January 24. 2024

Genetic effects of static and extremely low frequency (ELF) electromagnetic fields

This file contains 1.) the abstracts of studies on genetic effects (genetic damage and gene expression) of exposure to static and ELF EMF; 2.) a table summarizing the effects (more details of the experimental conditions are included; and 3.) a table on studies that reported effects at low flux densities ($\leq$ 0.01 mT).

The literature shows studies on static and ELF EMF genetic effects that reported effects **(E)** = 288 (84%) and no effect **(NE)** = 56 (16%).
Gene expression studies: 'effect' =173 (95%); 'no effect' = 10 (5%)

(E- Effect observed; NE- no effect observed) (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) Abdelhaliem E, Abdalla HM, Bolbol AA, Shehata RS. Assessment of protein and DNA polymorphisms in corn (Zea mays) under the effect of non-ionizing electromagnetic radiation. Caryologia [Internet] 75(4):49-66, 2023. (VO, AE, GT, GE)

Many reports highlight biological responses of crop plants after non-ionizing electromagnetic radiation (EMR) exposure based on the phenotypic and physiological levels. So, this study aimed to estimate genetic alterations in proteins, isozymes, and DNA banding patterns as well as the extent of nuclear DNA damage of economic corn (*Zea mays*) under the stress of EMR using accurate and reliable bioassays like sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), isozymes (Leucine- aminopeptidase, Esterases, Peroxidase, and Catalases), random amplified polymorphic DNA- polymerase chain reaction (RAPD-PCR), and Comet Assay, respectively. SDS-PAGE analysis showed distinct polymorphisms (96.66%) between EMR exposed and non-exposed corn seedlings depending on the number and type of bands, their intensities as well as molecular weight which ranged from (60.27 to 192.35 kDa), gain, and loss of bands. The four isozymes generated varies isozymatic polymorphisms based on relative front, zymogram number, and optical intensities. RAPD analysis generated 85 amplified DNA products with high polymorphism values ranged from 90.91 to 100% based on primers, band type, DNA sizes which ranged from 153 to 1008-bp, lose, gain, and intensity of DNA bands. Comet Assay scored highest extent of loosed DNA from nuclei (DNA damage) reached the value of (tailed ratio 20%) at EMR exposed corn nuclei for 5 days compared to non-exposed nuclei which reached the value of (tailed ratio 3%). This study concluded that each EMR exposure time had unique interaction with proteins, isozymes, and DNA of corn cells exhibiting wide range of genotoxic stress and subsequently, adversely effect on growth and yield of this sensitive crop plants.

(E) Agliassa C, Narayana R, Bertea 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 GA2ox, SOC1, AGL24, LFY, AP1, FD, 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 (F_1 - and F_2 -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) Ahmadi-Zeidabadi, M., Z. Akbarnejad, M. Esmaeeli, Y, Masoumi-Ardakani, L. Mohammadipoor-Ghasemabad, and H. Eskandary. Impact of extremely low-frequency electromagnetic field (100 Hz, 100 G) exposure on human glioblastoma U87 cells during Temozolomide administration. *Electromagn. Biol. Med* 38:198-209, 2019. (VT, AE, GE)

Glioblastoma multiforme (GBM) is a highly malignant brain tumor with an extremely dismal prognosis, a median survival is12 months. Temozolomide (TMZ) is an alkylating agent widely used to treat cancer, 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. Therefore, the present study was carried out to evaluate the potential synergistic effect of 100 µM TMZ and EMF (100 Hz, 100 G) on human glioma cell line U87 U87 cells with four experimental groups (I-IV) were exposed to ELF-EMF and TMZ for 120 and 144 h, as follows: (I) control; (II) ELF-EMF; (III) TMZ; (IV) ELF-PEMFs / TMZ. mRNA expression of genes such as (Nestin,CD133, Notch4 and GFAP) were investigated by Real-time PCR and western blot. We also evaluated, SOD activity, MDA and calcium concentration by ELISA assay. Co-treatment synergistically decreased the expression of Nestin,CD133, and Notch4 and increased the GFAP genes. We also observed an increase in Superoxide dismutase (SOD) activity, Malondialdehyde (MDA) and Ca2+concentration in comparison to controls.TMZ prevents cancer progression not only through the induction of cell death, but also by inducing differentiation in cancer cells. In addition, our data demonstrate ELF-EMF (100 Hz, 100 G) can

significantly enhance the effects of TMZ on human glioblastoma U87 cell. These findings may open new window for future studies.

