rate (SAR) of 4.0 W/kg for 1, 6, or 24 h. DNA damage was evaluated by quantification of γ H2AX foci, an early marker of DNA double-strand breaks. Cell cycle progression, cell proliferation, and cell viability were examined by flow cytometry, hemocytometer, and cell counting kit-8 assay, respectively. Results showed that exposure to RF-EMF at an SAR of 4.0 W/kg neither significantly induced γ H2AX foci formation in A172, U251, or SH-SY5Y cells, nor resulted in abnormal cell cycle progression, cell proliferation, or cell viability. Furthermore, prolonged incubation of these cells for up to 48 h after exposure did not significantly affect cellular behavior. Our data suggest that 1800 MHz RF-EMF exposure at 4.0 W/kg is unlikely to elicit DNA damage or abnormal cellular behaviors in neurogenic cells.

(NE) Su L, Yimaer A, Xu Z, Chen G. Effects of 1800 MHz RF-EMF exposure on DNA damage and cellular functions in primary cultured neurogenic cells. Int J Radiat Biol. 94(3):295-305, 2018. (VT, LE, GT)

PURPOSE: To systematically evaluate the effects of 1800 MHz radiofrequency electromagnetic fields (RF-EMF) exposure on DNA damage and cellular functions in primary cultured neurogenic cells. MATERIALS AND METHODS: The primary cultured astrocytes, microglia and cortical neurons were exposed to RF-EMF at a SAR of 4.0 W/kg. The DNA damage was evaluated by γ H2AX foci formation assay. The secretions of pro-inflammatory cytokines (TNF-

α , IL-6 and IL-1 β) in astrocytes and microglia, microglial phagocytic activity and neuronal development were examined by enzyme-linked immunosorbent assay, phagocytosis assay and immunofluorescent staining on microtubule-associated protein tau, microtubule-associated protein 2, postsynaptic density 95 and gephyrin, respectively. RESULTS: RF-EMF exposure did not significantly induce γ H2AX foci formation in three primary cultured neurogenic cells. Furthermore, RF-EMF exposure did not significantly affect the secretion of cytokines in astrocytes and microglia, and the morphological indicators of dendrites or synapses of cortical neurons. However, the exposure significantly reduced the phagocytic activity of microglia and inhibited the axon branch length and branch number of cortical neurons. CONCLUSIONS: Our data demonstrated that exposure to RF-EMF did not elicit DNA damage but inhibited the phagocytic ability of microglia and the axon branch length and branch number of cortical neurons.

(E) Sueiro-Benavides RA, Leiro-Vidal JM, Salas-Sánchez AA, Rodríguez-González JA, Ares-Pena FJ, López-Martín ME. Radiofrequency at 2.45 GHz increases toxicity, pro-inflammatory and pre-apoptotic activity caused by black carbon in the RAW 264.7 macrophage cell line. Sci Total Environ 2021 Apr 15;765:142681. (VT, AE, GE, LI)

Environmental factors such as air pollution by particles and/or electromagnetic fields (EMFs) are studied as harmful agents for human health. We analyzed whether the combined action of EMF with fine and coarse black carbon (BC) particles induced cell damage and inflammatory response in RAW 264.7 cell line macrophages exposed to 2.45 GHz in a gigahertz transverse electromagnetic (GTEM) chamber at sub-thermal specific absorption rate (SAR) levels. Radiofrequency (RF) dramatically increased BC-induced toxicity at high doses in the first 24 h and toxicity levels remained high 72 h later for all doses. The increase in macrophage phagocytosis induced after 24 h of RF and the high nitrite levels obtained by stimulation with lipopolysaccharide (LPS) endotoxin 24 and 72 h after radiation exposure suggests a prolongation of the innate and inflammatory immune response. The increase of proinflammatory cytokines tumor necrosis factor- α , after 24 h, and of interleukin-1 β and caspase-3, after 72 h, indicated activation of the pro-inflammatory response and the apoptosis pathways through the combined effect of radiation and BC. Our results indicate that the interaction of BC and RF modifies macrophage immune response, activates apoptosis, and accelerates cell toxicity, by which it can activate the induction of hypersensitivity reactions and autoimmune disorders.

(E) Sun C, Wei X, Fei Y, Su L, Zhao X, Chen G, Xu Z. Mobile phone signal exposure triggers a hormesis-like effect in Atm+/+ and Atm-/- mouse embryonic fibroblasts. Sci Rep. 6:37423, 2016. (VT, AE, GT) (compensatory effect)

Radiofrequency electromagnetic fields (RF-EMFs) have been classified by the International Agency for Research on Cancer as possible carcinogens to humans; however, this conclusion is based on limited epidemiological findings and lacks solid support from experimental studies. In particular, there are no consistent data regarding the genotoxicity of RF-EMFs. Ataxia telangiectasia mutated (ATM) is recognised as a chief guardian of genomic stability. To address the debate on whether RF-EMFs are genotoxic, we compared the effects of 1,800 MHz RF-EMF

exposure on genomic DNA in mouse embryonic fibroblasts (MEFs) with proficient ($Atm^{+/+}$) or deficient ($Atm^{-/-}$) ATM. In $Atm^{+/+}$ MEFs, RF-EMF exposure for 1 h at an average special absorption rate of 4.0 W/kg induced significant DNA single-strand breaks (SSBs) and activated the SSB repair mechanism. This effect reduced the DNA damage to less than that of the background level after 36 hours of exposure. In the $Atm^{-/-}$ MEFs, the same RF-EMF exposure for 12 h induced both SSBs and double-strand breaks and activated the two repair processes, which also reduced the DNA damage to less than the control level after prolonged exposure. The observed phenomenon is similar to the hormesis of a toxic substance at a low dose. To the best of our knowledge, this study is the first to report a hormesis-like effect of an RF-EMF.

(E) Sun LX, Yao K, He JL, Lu DQ, Wang KJ, Li HW.[Effect of acute exposure to microwave from mobile phone on DNA damage and repair of cultured human lens epithelial cells in vitro.] *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing ZaZhi*. 24:465-467, 2006a. [Article in Chinese] (VT, AE, GT)

OBJECTIVE: To investigate the DNA damage of human lens epithelial cells (LECs) caused by acute exposure to low-power 217 Hz modulated 1.8 GHz microwave radiation and DNA repair. **METHODS:** Cultured LECs were exposed to 217 Hz modulated 1.8 GHz microwave radiation at SAR (specific absorption rate) of 0, 1, 2, 3 and 4 W/kg for 2 hours in an sXc-1800 incubator and irradiate system. The DNA single strand breaks were detected with comet assay in sham-irradiated cells and irradiated cells incubated for varying periods: 0, 30, 60, 120 and 240 min after irradiation. Images of comets were digitized and analyzed using an Imagine-pro plus software, and the indexes used in this study were tail length (TL) and tail moment (TM). **RESULTS:** The difference in DNA-breaks between the exposure and sham exposure groups induced by 1 and 2 W/kg irradiation was not significant at every detect time ($P > 0.05$). As for the dosage of 3 and 4 W/kg there was difference in both groups immediately after irradiation ($P < 0.01$). At the time of 30 min after irradiation the difference went on at both group ($P < 0.01$). However, the difference disappeared after one hour's incubation in 3 W/kg group ($P > 0.05$), and existed in 4 W/kg group. **CONCLUSION:** No or repairable DNA damage was observed after 2 hour irradiation of 1.8 GHz microwave on LECs when SAR \leq 3 W/kg. The DNA damages caused by 4 W/kg irradiation were irreversible.

(E) Sun LX, Yao K, Jiang H, He JL, Lu DQ, Wang KJ, Li HW [DNA damage and repair induced by acute exposure of microwave from mobile phone on cultured human lens epithelial cells] *Zhonghua Yan Ke Za Zhi*. 42(12):1084-1088, 2006b. [Article in Chinese] (VT, AE, GT)

OBJECTIVE: To investigate the effects of acute exposure of low-power 217 Hz modulated 1.8 GHz microwave radiation on the DNA damage of human lens epithelial cells (hLECs) and repair. **METHODS:** Cultured hLECs were exposed to 217 Hz modulated 1.8 GHz microwave radiation at SAR (specific absorption rate) of 1.0, 2.0, 3.00 and 4.0 W/kg for 2 hours in an sXc-1800 incubator and irradiate system, the DNA single strand breaks were detected with comet assay (single-cell gel electrophoresis) in sham-irradiated cells and irradiated cells incubated for varying periods: 0, 30 and 60 minutes after irradiation. Images of comets were digitized and analyzed using an Imagine-pro plus software, and the indexes used in this study were tail length (TL) and tail moment (TM). BrdU was added into the medium with additional one hour incubation after

radiation, the cell proliferation rate was determined using a BrdU-kit. RESULTS: The difference of DNA-breaks between the exposure and sham exposure groups induced by 1.0 and 2.0 W/kg irradiation were not significant in each time points ($P > 0.05$); there were significant difference in both groups at the exposure dose of 3.0 and 4.0 W/kg immediately and at the time of 30 minutes after irradiation ($P < 0.01$); if the radiation exposure time was beyond one hour no differences were able to be detected in 3.0 W/kg group ($P > 0.05$) compared with control, but the evidence of significant DNA damage still existed in 4.0 W/kg group at the same time point. Cell proliferation rate had no significant difference when the application of SAR was ≤ 3.0 W/kg ($P > 0.05$), however the cell proliferation was decreased significantly at the dose of 4.0 W/kg irradiation ($P < 0.01$). CONCLUSIONS: No effective DNA damage was induced using comet assay after 2 hours irradiation of 1.8 GHz microwave on hLECs at the dose SAR ≤ 3.0 W/kg. 4.0 W/kg irradiation caused significantly DNA damage and inhibition of hLECs proliferation.

(E) Sun Y, Zong L, Gao Z, Zhu S, Tong J, Cao Y. Mitochondrial DNA damage and oxidative damage in HL-60 cells exposed to 900 MHz radiofrequency fields. *Mutat Res.* 797: 7-14, 2017. (VT, LE, GT, OX, GE, LI)

HL-60 cells, derived from human promyelocytic leukemia, were exposed to continuous wave 900MHz radiofrequency fields (RF) at $120\mu\text{W}/\text{cm}^2$ power intensity for 4h/day for 5 consecutive days to examine whether such exposure is capable of damaging the mitochondrial DNA (mtDNA) mediated through the production of reactive oxygen species (ROS). In addition, the effect of RF exposure was examined on 8-hydroxy-2'-deoxyguanosine (8-OHdG) which is a biomarker for oxidative damage and on the mitochondrial synthesis of adenosine triphosphate (ATP) which is the energy required for cellular functions. The results indicated a significant increase in ROS and significant decreases in mitochondrial transcription factor A, mtDNA polymerase gamma, mtDNA transcripts and mtDNA copy number in RF-exposed cells compared with those in sham-exposed control cells. In addition, there was a significant increase in 8-OHdG and a significant decrease in ATP in RF-exposed cells. The response in positive control cells exposed to gamma radiation (GR, which is also known to induce ROS) was similar to those in RF-exposed cells. Thus, the overall data indicated that RF exposure was capable of inducing mtDNA damage mediated through ROS pathway which also induced oxidative damage. Prior-treatment of RF- and GR-exposed cells with melatonin, a well-known free radical scavenger, reversed the effects observed in RF-exposed cells.

(E) Sykes PJ, McCallum BD, Bangay MJ, Hooker AM, Morley AA. Effect of exposure to 900 MHz radiofrequency radiation on intrachromosomal recombination in pKZ1 mice. *Radiat Res* 156(5):495-502, 2001. (VO, LE, GT)

Radiofrequency (RF) radiation emitted from mobile phones is not considered to be directly genotoxic, but it may have downstream effects on cellular DNA. We studied the effect of 4 W/kg pulsed 900 MHz RF radiation on somatic intrachromosomal recombination in the spleen in the pKZ1 recombination mutagenesis model. Somatic intrachromosomal recombination inversion events were detected in spleen tissue of pKZ1 mice by histochemical staining for E.

coli beta-galactosidase protein in cells in which the lacZ transgene has undergone an inversion event. pKZ1 mice were exposed daily for 30 min to plane-wave fields of 900 MHz with a pulse repetition frequency of 217 Hz and a pulse width of 0.6 ms for 1, 5 or 25 days. Three days after the last exposure, spleen sections were screened for DNA inversion events. There was no significant difference between the control and treated groups in the 1- and 5-day exposure groups, but there was a significant reduction in inversions below the spontaneous frequency in the 25-day exposure group. This observation suggests that exposure to RF radiation can lead to a perturbation in recombination frequency which may have implications for recombination repair of DNA. The biological significance of a reduction below the spontaneous frequency is not known. The number of mice in each treatment group in this study was small (n = 10 or n = 20). Therefore, repetition of this study with a larger number of animals is required to confirm these observations.

(NE) Takahashi S, Inaguma S, Cho Y-M, Imaida K, Wang J, Fujiwara O, Shirai T, Lack of mutation induction with exposure to 1.5 GHz electromagnetic near fields used for cellular phones in brains of big blue mice. Cancer Res 62:1956-1960, 2002. (VO, LE, GT)

The possible mutagenic potential of exposure to 1.5 GHz electromagnetic near field (EMF) was investigated using brain tissues of BigBlue mice (BBM). Male BBM were locally exposed to EMF in the head region at 2.0, 0.67, and 0 W/kg specific absorption rate for 90 min/day, 5 days/week, for 4 weeks. No gliosis or degenerative lesions were histopathologically noted in brain tissues, and no obvious differences in Ki-67 labeling and apoptotic indices of glial cells were evident among the groups. There was no significant variation in the frequency of independent mutations of the lacI trans gene in the brains. G:C to A:T transitions at CpG sites constituted the most prevalent mutations in all groups and at all time points. Deletion mutations were slightly increased in both the high and low EMF exposure groups as compared with the sham-exposed group, but the differences were not statistically significant. These findings suggest that exposure to 1.5 GHz EMF is not mutagenic to mouse brain cells and does not create any increased hazard with regard to brain tumor development.

(E) Tice RR, Hook GG, Donner M, McRee DI, Guy AW. Genotoxicity of radiofrequency signals. I. Investigation of DNA damage and micronuclei induction in cultured human blood cells. Bioelectromagnetics 23:113-126, 2002. (VT, AE, GT)

As part of a comprehensive investigation of the potential genotoxicity of radiofrequency (RF) signals emitted by cellular telephones, in vitro studies evaluated the induction of DNA and chromosomal damage in human blood leukocytes and lymphocytes, respectively. The signals were voice modulated 837 MHz produced by an analog signal generator or by a time division multiple access (TDMA) cellular telephone, 837 MHz generated by a code division multiple access (CDMA) cellular telephone (not voice modulated), and voice modulated 1909.8 MHz generated by a global system of mobile communication (GSM)-type personal communication systems (PCS) cellular telephone. DNA damage (strand breaks/alkali labile sites) was assessed in leukocytes using the alkaline (pH>13) single cell gel electrophoresis (SCG) assay. Chromosomal damage was evaluated in lymphocytes mitogenically stimulated to divide postexposure using

the cytochalasin B-binucleate cell micronucleus assay. Cells were exposed at $37\pm 1^\circ\text{C}$, for 3 or 24 h at average specific absorption rates (SARs) of 1.0-10.0 W/kg. Exposure for either 3 or 24 h did not induce a significant increase in DNA damage in leukocytes, nor did exposure for 3 h induce a significant increase in micronucleated cells among lymphocytes. However, exposure to each of the four RF signal technologies for 24 h at an average SAR of 5.0 or 10.0 W/kg resulted in a significant and reproducible increase in the frequency of micronucleated lymphocytes. The magnitude of the response (approximately four fold) was independent of the technology, the presence or absence of voice modulation, and the frequency (837 vs. 1909.8 MHz). This research demonstrates that, under extended exposure conditions, RF signals at an average SAR of at least 5.0 W/kg are capable of inducing chromosomal damage in human lymphocytes.

(E) Tiwari R, Lakshmi NK, Surender V, Rajesh AD, Bhargava SC, Ahuja YR. Combinative exposure effect of radio frequency signals from CDMA mobile phones and aphidicolin on DNA integrity. Electromagn Biol Med 27:418-425, 2008. (VT, AE, GT, IX)

The aim of present study is to assess DNA integrity on the effect of exposure to a radio frequency (RF) signal from Code Division Multiple Access (CDMA) mobile phones. Whole blood samples from six healthy male individuals were exposed for RF signals from a CDMA mobile phone for 1 h. Alkaline comet assay was performed to assess the DNA damage. The combinative exposure effect of the RF signals and APC at two concentrations on DNA integrity was studied. DNA repair efficiency of the samples was also studied after 2 h of exposure. The RF signals and APC (0.2 microg/ml) alone or in synergism did not have any significant DNA damage as compared to sham exposed. However, univariate analysis showed that DNA damage was significantly different among combinative exposure of RF signals and APC at 0.2 microg/ml ($p < 0.05$) and at 2 microg/ml ($p < 0.02$). APC at 2 microg/ml concentration also showed significant damage levels ($p < 0.05$) when compared to sham exposed. DNA repair efficiency also varied in a significant way in combinative exposure sets ($p < 0.05$). From these results, it appears that the repair inhibitor APC enhances DNA breaks at 2 microg/ml concentration and that the damage is possibly repairable. Thus, it can be inferred that the in vitro exposure to RF signals induces reversible DNA damage in synergism with APC.