(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 100μM 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.

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

(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 muT, 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) (60)Cobalt ((60)Co) 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:** <u>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.</u>

(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, 2007.(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, 2007. (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 (ZnCl(2), 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 CdCl (CdCl(2), 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 CdCl (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 JDT, Friedberg EC, Bittencourt-Oliveira MC, Cavalcante-Silva E, Schenberg ACG, Rodrigues TE, Garcia F, Louvison M, Paula CR, Mesa J, Moron MM, Maria DA, Genofre GC. Static electric fields interfere in the viability of cells exposed to ionising radiation. Int. J. Rad. Biol. 85:314-321, 2009. **(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 V · 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 V · 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 anti-body 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 169:84-98, 2021. (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 programed 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 form 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 muT 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) Bertea, 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 [^{18}F]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-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) and a strong static magnetic field (SMF) were evaluated both in isolation and in combination using the γH2AX assay detecting double-strand breaks in lymphocyte DNA. **METHODS:** Thirty-two healthy young volunteers allocated to three study arms were exposed to [^{18}F]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 [^{18}F]FDG within the samples and the time to monitor the γH2AX response. Absorbed doses to lymphocytes delivered in vivo and in vitro were estimated individually for each volunteer exposed to [^{18}F]FDG. γH2AX foci were scored automatically by immunofluorescence microscopy. **RESULTS:** Absorbed doses to lymphocytes exposed over 60 to 120 min to [^{18}F]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 γH2AX 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 [^{18}F]FDG and the SMF that may be of relevance for risk assessment of PET/MRI.

(E) Bułdak RJ, Polaniak R, Bułdak L, Zwirska-Korczala K, Skonieczna M, Monsiol A, Kukla M, Duława-Bułdak 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 pathophysiologic 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 methilene 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, Magazù S. Chromosome aberration in typical biological systems under exposure to low- and high-intensity magnetic fields. Electromagn Biol Med. 39(2):97-108, 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, Mariggiò 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, pregrowth 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 25(6):457-65, 2004. (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 40(1):49-64, 2021. (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 electromagnet 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*, *FN1*, 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) 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 29(28):43102-43113, 2022. (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 reducede 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)-β1 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-β1 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 genotoixcity 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, 2000. (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. 2000. (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 1α, 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 51(11):854-860, 2019. (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 1β (IL-1β), interleukin 2 (IL-2), interferon-γ (INF-γ) and transforming growth factor β (TGF-β) were evaluated, and the level of IL-1β mRNA expression was determined. Results: After ELF-EMF treatment, both IL-1β plasma level and IL-1β 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-1β 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-1β-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 10(5):266, 2020. (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*, *TNFα*, and *TP53*. We found that ELF-EMF significantly increased the expression of *BAX*, *CASP8*, *TNFα*, 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)
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 10(7):1840, 2021. (VT, AE, 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) 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, Lluis M Mir LM. Biological effects of ultrashort electric pulses in a Neuroblastoma cell line: the energy density role. Int J Radiat Biol 98(1):101-121, 2022. (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 42(5):357-370, 2021. (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.

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

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 O ^{6}-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 O^6-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 37(7):433-443, 2016. (AS, CE, GE)

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 μT. After exposure, blood was analyzed for leukocyte numbers (short-term and long-term) and adrenocorticotropic 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 ± lymphocytes. POMC expression and plasma adrenocorticotropic 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 ($P < or = .01$), while PUT decreased after treatment ($P < or = .01$); 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.