(E) Tkalec M, Malarić K, Pavlica M, Pevalek-Kozlina B, Vidaković-Cifrek Z. Effects of radiofrequency electromagnetic fields on seed germination and root meristematic cells of Allium Cepa L. Mutat Res 672(2):76-81, 2009 . (VO, AE, GT)

The effects of exposure to radiofrequency electromagnetic fields (RF-EMFs) on seed germination, primary root growth as well as mitotic activity and mitotic aberrations in root meristematic cells were examined in *Allium cepa* L. cv. Srebrnjak Majski. Seeds were exposed for 2h to EMFs of 400 and 900MHz at field strengths of 10, 23, 41 and 120Vm(-1). The effect of longer exposure time (4h) and field modulation was investigated at 23Vm(-1) as well. Germination rate and root length did not change significantly after exposure to radiofrequency fields under any of the treatment conditions. At 900MHz, exposures to EMFs of higher field strengths (41 and 120Vm(-1)) or to modulated fields showed a significant increase of the mitotic index compared with corresponding controls, while the percentage of mitotic abnormalities increased after all exposure treatments. On the other hand, at 400MHz the mitotic index increased only after exposure to modulated EMF. At this frequency, compared with the control

higher numbers of mitotic abnormalities were found after exposure to modulated EMF as well as after exposure to EMFs of higher strengths (41 and 120Vm(-1)). The types of aberration induced by the EMFs of both frequencies were quite similar, mainly consisting of lagging chromosomes, vagrants, disturbed anaphases and chromosome stickiness. Our results show that non-thermal exposure to the radiofrequency fields investigated here can induce mitotic aberrations in root meristematic cells of *A. cepa*. The observed effects were markedly dependent on the field frequencies applied as well as on field strength and modulation. Our findings also indicate that mitotic effects of RF-EMF could be due to impairment of the mitotic spindle.

(E) Tkalec M, Stambuk A, Srut M, Malarić K, Klobučar GI. Oxidative and genotoxic effects of 900 MHz electromagnetic fields in the earthworm *Eisenia fetida*. Ecotoxicol Environ Saf. 90:7-12, 2013. (VO, AE, GT, OX, WS)

Accumulating evidence suggests that exposure to radiofrequency electromagnetic field (RF-EMF) can have various biological effects. In this study the oxidative and genotoxic effects were investigated in earthworms *Eisenia fetida* exposed in vivo to RF-EMF at the mobile phone frequency (900MHz). Earthworms were exposed to the homogeneous RF-EMF at field levels of 10, 23, 41 and 120Vm(-1) for a period of 2h using a Gigahertz Transversal Electromagnetic (GTEM) cell. At the field level of 23Vm(-1) the effect of longer exposure (4h) and field modulation (80% AM 1kHz sinusoidal) was investigated as well. All exposure treatments induced significant genotoxic effect in earthworms coelomocytes detected by the Comet assay, demonstrating DNA damaging capacity of 900MHz electromagnetic radiation. Field modulation additionally increased the genotoxic effect. Moreover, our results indicated the induction of antioxidant stress response in terms of enhanced catalase and glutathione reductase activity as a result of the RF-EMF exposure, and demonstrated the generation of lipid and protein oxidative damage. Antioxidant responses and the potential of RF-EMF to induce damage to lipids, proteins and DNA differed depending on the field level applied, modulation of the field and duration of *E. fetida* exposure to 900MHz electromagnetic radiation. Nature of detected DNA lesions and oxidative stress as the mechanism of action for the induction of DNA damage are discussed.

(E) Tohidi F, Sadr-Nabavi A, Haghiri H, Fardid R, Rafatpanah H, Azimian H, Bahreyni-Toossi M. Long-term exposure to electromagnetic radiation from mobile phones can cause considerable changes in the balance of *Bax/Bcl2* mRNA expression in the hippocampus of mice. Electromagn Biol Med 40(1):131-137, 2021. (VO, LE, GE)

The aim of the present study was the investigation of the effects of mobile phones at different daily exposure times on the hippocampal expression of two apoptotic genes. Forty-eight male BALB/c mice were randomly divided into six groups with 8 animals in each group. Four experimental groups were respectively exposed to electromagnetic waves for 0.5, 1, 2 and 4 hours twice a day for 30 consecutive days. One experimental group was radiated for 4 hours once a day, while the control group did not receive any radiation during the experiment. The expression of both *Bax* and *Bcl2* mRNAs was upregulated in the mice exposed for one and two hours. Whilst the highest expressions were observed in the two-hours radiation in the exposed group, the expression of both studied genes was downregulated in animals with longer exposure to radiation in a duration-dependent manner. The highest ratio of *Bax/Bcl2* expression was observed in the mice that received radiation for four hours twice a day. These results revealed that mobile phone radiation can cause considerable changes in the balance of *Bax/Bcl2* mRNA expression in laboratory mice hippocampus.

(NE) Tomruk A, Guler G, Dincel AS. The influence of 1800 MHz GSM-like signals on hepatic oxidative DNA and lipid damage in nonpregnant, pregnant, and newly born rabbits. Cell Biochem Biophys 56:39-47, 2010. (VO, LE, GT, OX, DE)

The aim of our study is to evaluate the possible biological effects of whole-body 1800 MHz GSM-like radiofrequency (RF) radiation exposure on liver oxidative DNA damage and lipid peroxidation levels in nonpregnant, pregnant New Zealand White rabbits, and in their newly borns. Eighteen nonpregnant and pregnant rabbits were used and randomly divided into four groups which were composed of nine rabbits: (i) Group I (nonpregnant control), (ii) Group II (nonpregnant-RF exposed), (iii) Group III (pregnant control), (iv) Group IV (pregnant-RF exposed). Newborns of the pregnant rabbits were also divided into two groups: (v) Group V (newborns of Group III) and (vi) Group VI (newborns of Group III). 1800 MHz GSM-like RF radiation whole-body exposure (15 min/day for a week) was applied to Group II and Group IV. No significant differences were found in liver 8 OHdG/10 dG levels of exposure groups (Group II and Group IV) compared to controls (Group I and Group III). However, in Group II and Group IV malondialdehyde (MDA) and ferrous oxidation in xylenol orange (FOX) levels were increased compared to Group I ($P < 0.05$, Mann-Whitney). No significant differences were found in liver tissue of 8 OHdG/10 dG and MDA levels between Group VI and Group V ($P > 0.05$, Mann-Whitney) while liver FOX levels were found significantly increased in Group VI with respect to Group V ($P < 0.05$, Mann-Whitney). Consequently, the whole-body 1800 MHz GSM-like RF radiation exposure may lead to oxidative destruction as being indicators of subsequent reactions that occur to form oxygen toxicity in tissues.

(E) Trivino Pardo JC, Grimaldi S, Taranta M, Naldi I, Cinti C. Microwave electromagnetic field regulates gene expression in T-lymphoblastoid leukemia CCRF-CEM cell line exposed to 900 MHz. Electromagn Biol Med. 31(1):1-18, 2012. (VT, AE, GE)

Electric, magnetic, and electromagnetic fields are ubiquitous in our society, and concerns have been expressed regarding possible adverse effects of these exposures. Research on Extremely Low-Frequency (ELF) magnetic fields has been performed for more than two decades, and the methodology and quality of studies have improved over time. Studies have consistently shown increased risk for childhood leukemia associated with ELF magnetic fields. There are still inadequate data for other outcomes. More recently, focus has shifted toward Radio Frequencies (RF) exposures from mobile telephony. There are no persuasive data suggesting a health risk, but this research field is still immature with regard to the quantity and quality of available data. This technology is constantly changing and there is a need for continued research on this issue. To investigate whether exposure to high-frequency electromagnetic fields (EMF) could induce adverse health effects, we cultured acute T-lymphoblastoid leukemia cells (CCRF-CEM) in the presence of 900 MHz MW-EMF generated by a transverse electromagnetic (TEM) cell at short and long exposure times. We evaluated the effect of high-frequency EMF on gene expression and we identified functional pathways influenced by 900 MHz MW-EMF exposure.

(E) Trosic I. Multinucleated giant cell appearance after whole body microwave irradiation of rats. Int J Hyg Environ Health. 204(2-3):133-138, 2001. (VO, LE, GT)

Multinucleated giant cells are common for some chronic inflammatory processes in the lung.

These cells are formed by fusion of macrophages, but how the process relates to the kinetics of alveolar macrophage generation is not clear. This study investigated the influence of 2450 MHz microwave irradiation on alveolar macrophage kinetics and formation of multinucleated giant cells after whole body irradiation of rats. The range of electromagnetic radiation was selected as 2450 MHz microwaves at a power density of 5-15 mW/cm². A group of experimental animals was divided in four subgroups that received 2, 8, 13 and 22 irradiation treatments of two hours each. The animals were killed on experimental days 1, 8, 16, and 30. Free lung cell population was obtained by bronchoalveolar lavage. Cell response to the selected irradiation level was followed quantitatively, qualitatively and morphologically using standard laboratory methods. Total cell number retrieved by lavage slightly decreased in treated animals showing time- and dose-dependence. Cell viability did not significantly change in the irradiated animal group (G2) as compared with the control group (G1). Multinucleated cells significantly increased ($p < 0.01$) in treated animals. The elevation of the number of nuclei per cell was time- and dose-dependent. Macrophages with two nucleoli were more common in animals treated twice or eight times. Polynucleation, that is three and more nucleoli in a single cell, was frequently observed after 13 or 22 treatments. Binucleation and multinucleation of alveolar macrophages were sensitive time- and dose-dependent morphological indicators of pulmonary stress.

(E) Trosic I, Busljeta I. Frequency of micronucleated erythrocytes in rat bone marrow exposure to 2.45 GHz radiation. Physica Scripta T118: 168-170, 2005. (VO, LE, GT)

Wistar rats were exposed to 2.45 GHz continuous, radiofrequency microwave (RF/MW) field 2 hours daily, 7 days weekly, at power density 5–10mW/cm². Four subgroups were created in order to be irradiated 4, 16, 30 and 60 hours. Sham exposed controls were included in the study. Animals were euthanized on the final irradiation day of each treated subgroup. Bone marrow smears were examined to determine the extent of genotoxicity after the particular treatment time. Mann-Whitney test was used for statistical evaluation of data. In comparison to the sham exposed subgroups, the findings of polychromatic erythrocytes revealed significant differences for the 8th and 15th experimental day. Bone marrow erythrocyte maturation and/or proliferation initiated by subthermogenic RF/MW irradiation showed temporary disturbance. Thereafter, the frequency of micronucleated bone marrow red cells was significantly increased after 15 irradiation treatments. Comparison of micronucleus frequency data obtained after 2, 8 and 30 irradiation treatments did not reveal statistically significant differences between sham and treated subgroups. Under the applied experimental conditions, RF/MW irradiation initiates transitory cytogenetic effect manifested with micronucleus formation in erythropoietic cells.

(E) Trosic I, Busljeta I. Erythropoietic dynamic equilibrium in rats maintained after microwave irradiation. Exp Toxicol Pathol. 57(3):247-251, 2006. (VO, LE, GT)

The aim of study was to define influence of radiofrequency microwave (RF/MW) radiation on erythropoiesis in rats. The kinetics of polychromatic erythrocytes (PCEs) and micronucleated (MN) PCEs in the bone marrow (BM) and peripheral blood (PB) of rats during the intermittent subchronic experiment was followed. Rats were exposed 2h/day, 7 days/week to RF/MW of 2.45 GHz and whole-body specific absorption rate (SAR) of 1.25+/-0.36W/kg. Control animals

were included in the study. Each exposed and control group was killed on the final day of irradiation. Acridine-orange stained BM and blood smears were examined by fluorescence microscope. PCEs were obtained by inspection of 2000 BM and 1000 PB erythrocytes/slides. BMMNs and PBMNs frequency was obtained by observation of 1000 PCEs/slides. BMPCEs were increased on day 8 and 15, and PBPCs were elevated on days 2 and 8 ($p < 0.05$). The BMMN frequency was increased on experimental day 15, and MNPCEs in the PB was increased on day 8 ($p < 0.05$). Findings of BM and PBPCs or MNPCEs declined nearly to the control values until the end of the experiment. Such findings are considered to be indicators of radiation effects on BM erythropoiesis consequently reflected in the PB. Rehabilitated dynamic haemopoietic equilibrium in rats by the end of experiment indicates possibility of activation adaptation process in rats to the selected experimental conditions of subchronic RF/MW exposure.

(E) Trosic I, Busljeta I, Kasuba V, Rozgaj R. Micronucleus induction after whole-body microwave irradiation of rats. *Mutat Res* 521(1-2):73-79, 2002. (VO, LE, GT)

Adult male Wistar rats were exposed for 2h a day, 7 days a week for up to 30 days to continuous 2450 MHz radiofrequency microwave (rf/MW) radiation at a power density of 5-10 mW/cm². Sham-exposed rats were used as controls. After ether anesthesia, experimental animals were euthanized on the final irradiation day for each treated group. Peripheral blood smears were examined for the extent of genotoxicity, as indicated by the presence of micronuclei in polychromatic erythrocytes (PCEs). The results for the time-course of PCEs indicated significant differences ($P < 0.05$) for the 2nd, the 8th and the 15th day between control and treated subgroups of animals. Increased influx of immature erythrocytes into the peripheral circulation at the beginning of the experiment revealed that the proliferation and maturation of nucleated erythropoietic cells were affected by exposure to the 2450MHz radiofrequency radiation. Such findings are indicators of radiation effects on bone-marrow erythropoiesis and their subsequent effects in circulating red cells. The incidence of micronuclei/1000 PCEs in peripheral blood was significantly increased ($P < 0.05$) in the subgroup exposed to rf/MW radiation after eight irradiation treatments of 2h each in comparison with the sham-exposed control group. It is likely that an adaptive mechanism, both in erythrocytopoiesis and genotoxicity appeared in the rat experimental model during the subchronic irradiation treatment.

(E) Trosic I, Busljeta I, Modlic B. Investigation of the genotoxic effect of microwave irradiation in rat bone marrow cells: in vivo exposure. *Mutagenesis*. 19(5):361-364, 2004. (VO, LE, GT)

An in vivo mammalian cytogenetic test (the erythrocyte micronucleus assay) was used to investigate the extent of genetic damage in bone marrow red cells of rats exposed to radiofrequency/microwave (RF/MW) radiation. Wistar rats ($n = 40$) were exposed to a 2.45 GHz continuous RF/MW field for 2 h daily, 7 days a week, at a power density of 5-10 mW/cm². The whole body average specific absorption rate (SARs) was calculated to be 1.25 ± 0.36 (SE) W/kg. Four subgroups were irradiated for 4, 16, 30 and 60 h. Sham-exposed controls ($n = 24$) were included in the study. The animals of each treated subgroup were killed on the final day of irradiation. Bone marrow smears were examined to determine the extent of genotoxicity after particular treatment times. The results were statistically evaluated using non-parametric Mann-

Whitney and Kruskal-Wallis tests. In comparison with the sham-exposed subgroups, the findings of polychromatic erythrocytes (PCE) revealed significant differences ($P < 0.05$) for experimental days 8 and 15. The frequency of micronucleated PCEs was also significantly increased on experimental day 15 ($P < 0.05$). Pair-wise comparison of data obtained after 2, 8 and 30 irradiation treatments did not reveal statistically significant differences between sham-exposed and treated subgroups. Under the applied experimental conditions the findings revealed a transient effect on proliferation and maturation of erythropoietic cells in the rat bone marrow and the sporadic appearance of micronucleated immature bone marrow red cells.

(E) Trosić I, Pavčić I, Milković-Kraus S, Mladinić M, Zeljezić D. Effect of electromagnetic radiofrequency radiation on the rats' brain, liver and kidney cells measured by comet assay. Coll Antropol 35:1259-1264, 2011. (VO, LE, GT)

The goal of study was to evaluate DNA damage in rat's renal, liver and brain cells after in vivo exposure to radiofrequency/microwave (Rf/Mw) radiation of cellular phone frequencies range. To determine DNA damage, a single cell gel electrophoresis/comet assay was used. Wistar rats (male, 12 week old, approximate body weight 350 g) ($N = 9$) were exposed to the carrier frequency of 915 MHz with Global System Mobile signal modulation (GSM), power density of 2.4 W/m², whole body average specific absorption rate SAR of 0.6 W/kg. The animals were irradiated for one hour/day, seven days/week during two weeks period. The exposure set-up was Gigahertz Transversal Electromagnetic Mode Cell (GTEM--cell). Sham irradiated controls ($N = 9$) were apart of the study. The body temperature was measured before and after exposure. There were no differences in temperature in between control and treated animals. Comet assay parameters such as the tail length and tail intensity were evaluated. In comparison with tail length in controls (13.5 +/- 0.7 microm), the tail was slightly elongated in brain cells of irradiated animals (14.0 +/- 0.3 microm). The tail length obtained for liver (14.5 +/- 0.3 microm) and kidney (13.9 +/- 0.5 microm) homogenates notably differs in comparison with matched sham controls (13.6 +/- 0.3 microm) and (12.9 +/- 0.9 microm). Differences in tail intensity between control and exposed animals were not significant. The results of this study suggest that, under the experimental conditions applied, repeated 915 MHz irradiation could be a cause of DNA breaks in renal and liver cells, but not affect the cell genome at the higher extent compared to the basal damage.

(E) Tsybulin O, Sidorik E, Brieieva O, Buchynska L, Kyrlylenko S, Henshel D, Yakymenko I. GSM 900 MHz cellular phone radiation can either stimulate or depress early embryogenesis in Japanese quails depending on the duration of exposure. Int J Radiat Biol. 89(9):756-763, 2013. (VO, LE, LI, GT)

PURPOSE: Our study was designed to assess the effects of low intensity radiation of a GSM (Global System for Mobile communication) 900 MHz cellular phone on early embryogenesis in dependence on the duration of exposure. **MATERIALS AND METHODS:** Embryos of Japanese Quails were exposed in ovo to GSM 900 MHz cellular phone radiation during initial 38 h of brooding or alternatively during 158 h (120 h before brooding plus initial 38 h of brooding) discontinuously with 48 sec ON (average power density 0.25 μ W/cm²), specific absorption rate 3 μ W/kg) followed by 12 sec OFF intervals. A number of differentiated somites

were assessed microscopically. Possible DNA damage evoked by irradiation was assessed by an alkaline comet assay. **RESULTS:** Exposure to radiation from a GSM 900 MHz cellular phone led to a significantly altered number of differentiated somites. In embryos irradiated during 38 h the number of differentiated somites increased ($p < 0.001$), while in embryos irradiated during 158 h this number decreased ($p < 0.05$). The lower duration of exposure led to a significant ($p < 0.001$) decrease in a level of DNA strand breaks in cells of 38-h embryos, while the higher duration of exposure resulted in a significant ($p < 0.001$) increase in DNA damage as compared to the control. **CONCLUSION:** Effects of GSM 900 MHz cellular phone radiation on early embryogenesis can be either stimulating or deleterious depending on the duration of exposure.

(E) Usikalu MR, Obembe OO, Akinyemi ML, Zhu J. Short-duration exposure to 2.45 GHz microwave radiation induces DNA damage in Sprague Dawley rat's reproductive systems. African J Biotechnol. 2013; 12: 115-122.(VO, AE, GT, RP)

The genotoxic effects of 2.45 GHz microwave (MW) radiation on the testis and ovary of Sprague Dawley rats was investigated. The animals were exposed to varying levels of specific absorption rate (SAR) of 0 (control), 0.48, 0.95, 1.43, 1.91, 2.39, 2.90, 3.40, 3.80 and 4.30 Wkg^{-1} , for 10 min. The induction of DNA damages was assessed using DNA direct amplification of length polymorphisms (DALP) and validated with single cell gel electrophoresis (SCGE) comet assay for same cells at SAR 2.39 Wkg^{-1} . Potential damage at the organ level was assessed by histopathological study. The results show significant differences in the Olive moment and % DNA in the blood of the exposed animals when compared with the control ($p < 0.05$).

Hyperchromasia was observed in the ovary of the animals exposed to MW radiation. Also, there was reduction in the number of germ cells and cell disorganization in the testis of exposed group with increasing SARs. These results suggest that MW radiation has the potential to affect both male and female fertility adversely.

(E) Vafaei H, Kavari G, Izadi HR, Zahra Zare Dorahi ZZ, Dianatpour M, Daneshparvar A, Jamhiri I. (2020). Wi-Fi (2.4 GHz) affects anti-oxidant capacity, DNA repair genes expression and, apoptosis in pregnant mouse placenta. Iran J Basic Med Sci 23(6):833-840. (VO, LE, GE)

Objective(s): The placenta provides nutrients and oxygen to embryo and removes waste products from embryo's blood. As far as we know, the effects of exposure to Wi-Fi (2.4 GHz) signals on placenta have not been evaluated. Hence, we examined the effect of prenatal exposure to Wi-Fi signals on anti-oxidant capacity, expressions of CDKN1A, and GADD45a as well as apoptosis in placenta and pregnancy outcome. Materials and Methods: Pregnant mice were exposed to Wi-Fi signal (2.4 GHz) for 2 and 4 hr. Placenta tissues were examined to measure the MDA and SOD levels. To measure SOD, CDKN1A, GADD45a, Bax, and Bcl-2 expressions were compared by real-time PCR analysis. TUNEL assay was used to assess apoptosis in placenta tissues. The results were analyzed by one-way analysis of variance (ANOVA) using Prism version 6.0 software. Results: MDA and SOD levels had significantly increased in exposed Wi-Fi signal groups (P -value < 0.05). Also, quantitative PCR experiment showed that SOD mRNA expression

significantly increased in Wi-Fi signal groups. The data showed that CDKN1A and GADD45a genes were increased in Wi-Fi groups (P -value <0.05). The quantitative PCR and the TUNEL assay showed that apoptosis increased in Wi-Fi groups (P -value <0.05). Conclusion: Our results provide evidence that Wi-Fi signals increase lipid peroxidation, SOD activity (oxidative stress), apoptosis and CDKN1A and GADD45a overexpression in mice placenta tissue. However, further experimental studies are warranted to investigate other genes and aspects of pregnancy to determine the role of Wi-Fi radiation on fertility and pregnancy.

(NE) Valbonesi P, Franzellitti S, Piano A, Contin A, Biondi C, Fabbri E. Evaluation of HSP70 expression and DNA damage in cells of a human trophoblast cell line exposed to 1.8 GHz amplitude-modulated radiofrequency fields. Radiat Res 169:270-279, 2008. (VT, AE, GT, GE)

The aim of this study was to determine whether high-frequency electromagnetic fields (EMFs) could induce cellular effects. The human trophoblast cell line HTR-8/SVneo was used as a model to evaluate the expression of proteins (HSP70 and HSC70) and genes (HSP70A, B, C and HSC70) of the HSP70 family and the primary DNA damage response after nonthermal exposure to pulse-modulated 1817 MHz sinusoidal waves (GSM-217 Hz; 1 h; SAR of 2 W/kg). HSP70 expression was significantly enhanced by heat, which was applied as the prototypical stimulus. The HSP70A, B and C transcripts were differentially expressed under basal conditions, and they were all significantly induced above basal levels by thermal stress. Conversely, HSC70 protein and gene expression was not influenced by heat. Exposing HTR-8/SVneo cells to high-frequency EMFs did not change either HSP70 or HSC70 protein or gene expression. A significant increase in DNA strand breaks was caused by exposure to HO, which was used as a positive stimulus; however, no effect was observed after exposure of cells to high-frequency EMFs. Overall, no evidence was found that a 1-h exposure to GSM-217 Hz induced a HSP70-mediated stress response or primary DNA damage in HTR-8/SVneo cells. Nevertheless, further investigations on trophoblast cell responses after exposure to GSM signals of different types and durations are needed.

(E) Valbonesi P, Franzellitti S, Bersani F, Contin A, Fabbri E. Effects of the exposure to intermittent 1.8 GHz radio frequency electromagnetic fields on HSP70 expression and MAPK signaling pathways in PC12 cells. Int J Radiat Biol. 90(5):382-391, 2014. (VT, AE, GE, WS)

Purpose: We previously reported effects on heat shock protein 70 (HSP70) mRNA expression, a cytoprotective protein induced under stressful condition, in human trophoblast cells exposed to amplitude-modulated Global System for Mobile Communication (GSM) signals. In the present work the same experimental conditions were applied to the rat PC12 cells, in order to assess the stress responses mediated by HSP70 and by the Mitogen Activated Protein Kinases (MAPK) in neuronal-like cells, an interesting model to study possible effects of mobile phone frequencies exposure. Materials and methods: HSP70 gene expression level was evaluated by reverse transcriptase polymerase chain reaction, HSP70 protein expression and MAPK phosphorylation were assessed by Western blotting. PC12 cells were exposed for 4, 16 or 24 h to 1.8 GHz continuous wave signal (CW, carrier frequency without modulation) or to two different GSM

modulation schemes, GSM-217Hz and GSM-Talk (which generates temporal changes between two different GSM signals, active during talking or listening phases respectively, thus simulating a typical conversation). Specific adsorption rate (SAR) was 2 W/kg. Results: After PC12 cells exposure to the GSM-217Hz signal for 16 or 24 h, HSP70 transcription significantly increased, whereas no effect was observed in cells exposed to the CW or GSM-Talk signals. HSP70 protein expression and three different MAPK signaling pathways were not affected by the exposure to any of the three different 1.8 GHz signals. Conclusion: The positive effect on HSP70 mRNA expression, observed only in cells exposed to the GSM-217Hz signal, is a repeatable response previously reported in human trophoblast cells and now confirmed in PC12 cells. Further investigations towards a possible role of 1.8 GHz signal modulation are therefore advisable.

(NE) Valbonesi P, Franzellitti S, Bersani F, Contin A, Fabbri E. Activity and expression of acetylcholinesterase in PC12 cells exposed to intermittent 1.8 GHz 217-GSM mobile phone signal. Int J Radiat Biol. 92(1):1-10, 2016. (VT, AE, GE)

Purpose: Due to its role in learning, memory and in many neurodegenerative diseases, acetylcholinesterase (AChE) represents an interesting endpoint to assess possible targets of exposure to radiofrequency electromagnetic fields (RF-EMF) generated by mobile phones. We investigated possible alterations of enzymatic activity, gene and protein expression of AChE in neuronal-like cells exposed to a 1.8 GHz Global System for Mobile Communication (GSM) modulated signal (217-GSM). **Materials and methods:** Rat PC12 cells were exposed for 24 h to 1.8 GHz 217-GSM signal. Specific adsorption rate (SAR) was 2 W/kg. AChE enzyme activity was assessed spectrophotometrically by Ellman's method, mRNA expression level was evaluated by real time polymerase chain reaction, and protein expression was assessed by Western blotting. **Results:** AChE enzymatic activity increased of 1.4-fold in PC12 cells exposed to 217-GSM signal for 24 h, whilst AChE transcriptional or translational pathways were not affected. **Conclusion:** Our results provide the first evidence of effects on AChE activity after in vitro exposure of mammalian cells to the RF-EMF generated by GSM mobile phones, at the SAR value 2 W/kg. The obtained evidence promotes further investigations on AChE as a possible target of RF-EMF and confirm the ability of 1.8 GHz 217-GSM signal to induce biological effects in different mammalian cells.

(E) Vanishree, M., Manvikar V, Rudraraju A, Reddy KMP, Kumar NHP, and Quadri SJM. Significance of micronuclei in buccal smears of mobile phone users: A comparative study. J. Oral Maxillofacial Pathol. 22:448–452, 2018. (HU, LE, GT)

Aims and objectives: The present study was designed to evaluate the frequency of micronuclei (MN) in the buccal exfoliated cells of mobile phone users. In addition, comparison of MN frequency between high and low mobile phone users was also done. **Materials and methods:** A total of 30 male and 30 female participants between the age group of 20-28 years were selected from the Outpatient Department of Navodaya Dental College and Hospital, Raichur, Karnataka. The participants were divided into two groups: Group A - low mobile phone users and Group B - high mobile phone users. Cell sampling and preparation was done on the slide. All the slides were observed for a total of 1000 cells for the presence and number of MN in each cell. **Results:** There was a significant increase in the mean MN count in Group B in comparison to the

Group A. There was highly significant difference in the mean MN count of participants using (code division multiple access) CDMA than (global system for mobiles) GSM mobile phones. The MN mean count was found to be significantly increased in nonheadphone users in comparison to headphone users. In Group B, the MN count on the side of mobile phone use was found to be statistically significantly elevated in comparison to the opposite side. **Conclusion:** Mobile phone radiation even in the permissible range when used for longer duration can cause significant genotoxicity. The genotoxicity accentuates when mobile phones are frequently used on the same side which may be due to more amount of radiation and increase in the temperature. Headphone usage reduces the genotoxicity of mobile phone radiation to some extent.

(E) Varghese R, Majumdar A, Kumar G, Shukla A. Rats exposed to 2.45GHz of non-ionizing radiation exhibit behavioral changes with increased brain expression of apoptotic caspase 3. Pathophysiology. 25:19-30, 2018. (VO, LE, GE, OX)

In recent years there has been a tremendous increase in use of Wi-Fi devices along with mobile phones, globally. Wi-Fi devices make use of 2.4GHz frequency. The present study evaluated the impact of 2.45GHz radiation exposure for 4h/day for 45days on behavioral and oxidative stress parameters in female Sprague Dawley rats. Behavioral tests of anxiety, learning and memory were started from day 38. Oxidative stress parameters were estimated in brain homogenates after sacrificing the rats on day 45. In morris water maze, elevated plus maze and light dark box test, the 2.45GHz radiation exposed rats elicited memory decline and anxiety behavior. Exposure decreased activities of super oxide dismutase, catalase and reduced glutathione levels whereas increased levels of brain lipid peroxidation was encountered in the radiation exposed rats, showing compromised anti-oxidant defense. Expression of caspase 3 gene in brain samples were quantified which unraveled notable increase in the apoptotic marker caspase 3 in 2.45GHz radiation exposed group as compared to sham exposed group. No significant changes were observed in histopathological examinations and brain levels of TNF- α . Analysis of dendritic arborization of neurons showcased reduction in number of dendritic branching and intersections which corresponds to alteration in dendritic structure of neurons, affecting neuronal signaling. The study clearly indicates that exposure of rats to microwave radiation of 2.45GHz leads to detrimental changes in brain leading to lowering of learning and memory and expression of anxiety behavior in rats along with fall in brain antioxidant enzyme systems.

(E) Veerachari SB, Vasan SS. Mobile Phone Electromagnetic Waves and Its Effect on Human Ejaculated Semen: An in vitro Study. Int J Infertility Fetal Med 2012;3(1):15-21. (VT, AE, GT)

Mobile phones usage has seen an exponential growth recently. With this increasing demand, the amount of electromagnetic radiation (EMR) exposed is also increasing. Hence, we studied the effect of these radiations on ejaculated human semen and speculate the contribution of these harmful radiations in male infertility. Samples exposed to EMR showed a significant decrease in sperm motility and viability, increase in reactive oxygen species (ROS) and DNA fragmentation index (DFI) compared to unexposed group. We concluded that mobile phones emit electromagnetic waves which lead to oxidative stress in human semen and also cause changes in DNA fragmentation. We extrapolate these findings to speculate that these radiations may negatively affect spermatozoa and impair male fertility.

(E) Verma S, Keshri GK, Karmakar S, Mani KV, Chauhan S, Yadav A, Sharma M, Gupta A. Effects of Microwave 10 GHz Radiation Exposure in the Skin of Rats: An Insight on Molecular Responses. Radiat Res. 2021 Aug 18. doi: 10.1667/RADE-20-00155.1. (VO, LE, GE, OX)

Microwave (MW) radiation poses the risk of potential hazards on human health. The present study investigated the effects of MW 10 GHz exposure for 3 h/day for 30 days at power densities of 5.23 ± 0.25 and 10.01 ± 0.15 mW/cm² in the skin of rats. The animals exposed to 10 mW/cm² (corresponded to twice the ICNIRP-2020 occupational reference level of MW exposure for humans) exhibited significant biophysical, biochemical, molecular and histological alterations compared to sham-irradiated animals. Infrared thermography revealed an increase in average skin surface temperature by 1.8°C and standard deviation of 0.3°C after 30 days of 10 mW/cm² MW exposure compared to the sham-irradiated animals. MW exposure also led to oxidative stress (ROS, 4-HNE, LPO, AOPP), inflammatory responses (NFkB, iNOS/NOS2, COX-2) and metabolic alterations [hexokinase (HK), lactate dehydrogenase (LDH), citrate synthase (CS) and glucose-6-phosphate dehydrogenase (G6PD)] in 10 mW/cm² irradiated rat skin. A significant alteration in expression of markers associated with cell survival (Akt/PKB) and HSP27/p38MAPK-related stress-response signaling cascade was observed in 10 mW/cm² irradiated rat skin compared to sham-irradiated rat skin. However, MW-irradiated groups did not show apoptosis, evident by unchanged caspase-3 levels. Histopathological analysis revealed a mild cytoarchitectural alteration in epidermal layer and slight aggregation of leukocytes in 10 mW/cm² irradiated rat skin. Altogether, the present findings demonstrated that 10 GHz exposure in continuous-wave mode at 10 mW/cm² (3 h/day, 30 days) led to significant alterations in molecular markers associated with adaptive stress-response in rat skin. Furthermore, systematic scientific studies on more prevalent pulsed-mode of MW-radiation exposure for prolonged duration are warranted.

(NE) Verschaeve L, Heikkinen P, Verheyen G, Van Gorp U, Boonen F, Vander Plaetse F, Maes A, Kumlin T, Maki-Paakkanen J, Puranen L, Juutilainen J. Investigation of co-genotoxic effects of radiofrequency electromagnetic fields in vivo. Radiat Res 165:598-607, 2006. (VO, LE, GT, IX)

We investigated the possible combined genotoxic effects of radiofrequency (RF) electromagnetic fields (900 MHz, amplitude modulated at 217 Hz, mobile phone signal) with the drinking water mutagen and carcinogen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX). Female rats were exposed to RF fields for a period of 2 years for 2 h per day, 5 days per week at average whole-body specific absorption rates of 0.3 or 0.9 W/kg. MX was given in the drinking water at a concentration of 19 µg/ml. Blood samples were taken at 3, 6 and 24 months of exposure and brain and liver samples were taken at the end of the study (24 months). DNA damage was assessed in all samples using the alkaline comet assay, and micronuclei were determined in erythrocytes. We did not find significant genotoxic activity of MX in blood and liver cells. However, MX induced DNA damage in rat brain. Co-exposures to MX and RF radiation did not significantly increase the response of blood, liver and brain cells compared to MX exposure only. In conclusion, this 2-year animal study involving long-term exposures to RF radiation and MX did not provide any evidence for enhanced genotoxicity in rats exposed to RF radiation.

Verschaeve L. Genetic damage in subjects exposed to radiofrequency radiation. *Mutat Res.* 681(2-3):259-270 (2009). (Review)

Despite many research efforts and public debate there is still great concern about the possible adverse effects of radiofrequency (RF) radiation on human health. This is especially due to the enormous increase of wireless mobile telephones and other telecommunication devices throughout the world. The possible genetic effects of mobile phone radiation and other sources of radiofrequencies constitute one of the major points of concern. In the past several review papers were published on laboratory investigations that were devoted to in vitro and in vivo animal (cyto)genetic studies. However, it may be assumed that some of the most important observations are those obtained from studies with individuals that were exposed to relatively high levels of radiofrequency radiation, either as a result of their occupational activity or as frequent users of radiofrequency emitting tools. In this paper the cytogenetic biomonitoring studies of RF-exposed humans are reviewed. A majority of these studies do show that RF-exposed individuals have increased frequencies of genetic damage (e.g., chromosomal aberrations) in their lymphocytes or exfoliated buccal cells. However, most of the studies, if not all, have a number of shortcomings that actually prevents any firm conclusion. Radiation dosimetry was lacking in all papers, but some of the investigations were flawed by much more severe imperfections. Large well-coordinated multidisciplinary investigations are needed in order to reach any robust conclusion.

(E) Vian A, Roux D, Girard S, Bonnet P, Paladian F, Davies E, Ledoigt G. Microwave irradiation affects gene expression in plants. *Plant Signal Behav.* 1(2):67-70, 2006.(VO, AE, GE)

The physiological impact of nonionizing radiation has long been considered negligible. However, here we use a carefully calibrated stimulation system that mimics the characteristics (isotropy and homogeneity) of electromagnetic fields present in the environment to measure changes in a molecular marker (mRNA encoding the stress-related bZIP transcription factor), and show that low amplitude, short duration, 900 MHz EMF evokes the accumulation of this mRNA. Accumulation is rapid (peaking 5-15 min after stimulation) and strong (3.5-fold), and is similar to that evoked by mechanical stimulations.

(E) Vijayalaxmi, Frei, MR, Dusch, SJ, Guel, V, Meltz, ML, Jauchem, JR, Frequency of micronuclei in the peripheral blood and bone marrow of cancer-prone mice chronically exposed to 2450 MHz radiofrequency radiation. *Radiat Res* 147(4):495-500, 1997a. (VO, LE, GT)

C3H/HeJ mice, which are prone to mammary tumors, were exposed for 20 h/day, 7 days/week, over 18 months to continuous-wave 2450 MHz radiofrequency (RF) radiation in circularly polarized wave guides at a whole-body average specific absorption rate of 1.0 W/kg. Sham-exposed mice were used as controls. The positive controls were the sentinel mice treated with mitomycin C during the last 24 h before necropsy. At the end of the 18 months, all mice were necropsied. Peripheral blood and bone marrow smears were examined for the extent of genotoxicity as indicated by the presence of micronuclei in polychromatic erythrocytes (PCEs). The results indicate that the incidence of micronuclei/1,000 PCEs was not significantly different between groups exposed to RF radiation (62 mice) and sham-exposed groups (58 mice), and the

mean frequencies were 4.5 +/- 1.23 and 4.0 +/- 1.12 in peripheral blood and 6.1 +/- 1.78 and 5.7 +/- 1.60 in bone marrow, respectively. In contrast, the positive controls (7 mice) showed a significantly elevated incidence of micronuclei/1,000 PCEs in peripheral blood and bone marrow, and the mean frequencies were 50.9 +/- 6.18 and 55.2 +/- 4.65, respectively. When the animals with mammary tumors were considered separately, there were no significant differences in the incidence of micronuclei/1,000 PCEs between the group exposed to RF radiation (12 mice) and the sham-exposed group (8 mice), and the mean frequencies were 4.6 +/- 1.03 and 4.1 +/- 0.89 in peripheral blood and 6.1 +/- 1.76 and 5.5 +/- 1.51 in bone marrow, respectively. Thus there was no evidence for genotoxicity in mice prone to mammary tumors that were exposed chronically to 2450 MHz RF radiation compared with sham-exposed controls.

A correction was published in a subsequent issue of the journal, stating that there was actually a significant increase in micronucleus formation in peripheral blood and bone marrow cells after chronic exposure to the radiofrequency radiation. "Vijayalaxmi, Frei ,MR, Dusch, SJ, Guel, V, Meltz, ML, Jauchem, JR, Correction of an error in calculation in the article "Frequency of micronuclei in the peripheral blood and bone marrow of cancer-prone mice chronically exposed to 2450 MHz radiofrequency radiation" (Radiat. Res. 147, 495-500, 1997). Radiat Res 149(3):308, 1998 "

(NE) Vijayalaxmi, Mohan N, Meltz ML, Wittler MA. Proliferation and cytogenetic studies in human blood lymphocytes exposed in vitro to 2450 MHz radiofrequency radiation. Int J Radiat Biol. 1997b; 72: 751-757. (VT, AE, GT)

Aliquots of human peripheral blood collected from two healthy human volunteers were exposed in vitro to continuous wave 2450 MHz radiofrequency radiation (RFR), either continuously for a period of 90 min or intermittently for a total exposure period of 90 min (30 min on and 30 min off, repeated three times). Blood aliquots which were sham-exposed or exposed in vitro to 150 cGy gamma radiation served as controls. The continuous wave 2450 MHz RFR was generated with a net forward power of 34.5 W and transmitted from a standard gain rectangular antenna horn in a vertically downward direction. The mean power density at the position of the cells was 5.0 mW/cm². The mean specific absorption rate calculated by Finite Difference Time Domain analysis was 12.46 W/kg. Immediately after exposure, lymphocytes were cultured for 48 and 72 h to determine the incidence of chromosomal aberrations and micronuclei, respectively. Proliferation indices were also recorded. There were no significant differences between RFR-exposed and sham-exposed lymphocytes with respect to; (a) mitotic indices; (b) incidence of cells showing chromosome damage; (c) exchange aberrations; (d) acentric fragments; (e) binucleate lymphocytes, and (f) micronuclei, for either the continuous or intermittent RFR exposures. In contrast, the response of positive control cells exposed to 150 cGy gamma radiation was significantly different from RFR-exposed and sham-exposed lymphocytes. Thus, there is no evidence for an effect on mitogen-stimulated proliferation kinetics or for excess genotoxicity within 72 h in human blood lymphocytes exposed in vitro to 2450 MHz RFR.

(NE) Vijayalaxmi, Seaman RL, Belt ML, Doyle JM, Mathur SP, Prihoda TJ, Frequency of micronuclei in the blood and bone marrow cells of mice exposed to ultra-wideband

electromagnetic radiation. Int J Radiat Biol 75(1):115-120, 1999. (VO, AE, LI, GT)

PURPOSE: To investigate the extent of genetic damage in the peripheral blood and bone marrow cells of mice exposed to ultra-wideband electromagnetic radiation (UWBR). **MATERIALS AND METHODS:** CF-1 male mice were exposed to UWBR for 15 min at an estimated whole-body average specific absorption rate of 37 mWx kg(-1). Groups of untreated control and positive control mice injected with mitomycin C were also included in the study. After various treatments, half of the mice were killed at 18 h, and the other half at 24 h. Peripheral blood and bone marrow smears were examined to determine the extent of genotoxicity, as assessed by the presence of micronuclei (MN) in polychromatic erythrocytes (PCE). **RESULTS:** The percentages of PCE and the incidence of MN per 2000 PCE in both tissues in mice killed at 18 h were similar to the frequencies observed in mice terminated at 24 h. There were no significant differences in the percentage of PCE between control and the mice with or without UWBR exposure; the group mean values (+/- standard deviation) were in the range of 3.1+/-0.14 to 3.2+/-0.23 in peripheral blood, and 49.0+/-3.56 to 52.3+/-4.02 in bone marrow. The mean incidence of MN per 2000 PCE in control and in mice with or without UWBR exposure ranged from 7.7+/-2.00 to 9.7+/-2.54 in peripheral blood and 7.4+/-2.32 to 10.0+/-3.27 in bone marrow. Pairwise comparison of the data did not reveal statistically significant differences between the control and mice with or without UWBR exposure groups (excluding positive controls). **CONCLUSION:** Under the experimental conditions tested, there was no evidence for excess genotoxicity in peripheral blood or bone marrow cells of mice exposed to UWBR.

(NE) Vijayalaxmi, Leal BZ, Szilagyi M, Prihoda TJ, Meltz ML, Primary DNA damage in human blood lymphocytes exposed In vitro to 2450 MHz radiofrequency radiation. Radiat Res 153(4):479-486, 2000. (VT, AE, GT)

Human peripheral blood samples collected from three healthy human volunteers were exposed in vitro to pulsed-wave 2450 MHz radiofrequency (RF) radiation for 2 h. The RF radiation was generated with a net forward power of 21 W and transmitted from a standard gain rectangular antenna horn in a vertically downward direction. The average power density at the position of the cells in the flask was 5 mW/cm(2). The mean specific absorption rate, calculated by finite difference time domain analysis, was 2.135 (+/-0.005 SE) W/kg. Aliquots of whole blood that were sham-exposed or exposed in vitro to 50 cGy of ionizing radiation from a (137)Cs gamma-ray source were used as controls. The lymphocytes were examined to determine the extent of primary DNA damage (single-strand breaks and alkali-labile lesions) using the alkaline comet assay with three different slide-processing schedules. The assay was performed on the cells immediately after the exposures and at 4 h after incubation of the exposed blood at 37 +/- 1 degrees C to allow time for rejoining of any strand breaks present immediately after exposure, i.e. to assess the capacity of the lymphocytes to repair this type of DNA damage. At either time, the data indicated no significant differences between RF-radiation- and sham-exposed lymphocytes with respect to the comet tail length, fluorescence intensity of the migrated DNA in the tail, and tail moment. The conclusions were similar for each of the three different comet assay slide-processing schedules examined. In contrast, the response of lymphocytes exposed to ionizing radiation was significantly different from RF-radiation- and sham-exposed cells. Thus, under the experimental conditions tested, there is no evidence for induction of DNA single-

strand breaks and alkali-labile lesions in human blood lymphocytes exposed in vitro to pulsed-wave 2450 MHz radiofrequency radiation, either immediately or at 4 h after exposure.

(NE) Vijayalaxmi , Leal BZ, Meltz ML, Pickard WF, Bisht KS, Roti Roti JL , Straube WL, Moros EG, Cytogenetic studies in human blood lymphocytes exposed in vitro to radiofrequency radiation at a cellular telephone frequency (835.62 MHz, FDMA). Radiat Res 155(1):113-121, 2001a. (VO, AE, GT)

Freshly collected peripheral blood samples from four healthy human volunteers were diluted with RPMI 1640 tissue culture medium and exposed in sterile T-75 tissue culture flasks in vitro for 24 h to 835.62 MHz radiofrequency (RF) radiation, a frequency employed for customer-to-base station transmission of cellular telephone communications. An analog signal was used, and the access technology was frequency division multiple access (FDMA, continuous wave). A nominal net forward power of 68 W was used, and the nominal power density at the center of the exposure flask was 860 W/m². The mean specific absorption rate in the exposure flask was 4.4 or 5.0 W/kg. Aliquots of diluted blood that were sham-exposed or exposed in vitro to an acute dose of 1.50 Gy of gamma radiation were used as negative or positive controls. Immediately after the exposures, the lymphocytes were stimulated with a mitogen, phytohemagglutinin, and cultured for 48 or 72 h to determine the extent of genetic damage, as assessed from the frequencies of chromosomal aberrations and micronuclei. The extent of alteration in the kinetics of cell proliferation was determined from the mitotic indices in 48-h cultures and from the incidence of binucleate cells in 72-h cultures. The data indicated no significant differences between RF-radiation- and sham-exposed lymphocytes with respect to mitotic indices, incidence of exchange aberrations, excess fragments, binucleate cells, and micronuclei. In contrast, the response of the lymphocytes exposed to gamma radiation was significantly different from both RF-radiation- and sham-exposed cells for all of these indices. Thus, under the experimental conditions tested, there is no evidence for the induction of chromosomal aberrations and micronuclei in human blood lymphocytes exposed in vitro for 24 h to 835.62 MHz RF radiation at SARs of 4.4 or 5.0 W/kg.

(NE) Vijayalaxmi, Pickard WF, Bisht KS, Prihoda TJ, Meltz ML, LaRegina MC, Roti Roti JL, Straube WL, Moros EG. Micronuclei in the peripheral blood and bone marrow cells of rats exposed to 2450 MHz radiofrequency radiation. Int J Radiat Biol. 77(11):1109-1115, 2001b. (VT, AE, GT)

PURPOSE: To determine the incidence of micronuclei in peripheral blood and bone marrow cells of rats exposed continuously for 24 h to 2450 MHz continuous wave radiofrequency radiation (RFR) at an average whole-body specific absorption rate (SAR) of 12 W/kg. **MATERIALS AND METHODS:** Eight adult male Sprague-Dawley rats were exposed to 2450 MHz RFR in circularly polarized waveguides. Eight sham-exposed rats were kept in similar waveguides without the transmission of RFR. Four rats were treated with mitomycin-C (MMC) and used as positive controls. All rats were necropsied 24 h after the end of RFR and sham exposures, and after the 24h treatment with MMC. Peripheral blood and bone marrow smears were examined to determine the frequency of micronuclei (MN) in polychromatic erythrocytes (PCE). **RESULTS:**

The results indicated that the incidence of MN/2000 PCE were not significantly different between RFR- and sham-exposed rats. The group mean frequencies of MN in the peripheral blood were 2.3+/-0.7 in RFR-exposed rats and 2.1+/-0.6 in sham-exposed rats. In bone marrow cells, the average MN incidence was 3.8+/-1.0 in RFR-exposed rats and 3.4+/-0.7 in sham-exposed rats. The corresponding values in positive control rats treated with MMC were 23.5+/-4.7 in the peripheral blood and 33.8+/-7.4 in bone marrow cells. CONCLUSION: There was no evidence for the induction of MN in peripheral blood and bone marrow cells of rats exposed for 24 h to 2450 MHz continuous wave RFR at a whole body average SAR of 12 W/kg.

(NE) Vijayalaxmi, Bisht KS, Pickard WF, Meltz ML, Roti Roti JL, Moros EG. Chromosome damage and micronucleus formation in human blood lymphocytes exposed in vitro to radiofrequency radiation at a cellular telephone frequency (847.74 MHz, CDMA). Radiat Res 156(4):430-432, 2001c.(VT, AE, GT)

Peripheral blood samples collected from four healthy nonsmoking human volunteers were diluted with tissue culture medium and exposed in vitro for 24 h to 847.74 MHz radiofrequency (RF) radiation (continuous wave), a frequency employed for cellular telephone communications. A code division multiple access (CDMA) technology was used with a nominal net forward power of 75 W and a nominal power density of 950 W/m² (95 mW/cm²). The mean specific absorption rate (SAR) was 4.9 or 5.5 W/kg. Blood aliquots that were sham-exposed or exposed in vitro to an acute dose of 1.5 Gy of gamma radiation were included in the study as controls. The temperatures of the medium during RF-radiation and sham exposures in the Radial Transmission Line facility were controlled at 37 +/- 0.3 degrees C. Immediately after the exposures, lymphocytes were cultured at 37 +/- 1 degrees C for 48 or 72 h. The extent of genetic damage was assessed from the incidence of chromosome aberrations and micronuclei. The kinetics of cell proliferation was determined from the mitotic indices in 48-h cultures and from the incidence of binucleate cells in 72-h cultures. The data indicated no significant differences between RF-radiation-exposed and sham-exposed lymphocytes with respect to mitotic indices, frequencies of exchange aberrations, excess fragments, binucleate cells, and micronuclei. The response of gamma-irradiated lymphocytes was significantly different from that of both RF-radiation-exposed and sham-exposed cells for all of these indices. Thus there was no evidence for induction of chromosome aberrations and micronuclei in human blood lymphocytes exposed in vitro for 24 h to 847.74 MHz RF radiation (CDMA) at SARs of 4.9 or 5.5 W/kg.

(NE) Vijayalaxmi, Sasser LB, Morris JE, Wilson BW, Anderson LE. Genotoxic potential of 1.6 GHz wireless communication signal: in vivo two-year bioassay. Radiat Res 159(4):558-564, 2003.(VO, LE, GT)

Timed-pregnant Fischer 344 rats (from nineteenth day of gestation) and their nursing offspring (until weaning) were exposed to a far-field 1.6 GHz Iridium wireless communication signal for 2 h/day, 7 days/week. Far-field whole-body exposures

were conducted with a field intensity of 0.43 mW/cm² and whole-body average specific absorption rate (SAR) of 0.036 to 0.077 W/kg (0.10 to 0.22 W/kg in the brain). This was followed by chronic, head-only exposures of male and female offspring to a near-field 1.6 GHz signal for 2 h/day, 5 days/week, over 2 years. Near-field exposures were conducted at an SAR of 0.16 or 1.6 W/kg in the brain. Concurrent sham-exposed and cage control rats were also included in the study. At the end of 2 years, all rats were necropsied. Bone marrow smears were examined for the extent of genotoxicity, assessed from the presence of micronuclei in polychromatic erythrocytes. The results indicated that the incidence of micronuclei/2000 polychromatic erythrocytes were not significantly different between 1.6 GHz-exposed, sham-exposed and cage control rats. The group mean frequencies were 5.6 +/- 1.8 (130 rats exposed to 1.6 GHz at 0.16 W/kg SAR), 5.4 +/- 1.5 (135 rats exposed to 1.6 GHz at 1.6 W/kg SAR), 5.6 +/- 1.7 (119 sham-exposed rats), and 5.8 +/- 1.8 (100 cage control rats). In contrast, positive control rats treated with mitomycin C exhibited significantly elevated incidence of micronuclei/2000 polychromatic erythrocytes in bone marrow cells; the mean frequency was 38.2 +/- 7.0 (five rats). Thus there was no evidence for excess genotoxicity in rats that were chronically exposed to 1.6 GHz compared to sham-exposed and cage controls.

(NE) Vijayalaxmi, Logani MK, Bhanushali A, Marvin C, Ziskin MC, Prihoda TJ. Micronuclei in peripheral blood and bone marrow cells of mice exposed to 42 GHz electromagnetic millimeter waves. Radiat Res. 2004; 161: 341–345. (VO, LE, GT)

The genotoxic potential of 42.2 +/- 0.2 GHz electromagnetic millimeter-wave radiation was investigated in adult male BALB/c mice. The radiation was applied to the nasal region of the mice for 30 min/day for 3 consecutive days. The incident power density used was 31.5 +/- 5.0 mW/cm². The peak specific absorption rate was calculated as 622 +/- 100 W/kg. Groups of mice that were injected with cyclophosphamide (15 mg/kg body weight), a drug used in the treatment of human malignancies, were also included to determine if millimeter-wave radiation exposure had any influence on drug-induced genotoxicity. Concurrent sham-exposed and untreated mice were used as controls. The extent of genotoxicity was assessed from the incidence of micronuclei in polychromatic erythrocytes of peripheral blood and bone marrow cells collected 24 h after treatment. The results indicated that the incidence of micronuclei in 2000 polychromatic erythrocytes was not significantly different among untreated, millimeter wave-exposed, and sham-exposed mice. The group mean incidences were 6.0 +/- 1.6, 5.1 +/- 1.5 and 5.1 +/- 1.3 in peripheral blood and 9.1 +/- 1.1, 9.3 +/- 1.6 and 9.1 +/- 1.6 in bone marrow cells, respectively. Mice that were injected with cyclophosphamide exhibited significantly increased numbers of micronuclei, 14.6 +/- 2.7 in peripheral blood and 21.3 +/- 3.9 in bone marrow cells (P < 0.0001). The drug-induced micronuclei were not significantly different in millimeter wave-exposed and sham-exposed mice; the mean incidences were 14.3 +/- 2.8 and 15.4 +/- 3.0 in peripheral blood and 23.5 +/- 2.3 and 22.1 +/- 2.5 in bone marrow cells, respectively. Thus there was no evidence for the induction of genotoxicity in the peripheral blood and bone marrow cells of mice exposed to electromagnetic millimeter-wave radiation. Also, millimeter-wave radiation exposure did not influence cyclophosphamide-induced micronuclei in either type of cells.

(NE) Vijayalaxmi. Cytogenetic studies in human blood lymphocytes exposed in vitro to 2.45 GHz or 8.2 GHz radiofrequency radiation. Radiat Res 166, 532–538, 2006. (VT, AE, GT)

Peripheral blood samples collected from healthy human volunteers were exposed in vitro to 2.45 GHz or 8.2 GHz pulsed-wave radiofrequency (RF) radiation. The net forward power, average power density, mean specific absorption rate, and the temperature maintained during the 2-h exposure of the cells to 2.45 GHz or 8.2 GHz were, respectively, 21 W or 60 W, 5 mW/cm² or 10 mW/cm², 2.13 W/kg or 20.71 W/kg, and 36.9 ± 0.1°C or 37.5 ± 0.2°C. Aliquots of the same blood samples that were either sham-exposed or exposed in vitro to an acute dose of 1.5 Gy γ radiation were used as unexposed and positive controls, respectively. Cultured lymphocytes were examined to determine the extent of cytogenetic damage assessed from the incidence of chromosomal aberrations and micronuclei. Under the conditions used to perform the experiments, the levels of damage in RF-radiation-exposed and sham-exposed lymphocytes were not significantly different. Also, there were no significant differences in the response of unstimulated lymphocytes and lymphocytes stimulated with phytohemagglutinin when exposed to 8.2 GHz RF radiation. In contrast, the positive control cells that had been subjected to γ irradiation exhibited significantly more damage than RF-radiation- and sham-exposed lymphocytes.

Vijayalaxmi, Prihoda TJ. Comprehensive review of quality of publications and meta-analysis of genetic damage in mammalian cells exposed to non-ionizing radiofrequency fields. Radiat Res. 191(1):20-30, 2019. (Review)

There have been numerous published studies reporting on the extent of genetic damage observed in animal and human cells exposed in vitro and in vivo to non-ionizing radiofrequency fields (RF, electromagnetic waves that carry energy as they propagate in air and dense media). Overall, the data are inconsistent; while some studies have suggested significantly increased damage in cells exposed to RF energy compared to unexposed and/or sham-exposed control cells, others have not. Several variables in exposure conditions used in the experiments might have contributed to the controversy. In this comprehensive review, four specific quality control measures were used to determine the quality of 225 published studies in animal and human cells exposed in vitro and in vivo to RF energy, and the results from 2,160 tests with different sample sizes were analyzed. The four specific quality control measures were as follows: 1. "Blind" collection/analysis of the data to eliminate individual/observer "bias"; 2. Adequate description of "dosimetry" for independent replication/confirmation; 3. Inclusion of "positive controls" to confirm the outcomes; and 4. Inclusion of "sham-exposed controls" which are more appropriate to compare the data with those in RF exposure conditions. In addition, meta-analysis of the genetic damage in cells exposed to RF energy and control cells, thus far available in the RF literature database, was performed to obtain the "d" values, i.e., standardized mean difference between these two types of cells or the effect size. The relationship between d values and the above-mentioned quality control measures was ascertained. In addition, the correlation between the quality control measures and the conclusions reported in the publications (no significant difference between the cells exposed to RF energy and control cells; increased damage in former cells compared to the latter; increased, no significant difference and decreased damage in cells exposed to RF energy in the same experiment; or decreased damage in cells exposed to RF

energy) was examined. The overall conclusions were as follows: 1. When all four quality control measures were mentioned in the publication, the d values were smaller compared to those when one or more quality control measures were not mentioned in the investigation; 2. Based on the inclusion of quality control measures, the weighted outcome in cells exposed to RF energy (d values) indicated a very small effect, if any; 3. The number of published studies reporting no significant difference in genetic damage of cells exposed to RF energy, compared to that of control cells, increased with increased number of quality control measures employed in investigations; 4. The number of published studies reporting increased genetic damage in cells exposed to RF energy decreased with increased number of quality control measures; and 5. There was a "bias" towards the publications reporting increased genetic damage in cells exposed to RF energy even with very small sample size. Overall, the results from this study underscore the importance of including quality control measures in investigations so that the resulting data are useful, nationally and internationally, in evaluating "potential" health risks from exposure to RF energy

(E) Vilić, M., Tlak Gajger, I., Tucak, P., Štambuk, A., Šrut, M., Klobučar, G., Malarić, K., Žura Žaja, I., Pavelić, A., Manger, M., and Tkalec, M. Effects of short-term exposure to mobile phone radiofrequency (900 MHz) on the oxidative response and genotoxicity in honey bee larvae. J. Apic. Res. 56, 430–438, 2017. (VO, AE, GT, OX, WS)

Exposure of different animal species to radiofrequency electromagnetic fields (RF-EMF) could cause various biological effects such as oxidative stress, genotoxic effects and dysfunction of the immune system. However, there are a lack of results on oxidative stress response and genotoxicity in the honey bee (*Apis mellifera*) after exposure to RF-EMF. This study was performed to investigate the effects of exposure to RF-EMF on the activity of catalase, superoxide dismutase, glutathione *S*-transferase, lipid peroxidation level and DNA damage in honey bee larvae. Honey bee larvae were exposed to RF-EMF at 900 MHz and field levels of 10, 23, 41 and 120 V m⁻¹ for 2 h. At a field level of 23 V m⁻¹ the effect of 80% AM 1 kHz sinusoidal and 217 Hz modulation was investigated as well. Catalase activity and the lipid peroxidation level decreased significantly in the honey bee larvae exposed to the unmodulated field at 10 V m⁻¹ compared to the control. Superoxide dismutase and glutathione *S*-transferase activity in the honey bee larvae exposed to unmodulated fields were not statistically different compared to the control. DNA damage increased significantly in honey bee larvae exposed to modulated (80% AM 1 kHz sinus) field at 23 V m⁻¹ compared to the control and all other exposure groups. These results suggest that RF-EMF effects in honey bee larvae appeared only after exposure to a certain EMF conditions. The increase of the field level did not cause a linear dose-response in any of the measured parameters. Modulated RF-EMF produced more negative effects than the corresponding unmodulated field. Although honey bees in nature would not be exposed to such high field levels as used in our experiments, our results show the need for further intensive research in all stages of honey bee development.

(NE) Waldmann P, Bohnenberger S, Greinert R, Hermann-Then B, Heselich A, Klug SJ, Koenig J, Kuhr K, Kuster N, Merker M, Murbach M, Pollet D, Schadenboeck W, Scheidemann-Wesp U, Schwab B, Volkmer B, Weyer V, Blettner M. Influence of GSM signals on human peripheral lymphocytes: study of genotoxicity. Radiat Res. 179(2):243-253, 2013. (VT, AE, GT)

Exposure to radiofrequency (RF) electromagnetic fields (EMF) is continuously increasing worldwide. Yet, conflicting results of a possible genotoxic effect of RF EMF continue to be discussed. In the present study, a possible genotoxic effect of RF EMF (GSM, 1,800 MHz) in human lymphocytes was investigated by a collaboration of six independent institutes (institutes a, b, c, d, e, h). Peripheral blood of 20 healthy, nonsmoking volunteers of two age groups (10 volunteers 16-20 years old and 10 volunteers 50-65 years old) was taken, stimulated and intermittently exposed to three specific absorption rates (SARs) of RF EMF (0.2 W/kg, 2 W/kg, 10 W/kg) and sham for 28 h (institute a). The exposures were performed in a setup with strictly controlled conditions of temperature and dose, and randomly and automatically determined waveguide SARs, which were designed and periodically maintained by ITIS (institute h). Four genotoxicity tests with different end points were conducted (institute a): chromosome aberration test (five types of structural aberrations), micronucleus test, sister chromatid exchange test and the alkaline comet assay (Olive tail moment and % DNA). To demonstrate the validity of the study, positive controls were implemented. The genotoxicity end points were evaluated independently by three laboratories blind to SAR information (institute c = laboratory 1; institute d = laboratory 2; institute e = laboratory 3). Statistical analysis was carried out by institute b. Methods of primary statistical analysis and rules to adjust for multiple testing were specified in a statistical analysis plan based on a data review before unblinding. A linear trend test based on a linear mixed model was used for outcomes of comet assay and exact permutation test for linear trend for all other outcomes. It was ascertained that only outcomes with a significant SAR trend found by at least two of three analyzing laboratories indicated a substantiated suspicion of an exposure effect. On the basis of these specifications, none of the nine end points tested for SAR trend showed a significant and reproducible exposure effect. Highly significant differences between sham exposures and positive controls were detected by each analyzing laboratory, thus validating the study. In conclusion, the results show no evidence of a genotoxic effect induced by RF EMF (GSM, 1,800 MHz).

(E) Wang X, Liu C, Ma Q, Feng W, Yang L, Lu Y, Zhou Z, Yu Z, Li W, Zhang L. 8-oxoG DNA glycosylase-1 inhibition sensitizes Neuro-2a cells to oxidative DNA base damage induced by 900 MHz radiofrequency electromagnetic radiation. Cell Physiol Biochem. 37(3):1075-1088, 2015. (VT, AE, GT, OX)

BACKGROUND/AIMS: The purpose of this study was to explore the in vitro putative genotoxicity during exposure of Neuro-2a cells to radiofrequency electromagnetic fields (RF-EMFs) with or without silencing of 8-oxoG DNA glycosylase-1 (OGG1). **METHODS:** Neuro-2a cells treated with or without OGG1 siRNA were exposed to 900 MHz Global System for Mobile Communication (GSM) Talk signals continuously at a specific absorption rate (SAR) of 0, 0.5, 1 or 2 W/kg for 24 h. DNA strand breakage and DNA base damage were measured by the alkaline comet assay and a modified comet assay using formamidopyrimidine DNA glycosylase (FPG), respectively. Reactive oxygen species (ROS) levels and cell viability were monitored using the non-fluorescent probe 2, 7-dichlorofluorescein diacetate (DCFH-DA) and CCK-8 assay. **RESULTS:** Exposure to 900 MHz RF-EMFs with insufficient energy could induce oxidative DNA base damage in Neuro-2a cells. These increases were concomitant with similar increases in the generation of reactive oxygen species (ROS). Without OGG1 siRNA, 2 W/kg RF-EMFs induced oxidative DNA base damage in Neuro-2a cells. Interestingly, with OGG1

siRNA, RF-EMFs could cause DNA base damage in Neuro-2a cells as low as 1 W/kg. However, neither DNA strand breakage nor altered cell viability was observed. CONCLUSION: Even if further studies remain conducted we support the hypothesis that OGG1 is involved in the process of DNA base repair and may play a pivotal role in protecting DNA bases from RF-EMF induced oxidative damage.

(E) Wang Y, Jiang Z, Zhang L, Zhang Z, Liao Y, Cai P 3.5-GHz radiofrequency electromagnetic radiation promotes the development of *Drosophila melanogaster*. Environ Pollut 2022 Feb 1;294:118646. (VO, LE, LI, GE)

With the rapidly increasing popularity of 5G mobile technology, the effect of radiofrequency radiation on human health has caused public concern. This study explores the effects of a simulated 3.5 GHz radiofrequency electromagnetic radiation (RF-EMF) environment on the development and microbiome of flies under intensities of 0.1 W/m², 1 W/m² and 10 W/m². We found that the pupation percentages in the first 3 days and eclosion rate in the first 2 days were increased under exposure to RF-EMF, and the mean development time was shortened. In a study on third-instar larvae, the expression levels of the heat shock protein genes hsp22, hsp26 and hsp70 and humoral immune system genes AttC, TotC and TotA were all significantly increased. In the oxidative stress system, DuoX gene expression was decreased, sod2 and cat gene expression levels were increased, and SOD and CAT enzyme activity also showed a significant increase. According to the 16S rDNA results, the diversity and species abundance of the microbial community decreased significantly, and according to the functional prediction analysis, the genera Acetobacter and Lactobacillus were significantly increased. In conclusion, 3.5 GHz RF-EMF may enhance thermal stress, oxidative stress and humoral immunity, cause changes in the microbial community, and regulate the insulin/TOR and ecdysteroid signalling pathways to promote fly development.

(NE) Win-Shwe T-T, Ohtani S, Ushiyama A, Fujimaki H, Kunugita N. Can intermediate-frequency magnetic fields affect memory function-related gene expressions in hippocampus of C57BL/6J mice? J Toxicol Sci 38(2):169-176, 2013. (VO, LE, GE)

Recently, a cooking appliance based on the principle of electromagnetic induction has come to be used domestically on a widespread basis; this induction heating cooking hob mainly generates intermediate-frequency magnetic fields (IF-MF). However, whether electromagnetic fields originating from household appliances represent a health risk remains uncertain. We investigated the effect of IF-MF on the expressions of memory function-related genes and related transduction molecules in the mouse hippocampus. Male and female C57BL/6J mice were allotted to a control (sham-exposed), an exposure, or a recovery (one week after exposure) group and were exposed to IF-MF (21 kHz, 3.8 mT) one hour per day for 2 weeks. Twenty-four hour after final exposure, the expression levels of memory function-related genes and the mRNA levels for signal transduction pathway molecules in the hippocampi were examined using real-time RT-PCR. The relative mRNA expression levels of the N-methyl-D aspartate (NMDA) receptor subunits NR1, NR2A, and NR2B as well as transcription factors (calcium/calmodulin-dependent protein kinase (CaMK) -IV, cyclic AMP responsive element binding protein (CREB) -1) and neurotrophins (nerve growth factor (NGF), and brain-derived neurotrophic factors

(BDNF)) were not significantly altered in the IF-MF-exposed mice. We also examined the morphology of the hippocampus using a histological analysis, but no changes in the IF-MF-exposed mice were seen. This is the first in vivo study to show that IF-MF exposure did not affect the expression levels of memory function-related genes in the hippocampus of C57BL/6J mice. The present findings suggest that IF-MF exposure may not affect cognitive function in the present animal model.

(E) Win-Shwe TT, Ohtani S, Ushiyama A, Kunugita N. 2015. Early exposure to intermediate-frequency magnetic fields alters brain biomarkers without histopathological changes in adult mice. Int J Environ Res Public Health. 12(4):4406-4421. (VO, LE, GE, DE)

Recently we have reported that intermediate-frequency magnetic field (IF-MF) exposure transiently altered the mRNA expression levels of memory function-related genes in the hippocampi of adult male mice. However, the effects of IF-MF exposure during brain development on neurological biomarkers have not yet been clarified. In the present study, we investigated the effect of IF-MF exposure during development on neurological and immunological markers in the mouse hippocampus in 3- and 7-week-old male mice. Pregnant C57BL/6J mice were exposed to IF-MF (21 kHz, 3.8 mT) for one hour per day from organogenesis period day 7 to 17. At adolescence, some IF-MF-exposed mice were further divided into exposure, recovery, and sham-exposure groups. The adolescent-exposure groups were exposed again to IF-MF from postnatal day 27 to 48. The expression of mRNA in the hippocampi was examined using a real-time RT-PCR method, and microglia activation was examined by immunohistochemical analysis. The expression levels of NR1 and NR2B as well as transcription factors (CaMKIV, CREB1), inflammatory mediators (COX2, IL-1 b, TNF- α), and the oxidative stress marker heme-oxygenase (HO)-1 were significantly increased in the IF-MF-exposed mice, compared with the control group, in the 7-week-old mice, but not in the 3-week-old mice. Microglia activation was not different between the control and other groups. This study provides the first evidence that early exposure to IF-MF reversibly affects the NMDA receptor, its related signaling pathways, and inflammatory mediators in the hippocampus of young adult mice; these changes are transient and recover after termination of exposure without histopathological changes.

(E) Wu W, Yao K, Wang KJ, Lu DQ, He JL, Xu LH, Sun WJ. [Blocking 1800 MHz mobile phone radiation-induced reactive oxygen species production and DNA damage in lens epithelial cells by noise magnetic fields.] Zhejiang Da XueXueBao Yi Xue Ban 37:34-38, 2008. [Article in Chinese] (VT, AE, GT, IX, OX)

OBJECTIVE: To investigate whether the exposure to the electromagnetic noise can block reactive oxygen species (ROS) production and DNA damage of lens epithelial cells induced by 1800 MHz mobile phone radiation. **METHODS:** The DCFH-DA method and comet assay were used respectively to detect the intracellular ROS and DNA damage of cultured human lens epithelial cells induced by 4 W/kg 1800 MHz mobile phone radiation or/and 2microT electromagnetic noise for 24 h intermittently. **RESULT:** 1800 MHz mobile phone radiation at 4 W/kg for 24 h increased intracellular ROS and DNA damage significantly ($P < 0.05$). However, the ROS level and DNA damage of mobile phone radiation plus noise group were not significant enhanced ($P > 0.05$) as compared to sham exposure group. **Conclusion:** Electromagnetic noise can

block intracellular ROS production and DNA damage of human lens epithelial cells induced by 1800 MHz mobile phone radiation.

(E) Xu S, Zhong M, Zhang L, Zhou Z, Zhang W, Wang Y, Wang X, Li M, Chen Y, Chen C, He M, Zhang G, Yu Z. Exposure to 1800 MHz radiofrequency radiation induces oxidative damage to mitochondrial DNA in primary cultured neurons. Brain Res 1311:189-196. 2010. (VT, AE, GT, OX)

Increasing evidence indicates that oxidative stress may be involved in the adverse effects of radiofrequency (RF) radiation on the brain. Because mitochondrial DNA (mtDNA) defects are closely associated with various nervous system diseases and mtDNA is highly susceptible to oxidative stress, the purpose of this study was to determine whether radiofrequency radiation can cause oxidative damage to mtDNA. In this study, we exposed primary cultured cortical neurons to pulsed RF electromagnetic fields at a frequency of 1800 MHz modulated by 217 Hz at an average special absorption rate (SAR) of 2 W/kg. At 24h after exposure, we found that RF radiation induced a significant increase in the levels of 8-hydroxyguanine (8-OHdG), a common biomarker of DNA oxidative damage, in the mitochondria of neurons. Consistent with this finding, the copy number of mtDNA and the levels of mitochondrial RNA (mtRNA) transcripts showed an obvious reduction after RF exposure. Each of these mtDNA disturbances could be reversed by pretreatment with melatonin, which is known to be an efficient in the brain. Together, these results suggested that 1800 MHz RF radiation could cause oxidative damage to mtDNA in primary cultured neurons. Oxidative damage to mtDNA may account for the neurotoxicity of RF radiation in the brain.

(E) Xu S, Chen G, Chen C, Sun C, Zhang D, Murbach M, Kuster N, Zeng Q, Xu Z. Cell type-dependent induction of DNA damage by 1800 MHz radiofrequency electromagnetic fields does not result in significant cellular dysfunctions. PLoS One. 8(1):e54906, 2013. (VT, AE, GT, CS)

BACKGROUND: Although IARC clarifies radiofrequency electromagnetic fields (RF-EMF) as possible human carcinogen, the debate on its health impact continues due to the inconsistent results. Genotoxic effect has been considered as a golden standard to determine if an environmental factor is a carcinogen, but the currently available data for RF-EMF remain controversial. As an environmental stimulus, the effect of RF-EMF on cellular DNA may be subtle. Therefore, more sensitive method and systematic research strategy are warranted to evaluate its genotoxicity. **OBJECTIVES:** To determine whether RF-EMF does induce DNA damage and if the effect is cell-type dependent by adopting a more sensitive method γ H2AX foci formation; and to investigate the biological consequences if RF-EMF does increase γ H2AX foci formation. **METHODS:** Six different types of cells were intermittently exposed to GSM 1800 MHz RF-EMF at a specific absorption rate of 3.0 W/kg for 1 h or 24 h, then subjected to immunostaining with anti- γ H2AX antibody. The biological consequences in γ H2AX-elevated cell type were further explored with comet and TUNEL assays, flow cytometry, and cell growth assay. **RESULTS:** Exposure to RF-EMF for 24 h significantly induced γ H2AX foci formation in Chinese hamster lung cells and Human skin fibroblasts (HSFs), but not the other cells. However, RF-EMF-elevated γ H2AX foci formation in HSF cells did not result in detectable DNA fragmentation, sustainable cell cycle arrest, cell proliferation or viability change. RF-EMF

exposure slightly but not significantly increased the cellular ROS level. **CONCLUSIONS:** RF-EMF induces DNA damage in a cell type-dependent manner, but the elevated γ H2AX foci formation in HSF cells does not result in significant cellular dysfunctions.

(E) Yadav AS, Sharma MK. Increased frequency of micronucleated exfoliated cells among humans exposed in vivo to mobile telephone radiations. Mutat Res.650(2):175-180, 2008. (HU, LE, GT)

The health concerns have been raised following the enormous increase in the use of wireless mobile telephones throughout the world. This investigation had been taken, with the motive to find out whether mobile phone radiations cause any in vivo effects on the frequency of micronucleated exfoliated cells in the exposed subjects. A total of 109 subjects including 85 regular mobile phone users (exposed) and 24 non-users (controls) had participated in this study. Exfoliated cells were obtained by swabbing the buccal-mucosa from exposed as well as sex-age-matched controls. One thousand exfoliated cells were screened from each individual for nuclear anomalies including micronuclei (MN), karyolysis (KL), karyorrhexis (KH), broken egg (BE) and binucleated (BN) cells. The average daily duration of exposure to mobile phone radiations is 61.26 min with an overall average duration of exposure in term of years is 2.35 years in exposed subjects along with the 9.84 \pm 0.745 micronucleated cells (MNCs) and 10.72 \pm 0.889 total micronuclei (TMN) as compared to zero duration of exposure along with average 3.75 \pm 0.774 MNC and 4.00 \pm 0.808 TMN in controls. The means are significantly different in case of MNC and TMN at 0.01% level of significance. The mean of KL in controls is 13.17 \pm 2.750 and in exposed subjects is 13.06 \pm 1.793. The value of means of KH in exposed subjects (1.84 \pm 0.432) is slightly higher than in controls (1.42 \pm 0.737). Mean frequency of broken egg is found to be more in exposed subjects (0.65 \pm 0.276) as compared to controls (0.50 \pm 0.217). Frequency of presence of more than one nucleus in a cell (binucleated) is also higher in exposed (2.72 \pm 0.374) in comparison to controls (0.67 \pm 0.231). Although there is a slight increase in mean frequency of KH, BE and BN in exposed subjects but the difference is not found statistically significant. Correlation between 0-1, 1-2, 2-3 and 3-4 years of exposure and the frequency of MNC and TMN has been calculated and found to be positively correlated.

Yahyazadeh A, Deniz ÖG, Kaplan AA, Altun G, Yurt KK, Davis D. The genomic effects of cell phone exposure on the reproductive system. Environ Res. 167:684-693, 2018. (review)

Humans are exposed to increasing levels of electromagnetic fields (EMF) at various frequencies as technology advances. In this context, improving understanding of the biological effects of EMF remains an important, high priority issue. Although a number of studies in this issue and elsewhere have focused on the mechanisms of the oxidative stress caused by EMF, the precise understanding of the processes involved remains to be elucidated. Due to unclear results among the studies, the issue of EMF exposure in the literature should be evaluated at the genomic level on the reproductive system. Based on this requirement, a detail review of recently published studies is necessary. The main objectives of this study are to show differences between negative and positive effect of EMF on the reproductive system of animal and human. Extensive review of literature has been made based on well known data bases like Web of Science, PubMed, MEDLINE, Google Scholar, Science Direct, Scopus. This paper reviews the current literature

and is intended to contribute to a better understanding of the genotoxic effects of EMF emitted from mobile phones and wireless systems on the human reproductive system, especially on fertility. The current literature reveals that mobile phones can affect cellular functions via non-thermal effects. Although the cellular targets of global system for mobile communications (GSM)-modulated EMF are associated with the cell membrane, the subject is still controversial. Studies regarding the genotoxic effects of EMF have generally focused on DNA damage. Possible mechanisms are related to ROS formation due to oxidative stress. EMF increases ROS production by enhancing the activity of nicotinamide adenine dinucleotide (NADH) oxidase in the cell membrane. Further detailed studies are needed to elucidate DNA damage mechanisms and apoptotic pathways during oogenesis and spermatogenesis in germ cells exposed to EMF.

(E) Yakymenko I, Burlaka A, Tsybulin I, Brieieva I, Buchynska L, Tsehmistrenko I, Chekhun F. Oxidative and mutagenic effects of low intensity GSM 1800 MHz microwave radiation. Exp Oncol. 40(4):282-287,2018. (VO, LE, GT, OX)

AIM: Despite a significant number of epidemiological studies on potential carcinogenicity of microwave radiation (MWR) from wireless devices and a bulk of experimental studies on oxidative and mutagenic effects of low intensity MWR, the discussion on potential carcinogenicity of low intensity MWR is going on. This study aims to assess oxidative and mutagenic effects of low intensity MWR from a typical commercial model of a modern smartphone. **MATERIALS AND METHODS:** The model of developing quail embryos has been used for the assessment of oxidative and mutagenic effects of Global System for Mobile communication (GSM) 1800 MHz MWR from a commercial model of smartphone. The embryos were exposed in ovo to 0.32 $\mu\text{W}/\text{cm}^2$, discontinuously - 48 s - On, 12 s - Off, during 5 days before and 14 days through the incubation period. **RESULTS:** The exposure of quail embryos before and during the incubation period to low intensity GSM 1800 MHz has resulted in expressive statistically significant oxidative effects in embryonic cells, including a 2-fold increase in superoxide generation rate and 85% increase in nitrogen oxide generation rate, damages of DNA integrity and oxidative damages of DNA (up to twice increased levels of 8-oxo-dG in cells of 1-day old chicks from the exposed embryos). Finally, the exposure resulted in a significant, almost twice, increase of embryo mortality. **CONCLUSION:** The exposure of model biological system to low intensity GSM 1800 MHz MWR resulted in significant oxidative and mutagenic effects in exposed cells, and thus should be recognized as a significant risk factor for living cells.

(E) Yan JG, Agresti M, Zhang LL, Yan Y, Matloub HS. Upregulation of specific mRNA levels in rat brain after cell phone exposure. Electromagn Biol Med. 27(2):147-154, 2008. (VO, LE, GE)

Adult Sprague-Dawley rats were exposed to regular cell phones for 6 h per day for 126 days (18 weeks). RT-PCR was used to investigate the changes in levels of mRNA synthesis of several injury-associated proteins. Calcium ATPase, Neural Cell Adhesion Molecule, Neural Growth Factor, and Vascular Endothelial Growth Factor were evaluated. The results showed statistically significant mRNA up-regulation of these proteins in the brains of rats exposed to cell phone radiation. These results indicate that relative chronic exposure to cell phone microwave radiation may result in cumulative injuries that could eventually lead to clinically significant neurological damage.

(E) Yang X-S, He G-L, Hao Y-T, Xiao Y, Chen C-H, Zhang G-B, Yu Z-P. Exposure to 2.45 GHz electromagnetic fields elicits an HSP-related stress response in rat hippocampus. Brain Res Bull 88(4):371-378, 2012. (VO, AE, GE)

The issue of possible neurobiological effects of the electromagnetic field (EMF) exposure is highly controversial. To determine whether electromagnetic field exposure could act as an environmental stimulus capable of producing stress responses, we employed the hippocampus, a sensitive target of electromagnetic radiation, to assess the changes in its stress-related gene and protein expression after EMF exposure. Adult male Sprague-Dawley rats with body restrained were exposed to a 2.45 GHz EMF at a specific absorption rate (SAR) of 6 W/kg or sham conditions. cDNA microarray was performed to examine the changes of gene expression involved in the biological effects of electromagnetic radiation. Of 2048 candidate genes, 23 upregulated and 18 downregulated genes were identified. Of these differential expression genes, two heat shock proteins (HSP), HSP27 and HSP70, are notable because expression levels of both proteins are increased in the rat hippocampus. Result from immunocytochemistry revealed that EMF caused intensive staining for HSP27 and HSP70 in the hippocampus, especially in the pyramidal neurons of cornu ammonis 3 (CA3) and granular cells of dentate gyrus (DG). The gene and protein expression profiles of HSP27 and HSP70 were further confirmed by reverse transcription polymerase chain reaction (RT-PCR) and Western blot. Our data provide direct evidence that exposure to electromagnetic fields elicits a stress response in the rat hippocampus.

(E) Yao K, Wang KJ, Sun ZH, Tan J, Xu W, Zhu LJ, Lu de Q. Low power microwave radiation inhibits the proliferation of rabbit lens epithelial cells by upregulating P27Kip1 expression. Mol Vis. 10:138-143, 2004. (VT, AE, GE)

PURPOSE: The goal of this study was to examine the effects of low power microwave radiation (<10 mW/cm²) on the proliferation of cultured rabbit lens epithelial cells (RLEC). **METHODS:** Cultured RLEC were exposed to continuous microwave radiation at a frequency of 2,450 MHz and power densities of 0.10, 0.25, 0.50, 1.00, and 2.00 mW/cm² for 8 h. Cell morphologic changes were observed under a phase-contrast microscope. Cell viability was measured using the MTT assay and cell cycle analysis was measured using flow cytometry. After exposure to 2.00 mW/cm² microwave radiation for 4, 6, and 8 h, the expression of cell cycle-regulatory proteins, P21WAF1 and P27Kip1, was examined using western blot analysis. Finally, the levels of P21WAF1 and P27Kip1 mRNA were analyzed by reverse transcription-polymerase chain reaction (RT-PCR). **RESULTS:** After 8 h of radiation treatment, cells treated with 0.50, 1.00, and 2.00 mW/cm² microwave radiation exhibited decreased cell viability, increased cell condensation and an inhibition of DNA synthesis. RLEC showed significant G0/G1 arrest. No obvious changes could be detected in the 0.10 and 0.25 mW/cm² microwave treatment groups. Protein expression of P27Kip1 was markedly increased after microwave radiation. However, the mRNA levels were unchanged. On the other hand, there were no detectable differences in P21WAF1 protein expression and mRNA levels between microwave treatment and control groups. **CONCLUSIONS:** This study suggests that low power microwave radiation higher than 0.50 mW/cm² can inhibit lens epithelial cell proliferation, and increase the expression of P27Kip1. These effects may account for the decline of lens epithelial proliferation after

exposure to microwave radiation.

(E) Yao K, Wu W, Wang K, Ni S, Ye P, Yu Y, Ye J, Sun L. Electromagnetic noise inhibits radiofrequency radiation-induced DNA damage and reactive oxygen species increase in human lens epithelial cells. Mol Vis 14:964-969, 2008. (VT, AE, GT, IX, OX)

PURPOSE: The goal of this study was to investigate whether superposing of electromagnetic noise could block or attenuate DNA damage and intracellular reactive oxygen species (ROS) increase of cultured human lens epithelial cells (HLECs) induced by acute exposure to 1.8 GHz radiofrequency field (RF) of the Global System for Mobile Communications (GSM).

METHODS: An sXc-1800 RF exposure system was used to produce a GSM signal at 1.8 GHz (217 Hz amplitude-modulated) with the specific absorption rate (SAR) of 1, 2, 3, and 4 W/kg. After 2 h of intermittent exposure, the ROS level was assessed by the fluorescent probe, 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). DNA damage to HLECs was examined by alkaline comet assay and the phosphorylated form of histone variant H2AX (gammaH2AX) foci formation assay. RESULTS: After exposure to 1.8 GHz RF for 2 h, HLECs exhibited significant intracellular ROS increase in the 2, 3, and 4 W/kg groups. RF radiation at the SAR of 3 W/kg and 4 W/kg could induce significant DNA damage, examined by alkaline comet assay, which was used to detect mainly single strand breaks (SSBs), while no statistical difference in double strand breaks (DSBs), evaluated by gammaH2AX foci, was found between RF exposure (SAR: 3 and 4 W/kg) and sham exposure groups. When RF was superposed with 2 muT electromagnetic noise could block RF-induced ROS increase and DNA damage. CONCLUSIONS: DNA damage induced by 1.8 GHz radiofrequency field for 2 h, which was mainly SSBs, may be associated with the increased ROS production. Electromagnetic noise could block RF-induced ROS formation and DNA damage.

(E) Ye W, Wang F, Zhang W, Fang N, Zhao W, Wang J. Effect of Mobile Phone Radiation on Cardiovascular Development of Chick Embryo. Anat Histol Embryol. 45(3):197-208, 2016. (VO, LE, GT, DE)

The biological effects on cardiovascular development of chicken embryos were examined after radiation exposure using mobile phone (900 MHz; specific absorption rate ~1.07 W/kg) intermittently 3 h per day during incubation. Samples were selected by morphological and histological methods. The results showed the rate of embryonic mortality and cardiac deformity increased significantly in exposed group ($P < 0.05$). No any histological pathological changes were observed on Day 5-7 (D5-D7) of incubation. A higher distribution of lipid droplets was unexpectedly present in myocardial tissue from the exposure groups on D10-D13. Soon afterwards, myofibril disruption, atrioventricular valve focal necrosis, mitochondria vacuolization and atrial natriuretic peptide (ANP) decrease appeared on D15-D21 of incubation. Comet assay data showed the haemocyte mean tail in the exposed group was significantly larger than that of the control ($P < 0.01$). The arterial vascular wall of exposed group was thicker ($P < 0.05$) than that of the control on D13, which was reversed to normal in later stages. Our findings suggest that long-term exposure of MPR may induce myocardium pathological changes, DNA damage and increased mortality; however, there was little effect on vascular development.

(NE) Yildirim MS, Yildirim A, Zamani AG, Okudan N. Effect of mobile phone station on micronucleus frequency and chromosomal aberrations in human blood cells. Genet Couns. 21(2):243-251, 2010. (HU, LE, GT)

The use of mobile telephones has rapidly increased worldwide as well as the number of mobile phone base stations that lead to rise low level radiofrequency emissions which may in turn have possible harm for human health. The national radiation protection board has published the known effects of radio waves exposure on humans living close to mobile phone base stations. However, several studies have claimed that the base station has detrimental effects on different tissues. In this study, we aimed to evaluate the effects of mobile phone base stations on the micronucleus (MN) frequency and chromosomal aberrations on blood in people who were living around mobile phone base stations and healthy controls. Frequency of MN and chromosomal aberrations in study and control groups was 8.96 +/- 3.51 and 6.97 +/- 1.52 (p: 0.16); 0.36 +/- 0.31 and 0.75 +/- 0.61 (p: 0.07), respectively. Our results show that there was not a significant difference of MN frequency and chromosomal aberrations between the two study groups. The results claim that cellular phones and their base stations do not produce important carcinogenic changes.

(E) Zalata, A., A. Z. El-Samanoudy, D. Shaalan, Y. El-Baiomy, and T. Mostafa. In vitro effect of cell phone radiation on motility, DNA fragmentation and clusterin gene expression of sperm. Int J Fertil Steril. 9(1):129-136, 2015. (VT, AE, GT, GE, RP)

Background: Use of cellular phones that emits radiofrequency electromagnetic field (RF-EMF) has been increased exponentially and became a part of everyday life. This study aimed to investigate the effects of RF-EMF radiation emitted from cellular phones on sperm motility variables, sperm DNA fragmentation and clusterin (CLU) gene expression. Materials and Methods: 124 semen samples were grouped into; normozoospermia (N, n=26), asthenozoospermia (A, n=32), asthenoteratozoospermia (AT, n=31) and oligoasthenoteratozoospermia (OAT, n=35). Semen samples were divided into two aliquots; samples not exposed to cell phone and samples exposed to cell phone radiation (850 MHz, maximum power < 1 watt; SAR 1.46 W/kg at 10 cm distance) for 1 hr. Before and immediately after exposure both aliquots were subjected to assessment of sperm motility, acrosin activity, sperm DNA fragmentation and CLU gene expression. Statistical differences were analyzed using paired t-student test for comparisons where P< 0.05 was set as significant. Results: There was significant decrease in sperm motility, sperm linear velocity, sperm linearity index, sperm acrosin activity and significant increase in sperm DNA fragmentation percent, CLU gene expression and CLU protein levels in the exposed semen samples to RF-EMF compared with non- exposed samples in OAT > AT > A > N groups (P<0.05). Conclusions: Cell phone emissions have a negative impact on exposed sperm motility indices, sperm acrosin activity, sperm DNA fragmentation and CLU gene expression especially in OAT cases.

(NE) Zeni, O., Schiavoni, A. S., Sannino, A., Antolini, A., Forigo, D., Bersani, F. and Scarfi, M. R. Lack of Genotoxic Effects (Micronucleus Induction) in Human Lymphocytes Exposed In Vitro to 900 MHz Electromagnetic Fields. Radiat. Res. 160, 152-158, 2003. (VT, AE, GT)

In the present study, we investigated the induction of genotoxic effects in human peripheral blood lymphocytes after exposure to electromagnetic fields used in mobile communication

systems (frequency 900 MHz). For this purpose, the incidence of micronuclei was evaluated by applying the cytokinesis-block micronucleus assay. Cytotoxicity was also investigated using the cytokinesis-block proliferation index. The experiments were performed on peripheral blood from 20 healthy donors, and several conditions were tested by varying the duration of exposure, the specific absorption rate (SAR), and the signal [continuous-wave (CW) or GSM (Global System of Mobile Communication) modulated signal]. The following exposures were carried out: (1) CW intermittent exposure (SAR = 1.6 W/kg) for 6 min followed by a 3-h pause (14 on/off cycles); (2) GSM signal, intermittent exposure as described in (1); (3) GSM signal, intermittent exposure as described in (1) 24 h before stimulation with phytohemagglutinin (8 on/off cycles); (4) GSM signal, intermittent exposure (SAR = 0.2 W/kg) 1 h per day for 3 days. The SARs were estimated numerically. No statistically significant differences were detected in any case in terms of either micronucleus frequency or cell cycle kinetics.

(NE) Zeni O, Romano M, Perrotta A, Lioi MB, Barbieri R, d'Ambrosio G, Massa R, Scarfi MR. Evaluation of genotoxic effects in human peripheral blood leukocytes following an acute in vitro exposure to 900 MHz radiofrequency fields. Bioelectromagnetics. 26(4):258-265, 2005. (VT, AE, GT)

Human peripheral blood leukocytes from healthy volunteers have been employed to investigate the induction of genotoxic effects following 2 h exposure to 900 MHz radiofrequency radiation. The GSM signal has been studied at specific absorption rates (SAR) of 0.3 and 1 W/kg. The exposures were carried out in a waveguide system under strictly controlled conditions of both dosimetry and temperature. The same temperature conditions (37.0 ± 0.1 degrees C) were realized in a second waveguide, employed to perform sham exposures. The induction of DNA damage was evaluated in leukocytes by applying the alkaline single cell gel electrophoresis (SCGE)/comet assay, while structural chromosome aberrations and sister chromatid exchanges were evaluated in lymphocytes stimulated with phytohemagglutinin. Alterations in kinetics of cell proliferation were determined by calculating the mitotic index. Positive controls were also provided by using methyl methanesulfonate (MMS) for comet assay and mitomycin-C (MMC), for chromosome aberration, or sister chromatid exchange tests. No statistically significant differences were detected in exposed samples in comparison with sham exposed ones for all the parameters investigated. On the contrary, the positive controls gave a statistically significant increase in DNA damage in all cases, as expected. Thus the results obtained in our experimental conditions do not support the hypothesis that 900 MHz radiofrequency field exposure induces DNA damage in human peripheral blood leukocytes in this range of SAR.

(NE) Zeni O, Gallaerano GP, Perrotta A, Romano, Sannino A, Sarti M, et al. Cytogenetic observations in human peripheral blood leukocytes following in vitro exposure to THz radiation: A pilot study. Health Phy. 2007; 92: 349-57. (VT, AE, GT)

Emerging technologies are considering the possible use of Terahertz radiation in different fields ranging from telecommunications to biology and biomedicine. The study of the potential effects of Terahertz radiation on biological systems is therefore an important issue in order to safely develop a variety of applications. This paper describes a pilot study devoted to determine if

Terahertz radiation could induce genotoxic effects in human peripheral blood leukocytes. For this purpose, human whole blood samples from healthy donors were exposed for 20 min to Terahertz radiation. Since, to our knowledge, this is the first study devoted to the evaluation of possible genotoxic effects of such radiation, different electromagnetic conditions were considered. In particular, the frequencies of 120 and 130 GHz were chosen: the first one was tested at a specific absorption rate (SAR) of 0.4 mW g⁻¹, while the second one was tested at SAR levels of 0.24, 1.4, and 2 mW g⁻¹. Chromosomal damage was evaluated by means of the cytokinesis block micronucleus technique, which also gives information on cell cycle kinetics. Moreover, human whole blood samples exposed to 130 GHz at SAR levels of 1.4 and 2 mW g⁻¹ were also tested for primary DNA damage by applying the alkaline comet assay immediately after exposure. The results obtained indicate that THz exposure, in the explored electromagnetic conditions, is not able to induce either genotoxicity or alteration of cell cycle kinetics in human blood cells from healthy subjects.

(NE) Zeni O, Schiavoni A, Perrotta A, Forigo D, Deplano M, Scarfi MR. Evaluation of genotoxic effects in human leukocytes after in vitro exposure to 1950 MHz UMTS radiofrequency field. Bioelectromagnetics 29:177-184, 2008. (VT, AE, GT)

In the present study the third generation wireless technology of the Universal Mobile Telecommunication System (UMTS) signal was investigated for the induction of genotoxic effects in human leukocytes. Peripheral blood from six healthy donors was used and, for each donor, intermittent exposures (6 min RF on, 2 h RF off) at the frequency of 1950 MHz were conducted at a specific absorption rate of 2.2 W/kg. The exposures were performed in a transverse electro magnetic (TEM) cell hosted in an incubator under strictly controlled conditions of temperature and dosimetry. Following long duration intermittent RF exposures (from 24 to 68 h) in different stages of the cell cycle, micronucleus formation was evaluated by applying the cytokinesis block micronucleus assay, which also provides information on cell division kinetics. Primary DNA damage (strand breaks/alkali labile sites) was also investigated following 24 h of intermittent RF exposures, by applying the alkaline single cell gel electrophoresis (SCG)/comet assay. Positive controls were included by treating cell cultures with Mitomycin-C and methylmethanesulfonate for micronucleus and comet assays, respectively. The results obtained indicate that intermittent exposures of human lymphocytes in different stages of cell cycle do not induce either an increase in micronucleated cells, or change in cell cycle kinetics; moreover, 24 h intermittent exposures also fail to affect DNA structure of human leukocytes soon after the exposures, likely indicating that repairable DNA damage was not induced.

(E) Zeni O, Sannino A, Romeo S, Massaa R, Sarti M, Reddy AB, et al. Induction of an adaptive response in human blood lymphocytes exposed to radiofrequency fields: Influence of the universal mobile telecommunication system (UMTS) signal and the specific absorption rate. Mutat Res. 2012a; 747: 29-35. (VT, AE, GT, IX)

The induction of an adaptive response (AR) was examined in human peripheral blood lymphocytes exposed to non-ionizing radiofrequency fields (RF). Cells from nine healthy human volunteers were stimulated for 24h with phytohaemagglutinin and then exposed for 20h to an

adaptive dose (AD) of a 1950MHz RF UMTS (universal mobile telecommunication system) signal used for mobile communications, at different specific absorption rates (SAR) of 1.25, 0.6, 0.3, and 0.15W/kg. This was followed by treatment of the cells at 48h with a challenge dose (CD) of 100ng/ml mitomycin C (MMC). Lymphocytes were collected at the end of the 72h total culture period. The cytokinesis-block method was used to record the frequency of micronuclei (MN) as genotoxicity end-point. When lymphocytes from six donors were pre-exposed to RF at 0.3W/kg SAR and then treated with MMC, these cells showed a significant reduction in the frequency of MN, compared with the cells treated with MMC alone; this result is indicative of induction of AR. The results from our earlier study indicated that lymphocytes that were stimulated for 24h, exposed for 20h to a 900MHz RF GSM (global system for mobile communication) signal at 1.25W/kg SAR and then treated with 100ng/ml MMC, also exhibited AR. These overall data suggest that the induction of AR depends on RF frequency, type of the signal and SAR. Further characterization of RF-induced AR is in progress.

(NE) Zeni O, Sannino A, Sarti M, Romeo S, Massa R, Scarfi MR. Radiofrequency radiation at 1950 MHz (UMTS) does not affect key cellular endpoints in neuron-like PC12 Cells. Bioelectromagnetics. 2012b; 33: 497-507. (VT, AE, GT)

In this study, rat pheochromocytoma (PC12) cells were exposed, as a model of neuron-like cells, to 1950 MHz radiofrequency (RF) radiation with a signal used by the 3G wireless technology of the Universal Mobile Telecommunications System (UMTS) to assess possible adverse effects. RF exposure for 24 h at a specific absorption rate (SAR) of 10 W/kg was carried out in a waveguide system under accurately controlled environmental and dosimetric parameters. DNA integrity, cell viability, and apoptosis were investigated as cellular endpoints relevant for carcinogenesis and other diseases of the central nervous system. Very sensitive biological assays were employed to assess the effects immediately after RF exposure and 24 h later, as demonstrated by the cellular response elicited in PC12 cells using positive control treatments provided for each assay. In our experimental conditions, 24 h of RF exposure at a carrier frequency and modulation scheme typical of a UMTS signal was not able to elicit any effect in the selected cellular endpoints in undifferentiated PC12 cells, despite the application of a higher SAR value than those applied in the majority of the studies reported in the literature.

(E) Zeni O, Romeo S, Sannino A, Palumbo R, Scarfi MR. Evidence of bystander effect induced by radiofrequency radiation in a human neuroblastoma cell line. Environ Res 2021 Feb 26;196:110935. (VT, AE, GT)

In previous studies we demonstrated that radiofrequency (RF) electromagnetic fields (EMF) is able to reduce DNA damage induced by a subsequent treatment with genotoxic agents, resembling the adaptive response, a phenomenon well known in radiobiology. In this study we report on the capability of the culture medium from SH-SY5Y neuroblastoma cells exposed to 1950 MHz to elicit, in recipient non-exposed cells, a reduction of menadione-induced DNA damage ($P < 0.05$; comet assay), indicating the capability of non-ionizing radiation to elicit a bystander effect. A comparable reduction was also detected in cultures directly exposed to the same EMF conditions ($P < 0.05$), confirming the adaptive response. In the same exposure conditions, we also evidenced an increase of heat shock protein 70 (hsp70) in culture medium of

cells exposed to RF with respect to sham exposed ones ($P < 0.05$; western blot analysis), while no differences were detected in the intracellular content of hsp70. On the whole, our results evidence a protective effect of RF against menadione-induced DNA damage in directly and non-directly exposed cells, and suggest hsp70 pathway to be investigated as one of the potential candidate underpinning the interaction between RF exposure and biological systems.

(E) Zhang DY, Xu ZP, Chiang H, Lu DQ, Zeng QL. [Effects of GSM 1800 MHz radiofrequency electromagnetic fields on DNA damage in Chinese hamster lung cells.] Zhonghua Yu Fang Yi XueZaZhi 40:149-152, 2006. [Article in Chinese] (VT, AE, GT)

OBJECTIVE: To study the effects of GSM 1800 MHz radiofrequency electromagnetic fields (RF EMF) on DNA damage in Chinese hamster lung (CHL) cells. METHODS: The cells were intermittently exposed or sham-exposed to GSM 1800 MHz RF EMF (5 minutes on/10 minutes off) at a special absorption rate (SAR) of 3.0 W/kg for 1 hour or 24 hours. Meanwhile, cells exposed to 2-acetaminofluorene, a DNA damage agent, at a final concentration of 20 mg/L for 2 hours were used as positive control. After exposure, cells were fixed by using 4% paraformaldehyde and processed for phosphorylated form of H2AX (gammaH2AX) immunofluorescence measurement. The primary antibody used for immunofluorescence was mouse monoclonal antibody against gammaH2AX and the secondary antibody was fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG. Nuclei were counterstained with 4, 6-diamidino-2-phenylindole (DAPI). The gammaH2AX foci and nuclei were visualized with an Olympus AX70 fluorescent microscope. Image Pro-Plus software was used to count the gammaH2AX foci in each cell. For each exposure condition, at least 50 cells were selected to detect gammaH2AX foci. Cells were classified as positive when more than five foci were detected. The percentage of gammaH2AX foci positive cells was adopted as the index of DNA damage. RESULTS: The percentage of gammaH2AX foci positive cell of 1800 MHz RF EMF exposure for 24 hours (37.9 +/- 8.6)% or 2-acetylaminofluorene exposure (50.9 +/- 9.4)% was significantly higher compared with the sham-exposure (28.0 +/- 8.4)%. However, there was no significant difference between the sham-exposure and RF EMF exposure for 1 hour (31.8 +/- 8.7)%. CONCLUSION: 1800 MHz RF EMF (SAR, 3.0 W/kg) for 24 hours might induce DNA damage in CHL cells.

(E) Zhang MB, He JL, Jin LF, Lu DQ. Study of low-intensity 2450-MHz microwave exposure enhancing the genotoxic effects of mitomycin C using micronucleus test and comet assay in vitro. Biomed Environ Sci 15(4):283-290, 2002. (VT, AE, GT, IX)

OBJECTIVE: To determine the interaction between 2450-MHz microwaves (MW) radiation and mitomycin C (MMC). METHODS: The synergistic genotoxic effects of low-intensity 2450-MHz microwave and MMC on human lymphocytes were studied using single cell gel electrophoresis (SCGE) assay (comet assay) and cytokinesis-blocked micronucleus (CBMN) test in vitro. The whole blood cells from a male donor and a female donor were either only exposed to 2450-MHz microwaves (5.0 mW/cm²) for 2 h or only exposed to MMC (0.0125 microgram/mL, 0.025 microgram/mL and 0.1 microgram/mL) for 24 h; and the samples were exposed to MMC for 24 h after exposure to MW for 2 h. RESULTS: In the comet assay, the comet lengths (29.1 microns and 25.9 microns) of MW were not significantly longer than those (26.3 microns and 24.1

microns) of controls ($P > 0.05$). The comet lengths (57.4 microns, 68.9 microns, 91.4 microns, 150.6 microns, 71.7 microns, 100.1 microns, 145.1 microns) of 4 MMC groups were significantly longer than those of controls ($P < 0.01$). The comet lengths (59.1 microns, 92.3 microns, 124.5 microns, 182.7 microns and 57.4 microns, 85.5 microns, 137.5 microns, 178.3 microns) of 4 MW plus MMC groups were significantly longer than those of controls too ($P < 0.01$). The comet lengths of MW plus MMC groups were significantly longer than those of the corresponding MMC doses ($P < 0.05$ or $P < 0.01$) when the doses of MMC were ≥ 0.025 microgram/mL. In the CBMN, the micronucleated cell (MNC) rates of MW were 5@1000 and 6@1000, which showed no difference compared with those (4@1000 and 4@1000) of controls ($P > 0.05$). The MNC rates of 4 MMC groups were 8@1000, 9@1000, 14@1000, 23@1000 and 8@1000, 8@1000, 16@1000, 30@1000 respectively. When the doses of MMC were ≥ 0.05 microgram/mL, MNC rates of MMC were higher than those of controls ($P < 0.05$). MNC rates of 4 MW plus MMC groups were 12@1000, 13@1000, 20@1000, 32@1000 and 8@1000, 9@1000, 23@1000, 40@1000. When the doses of MMC were ≥ 0.05 microgram/mL, MNC rates of MW plus MMC groups were much higher than those of controls ($P < 0.01$). MNC rates of 4 MW plus MMC groups were not significantly higher than those of the corresponding MMC doses. **CONCLUSION:** The low-intensity 2450-MHz microwave radiation cannot induce DNA and chromosome damage, but can increase DNA damage effect induced by MMC in comet assay.

(E) Zhang SZ, Yao GD, Lu DQ, Chiang H, Xu ZP. [Effect of 1.8 GHz radiofrequency electromagnetic fields on gene expression of rat neurons]. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 26(8):449-452, 2008. [Article in Chinese] (VT, AE, GE, WS)

OBJECTIVE: To investigate the changes of gene expression in rat neuron induced by 1.8 GHz radiofrequency electromagnetic fields (RF EMF) to screen for RF EMF-responsive genes and the effect of different exposure times and modes on the gene expression in neuron. **METHODS:** Total RNA was extracted immediately and purified from the primary culture of neurons after intermittent exposed or sham-exposed to a frequency of 1.8 GHz RF EMF for 24 hours at an average special absorption rate (SAR) of 2 W/kg. Affymetrix Rat Neurobiology U34 array was applied to investigate the changes of gene expression in rat neuron. Differentially expressed genes (Egr-1, Mbp and Plp) were further confirmed by semi-quantitative reverse transcription polymerase chain reaction (RT PCR). The expression levels of Egr-1, Mbp and Plp were observed at different exposure times (6, 24 h) and modes (intermittent and continuous exposure). **RESULTS:** Among 1200 candidate genes, 24 up-regulated and 10 down-regulated genes were found by using Affymetrix microarray suite software 5.0 which are associated with multiple cellular functions (cytoskeleton, signal transduction pathway, metabolism, etc.) after functional classification. Under 24 h and 6 h intermittent exposure, Egr-1 and Plp in experiment groups showed statistic significance ($P < 0.05$) compared with the control groups, while expression of Mbp did not change significantly ($P > 0.05$). After 24 h continuous exposure, Egr-1 and Mbp in experiment groups showed statistic significance ($P < 0.05$) compared with the control group, while expression of Plp did not change significantly ($P > 0.05$). Under the same exposure mode 6 h, expression of all the 3 genes did not change significantly. Different times (6, 24 h) and modes (intermittent and continuous exposure) of exposure exerted remarkable different influences on the expression of Egr-1, Mbp, Plp genes ($P < 0.01$). **CONCLUSION:** The changes of many genes transcription were involved in the effect of 1.8 GHz RF EMF on rat neurons; Down-regulation of

Egr-1 and up-regulation of Mbp, Plp indicated the negative effects of RF EMF on neurons; The effect of RF intermittent exposure on gene expression was more obvious than that of continuous exposure; The effect of 24 h RF exposure (both intermittent and continuous) on gene expression was more obvious than that of 6 h (both intermittent and continuous).

(E) Zhao J, Hu E, Shang S, Wu D, Li P, Zhang P, Tan D, Lu X. Study of the effects of 3.1 THz radiation on the expression of recombinant red fluorescent protein (RFP) in E. coli. Biomed Opt Express. 2020 Jun 22;11(7):3890-3899. doi: 10.1364/BOE.392838. (VO, AE, GE)

In recent years, many studies have been conducted to investigate the non-thermal effects of THz radiation on different organisms, but further studies are needed to fully elucidate the effects, especially on the molecular level. In this study, we explored the effects of at 3.1 THz radiation on protein expression in Escherichia coli (E. coli) using red fluorescent protein as a reporter molecule. After 8 hours of continuous THz irradiation of bacteria on LB (Luria-Bertani) solid plates at an average power of 33 mW/cm² and 10 Hz pulse repetition frequency, we found that the plasmid copy number, protein expression and fluorescence intensity of bacteria from the irradiated area were 3.8-, 2.7-, and 3.3 times higher than in bacteria from the un-irradiated area, respectively. These findings suggest that plasmid replication changed significantly in bacteria exposed to 3.1 THz radiation, resulting in increased protein expression as evidenced by increased fluorescence intensity of the RFP reporter.

(E) Zhao R, Zhang S, Xu Z, Ju L, Lu D, Yao G. Studying gene expression profile of rat neuron exposed to 1800 MHz radiofrequency electromagnetic fields with cDNA microassay. Toxicology 235:167-175, 2007. (VT, AE, GE)

A widespread use of mobile phone (MP) evokes a growing concern for their possible adverse effects on human, especially the brain. Gene expression is a unique way of characterizing how cells and organism adapt to changes in the external environment, so the aim of this investigation was to determine whether 1800 MHz radiofrequency electromagnetic fields (RF EMF) can influence the gene expression of neuron. Affymetrix Rat Neurobiology U34 array was applied to investigate the changes of gene expression in rat neuron after exposed to the pulsed RF EMF at a frequency of 1800 MHz modulated by 217 Hz which is commonly used in MP. Among 1200 candidate genes, 24 up-regulated genes and 10 down-regulated genes were identified after 24-h intermittent exposure at an average special absorption rate (SAR) of 2 W/kg, which are associated with multiple cellular functions (cytoskeleton, signal transduction pathway, metabolism, etc.) after functional classification. The results were further confirmed by quantitative real-time polymerase chain reaction (RT PCR). The present results indicated that the gene expression of rat neuron could be altered by exposure to RF EMF under our experimental conditions.

(E) Zhao TY, Zou SP, Knapp PE. Exposure to cell phone radiation up-regulates apoptosis genes in primary cultures of neurons and astrocytes. Neurosci Lett. 412(1):34-38, 2007. (VT, AE, GE, CS)

The health effects of cell phone radiation exposure are a growing public concern. This study investigated whether expression of genes related to cell death pathways are dysregulated in primary cultured neurons and astrocytes by exposure to a working Global System for Mobile Communication (GSM) cell phone rated at a frequency of 1900MHz. Primary cultures were exposed to cell phone emissions for 2h. We used array analysis and real-time RT-PCR to show up-regulation of caspase-2, caspase-6 and Asc (apoptosis associated speck-like protein containing a card) gene expression in neurons and astrocytes. Up-regulation occurred in both "on" and "stand-by" modes in neurons, but only in "on" mode in astrocytes. Additionally, astrocytes showed up-regulation of the Bax gene. The effects are specific since up-regulation was not seen for other genes associated with apoptosis, such as caspase-9 in either neurons or astrocytes, or Bax in neurons. The results show that even relatively short-term exposure to cell phone radiofrequency emissions can up-regulate elements of apoptotic pathways in cells derived from the brain, and that neurons appear to be more sensitive to this effect than astrocytes.

(NE) Zhijian C, Xiaoxue L, Yezhen L, Deqiang L, Shijie C, Lifen J, Jianlin L, Jiliang H. Influence of 1.8-GHz (GSM) radiofrequency radiation (RFR) on DNA damage and repair induced by X-rays in human leukocytes in vitro. Mutat Res. 677(1-2):100-104, 2009. (VT, AE, GT, IX)

In the present study, the in vitro comet assay was used to determine whether 1.8-GHz radiofrequency radiation (RFR) can influence DNA repair in human leukocytes exposed to X-rays. The specific energy absorption rate (SAR) of 2 W/kg (the current European safety limit) was applied. The leukocytes from four young healthy donors were intermittently exposed to RFR for 24 h (fields on for 5 min, fields off for 10 min), and then irradiated with X-rays at doses of 0.25, 0.5, 1.0 and 2.0 Gy. DNA damage to human leukocytes was detected using the comet assay at 0, 15, 45, 90, 150 and 240 min after exposure to X-rays. Using the comet assay, the percent of DNA in the tail (% tail DNA) served as the indicator of DNA damage; the DNA repair percentage (DRP) served as the indicator of the DNA repair speed. The results demonstrated that (1) the DNA repair speeds of human leukocytes after X-ray exposure exhibited individual differences among the four donors; (2) the intermittent exposures of 1.8-GHz RFR at the SAR of 2 W/kg for 24 h did not directly induce DNA damage or exhibit synergistic effects with X-rays on human leukocytes.

(NE) Zhijian C, Xiaoxue L, Yezhen L, Shijie C, Lifen J, Jianlin L, Deqiang L, Jiliang H. Impact of 1.8-GHz radiofrequency radiation (RFR) on DNA damage and repair induced by doxorubicin in human B-cell lymphoblastoid cells. Mutat Res. 695(1-2):16-21, 2010. (VT, AE, GT, IX)

In the present in vitro study, a comet assay was used to determine whether 1.8-GHz radiofrequency radiation (RFR, SAR of 2W/kg) can influence DNA repair in human B-cell lymphoblastoid cells exposed to doxorubicin (DOX) at the doses of 0microg/ml, 0.05microg/ml, 0.075microg/ml, 0.10microg/ml, 0.15microg/ml and 0.20microg/ml. The combinative exposures to RFR with DOX were divided into five categories. DNA damage was detected at 0h, 6h, 12h, 18h and 24h after exposure to DOX via the comet assay, and the percent of DNA in the tail (% tail DNA) served as the indicator of DNA damage. The results demonstrated that (1) RFR could

not directly induce DNA damage of human B-cell lymphoblastoid cells; (2) DOX could significantly induce DNA damage of human B-cell lymphoblastoid cells with the dose-effect relationship, and there were special repair characteristics of DNA damage induced by DOX; (3) E-E-E type (exposure to RFR for 2h, then simultaneous exposure to RFR and DOX, and exposure to RFR for 6h, 12h, 18h and 24h after exposure to DOX) combinative exposure could obviously influence DNA repair at 6h and 12h after exposure to DOX for four DOX doses (0.075microg/ml, 0.10microg/ml, 0.15microg/ml and 0.20microg/ml) in human B-cell lymphoblastoid cells.

(NE) Ziemann C, Brockmeyer H, Reddy SB, Vijayalaxmi, Prihoda TJ, Kuster N, Tillmann T, Dasenbrock C. Absence of genotoxic potential of 902 MHz (GSM) and 1747 MHz (DCS) wireless communication signals: In vivo two-year bioassay in B6C3F1 mice. Int J Radiat Biol. 85(5):454-464, 2009. (VO, LE, GT)

PURPOSE: The aim of the present investigation was to determine the incidence of micronuclei in peripheral blood erythrocytes of B6C3F1 mice that had been chronically exposed to radiofrequencies (RF) used for mobile communication. **MATERIALS AND METHODS:** 'Ferris wheels' were used to expose tube-restrained male and female mice to simulated environmental RF signals of the Global System for Mobile Communications (GSM, 902 MHz) or Digital Cellular System (DCS, 1747 MHz). RF signals were applied to the mice for 2 hours/day on 5 days/week for two years, at maximal whole-body-averaged specific absorption rates of 0.4, 1.3, and 4.0 W/kg body weight. Concurrent sham-exposed mice, cage controls, and positive controls injected with mitomycin C were included in this investigation. At necropsy, peripheral blood smears were prepared, and coded slides were stained using May-Grunwald-Giemsa or acridine orange. The incidence of micronuclei was recorded for each mouse in 2000 polychromatic and 2000 normochromatic erythrocytes. **RESULTS:** There were no significant differences in the frequency of micronuclei between RF-exposed, sham-exposed, and cage control mice, irrespective of the staining/counting method used. Micronuclei were, however, significantly increased in polychromatic erythrocytes of the positive control mice. **CONCLUSIONS:** In conclusion, the data did not indicate RF-induced genotoxicity in mice after two years of exposure.

(E) Zong C, Ji Y, He Q, Zhu S, Qin F, Tong J, et al. Adaptive response in mice exposed to 900 MHz radiofrequency fields: Bleomycin-induced DNA and oxidative damage/repair. Int J Radiat Biol. 91: 270-276, 2015. (VO, LE, GT, IX, OX, LI)

Purpose: To determine whether mice exposed to radiofrequency fields (RF) and then injected with a radiomimetic drug, bleomycin (BLM), exhibit adaptive response and provide some mechanistic evidence for such response. **Materials and methods:** Adult mice were exposed to 900 MHz RF at 120 μ W/cm² power density for 4 hours/day for 7 days. Immediately after the last exposure, some mice were sacrificed while the others were injected with BLM 4 h later. In each animal: (i) The primary DNA damage and BLM-induced damage as well as its repair kinetics were determined in blood leukocytes; and (ii) the oxidative damage was determined from malondialdehyde (MDA) levels and the antioxidant status was assessed from superoxide dismutase (SOD) levels in plasma, liver and lung tissues. **Results:** There were no indications for increased DNA and oxidative damages in mice exposed to RF alone in contrast to those treated

with BLM alone. Mice exposed to RF+ BLM showed significantly: (a) reduced BLM-induced DNA damage and that remained after each 30, 60, 90, 120 and 150 min repair time, and (b) decreased levels of MDA in plasma and liver, and increased SOD level in the lung.

Conclusions: The overall data suggested that RF exposure was capable of inducing adaptive response and mitigated BLM- induced DNA and oxidative damages by activating certain cellular processes.

(E) Zothansiana, Zosangzuali M, Lalramdinpuii M, Jagetia GC. Impact of radiofrequency radiation on DNA damage and antioxidants in peripheral blood lymphocytes of humans residing in the vicinity of mobile phone base stations. Electromagn Biol Med. 2017;36(3):295-305. (HU, LE, GT, OX)

Radiofrequency radiations (RFRs) emitted by mobile phone base stations have raised concerns on its adverse impact on humans residing in the vicinity of mobile phone base stations. Therefore, the present study was envisaged to evaluate the effect of RFR on the DNA damage and antioxidant status in cultured human peripheral blood lymphocytes (HPBLs) of individuals residing in the vicinity of mobile phone base stations and comparing it with healthy controls. The study groups matched for various demographic data including age, gender, dietary pattern, smoking habit, alcohol consumption, duration of mobile phone use and average daily mobile phone use. The RF power density of the exposed individuals was significantly higher ($p < 0.0001$) when compared to the control group. The HPBLs were cultured and the DNA damage was assessed by cytokinesis blocked micronucleus (MN) assay in the binucleate lymphocytes. The analyses of data from the exposed group (n = 40), residing within a perimeter of 80 m of mobile base stations, showed significantly ($p < 0.0001$) higher frequency of micronuclei when compared to the control group, residing 300 m away from the mobile base station/s. The analysis of various antioxidants in the plasma of exposed individuals revealed a significant attrition in glutathione (GSH) concentration ($p < 0.01$), activities of catalase (CAT) ($p < 0.001$) and superoxide dismutase (SOD) ($p < 0.001$) and rise in lipid peroxidation (LOO) when compared to controls. Multiple linear regression analyses revealed a significant association among reduced GSH concentration ($p < 0.05$), CAT ($p < 0.001$) and SOD ($p < 0.001$) activities and elevated MN frequency ($p < 0.001$) and LOO ($p < 0.001$) with increasing RF power density.

(E) Zotti-Martelli L, Peccatori M, Scarpato R, Migliore L, Induction of micronuclei in human lymphocytes exposed in vitro to microwave radiation. *Mutat Res* 472(1-2):51-58, 2000. (VT, AE, GT)

Increasing applications of electromagnetic fields are of great concern with regard to public health. Several in vitro studies have been conducted to detect effects of microwave exposure on the genetic material leading to negative or questionable results. The micronucleus (MN) assay which is proved to be a useful tool for the detection of radiation exposure-induced cytogenetic damage was used in the present study to investigate the genotoxic effect of microwaves in human peripheral blood lymphocytes in vitro exposed in G(0) to electromagnetic fields with different frequencies (2.45 and 7.7GHz) and power density (10, 20 and

30mW/cm²) for three times (15, 30 and 60min). The results showed for both radiation frequencies an induction of micronuclei as compared to the control cultures at a power density of 30mW/cm² and after an exposure of 30 and 60min. Our study would indicate that microwaves are able to cause cytogenetic damage in human lymphocytes mainly for both high power density and long exposure time.

(E) Zotti-Martelli L, Peccatori M, Maggini V, Ballardini M, Barale R. Individual responsiveness to induction of micronuclei in human lymphocytes after exposure in vitro to 1800-MHz microwave radiation. *Mutat Res.* 582(1-2):42-52, 2005. (VT, AE, GT)

The widespread application of microwaves is of great concern in view of possible consequences for human health. Many in vitro studies have been carried out to detect possible effects on DNA and chromatin structure following exposure to microwave radiation. The aim of this study is to assess the capability of microwaves, at different power densities and exposure times, to induce genotoxic effects as evaluated by the in vitro micronucleus (MN) assay on peripheral blood lymphocytes from nine different healthy donors, and to investigate also the possible inter-individual response variability. Whole blood samples were exposed for 60, 120 and 180 min to continuous microwave radiation with a frequency of 1800 MHz and power densities of 5, 10 and 20 mW/cm². Reproducibility was tested by repeating the experiment 3 months later. Multivariate analysis showed that lymphocyte proliferation indices were significantly different among donors ($p < 0.004$) and between experiments ($p < 0.01$), whereas the applied power density and the exposure time did not have any effect on them. Both spontaneous and induced MN frequencies varied in a highly significant way among donors ($p < 0.009$) and between experiments ($p < 0.002$), and a statistically significant increase of MN, although rather low, was observed dependent on exposure time ($p = 0.0004$) and applied power density ($p = 0.0166$). A considerable decrease in spontaneous and induced MN frequencies was measured in the second experiment. The results show that microwaves are able to induce MN in short-time exposures to medium power density fields. Our data analysis highlights a wide inter-individual variability in the response, which was confirmed to be a characteristic reproducible trait by means of the second experiment.

(NE) Zuo WQ, Hu YJ, Yang Y, Zhao XY, Zhang YY, Kong W, Kong WJ. Sensitivity of spiral ganglion neurons to damage caused by mobile phone electromagnetic radiation will increase in lipopolysaccharide-induced inflammation in vitro model. *J Neuroinflammation.* 12(1):105,2015. (VT, AE, GT, IX)

BACKGROUND: With the increasing popularity of mobile phones, the potential hazards of radiofrequency electromagnetic radiation (RF-EMR) on the auditory system remain unclear. Apart from RF-EMR, humans are also exposed to various physical and chemical factors. We established a lipopolysaccharide (LPS)-induced inflammation in vitro model to investigate whether the possible sensitivity of spiral ganglion neurons to damage caused by mobile phone electromagnetic radiation (at specific absorption rates: 2, 4 W/kg) will increase. METHODS: Spiral ganglion neurons (SGN) were obtained from neonatal (1- to 3-day-old) Sprague Dawley® (SD) rats. After the SGN were treated with different concentrations (0, 20, 40, 50, 100, 200, and 400 µg/ml) of LPS, the Cell Counting Kit-8 (CCK-8) and alkaline comet assay were used to

quantify cellular activity and DNA damage, respectively. The SGN were treated with the moderate LPS concentrations before RF-EMR exposure. After 24 h intermittent exposure at an absorption rate of 2 and 4 W/kg, DNA damage was examined by alkaline comet assay, ultrastructure changes were detected by transmission electron microscopy, and expression of the autophagy markers LC3-II and Beclin1 were examined by immunofluorescence and confocal laser scanning microscopy. Reactive oxygen species (ROS) production was quantified by the dichlorofluorescein-diacetate assay. RESULTS: LPS (100 µg/ml) induced DNA damage and suppressed cellular activity ($P < 0.05$). LPS (40 µg/ml) did not exhibit cellular activity changes or DNA damage ($P > 0.05$); therefore, 40 µg/ml was used to pretreat the concentration before exposure to RF-EMR. RF-EMR could not directly induce DNA damage. However, the 4 W/kg combined with LPS (40 µg/ml) group showed mitochondria vacuoles, karyopyknosis, presence of lysosomes and autophagosome, and increasing expression of LC3-II and Beclin1. The ROS values significantly increased in the 4 W/kg exposure, 4 W/kg combined with LPS (40 µg/ml) exposure, and H₂O₂ groups ($P < 0.05, 0.01$). CONCLUSIONS: Short-term exposure to radiofrequency electromagnetic radiation could not directly induce DNA damage in normal spiral ganglion neurons, but it could cause the changes of cellular ultrastructure at special SAR 4.0 W/kg when cells are in fragile or micro-damaged condition. It seems that the sensitivity of SGN to damage caused by mobile phone electromagnetic radiation will increase in a lipopolysaccharide-induced inflammation in vitro model.