Dhiman SK, Galland P. Effects of weak static magnetic fields on the gene expression of seedlings of Arabidopsis thaliana. J Plant Physiol 231:9-18, 2018. (review)

Magnetic-field reception of animals and plants is currently discussed in the framework of a cryptochrome-based radical-pair mechanism. Efforts to unravel magnetoreception in plants suffered historically from several shortcomings, most prominently, the conspicuous absence of detailed stimulus-response relationships. To determine the sensitivity of seedlings of Arabidopsis thaliana to weak static magnetic fields we generated stimulus-response curves between near zero and 188 μT for the transcript levels of the genes rbcl, cab4, pal4 and ef1. The moderate magneto-responsiveness of dark-grown seedlings was greatly enhanced under blue light, and for rbcl and pal4 also under red light. The stimulus-response curves obtained under blue light of constant photon-fluence rate displayed multiple maxima and thus a pattern fundamentally different from that prevalent in plant and animal physiology. A double mutant lacking cryptochromes 1 and 2 displayed altered stimulus-response curves without losing, however, magneto-responsiveness completely. A reversal of the magnetic field direction substantially affected the gene expression and the quantity of CAB-protein (chlorophyll a,b-binding protein). The majority of our results are at variance with the notion of cryptochromes acting as the only magnetic-field sensors. They do not, however, exclude the possibility that cryptochromes participate in the magnetic field reception of Arabidopsis. The findings have the unexpected implication that cryptochrome- and phytochrome-mediated plant responses can be modulated by the strength and the orientation of the local geomagnetic field.

(E) Di G, Xiang J, Dong L, Wu J. Testosterone synthesis in testicular Leydig cells after long-term exposure to a static electric field (SEF). Toxicology 458:152836, 2021. (VO, LE, GE)

China's clean energy and resources are mainly located in the west and north while electric load center is concentrated in the middle and east. Thus, these resources and energy need to be converted into electrical energy in situ and transported to electric load center through ultra-high voltage direct current (UHVDC) transmissions. China has built 25,000 km UHVDC transmission lines of 800 kV and 1100 kV, near which the impact of electric field on health has attracted public attention. Previous studies showed that time-varying electromagnetic field exposure could disturb testosterone secretion. To study the effect of non-time-varying electric field caused by direct current transmission lines on testosterone synthesis, male ICR mice were continually (24 h/d) exposed to static electric field of 56.3 ± 1.4 kV/m. Results showed that on the 3rd day of exposure and on the 7th day after ceasing the exposure of 28 d, serum testosterone level and testicular oxidative stress indicators didn't change significantly. On the 28th day of exposure, serum testosterone levels, testicular glutathione peroxidase (GSH-Px) activity, the mRNA and protein levels of testicular StAR, PBR, CYP11A1 decreased significantly, and testicular

malondialdehyde (MDA) content increased significantly. Meanwhile, electron-dense edges and vacuolation appeared in lipid droplets of Leydig cells. The gap between inner mitochondrial membrane (IMM) and outer mitochondrial membrane (OMM) enlarged, which would cause the swelling of mitochondria, the rupture and deficiency of mitochondrial membranes. Analysis showed that testicular oxidative stress could induce the damage of mitochondrial structure in Leydig cells, which would decrease the rate of cholesterol transport from cytoplasm to mitochondria. Since cholesterol is the necessary precursor of testosterone synthesis, testosterone synthesis was inhibited. The decrease of the mRNA and protein expression levels of StAR and PBR in testes could diminish the cholesterol transported from OMM to IMM. The decrease of the mRNA and protein expression levels of CYP11A1 could reduce the pregnenolone required in testosterone synthesis and inhibit testosterone synthesis consequently.

(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±SEM: 6.10±0.39) compared to the control group (4.45±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±0.21) compared to controls (4.89±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. 29(3):4093-4100, 2022. (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-κB (NF-κB) 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-κB, 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 22(12):6438, 2021. (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, Kozlowska W, Paukszto 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 22(14):7322, 2021. (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 suspectibility of mammals to the genotoxicity potential of ELF-MF.

(E) Erdal ME, Yılmaz SG, Gürgül S, Uzun C, Derici 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 ± 2Vpp 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 (ZnSO4) on the osteogenic differentiation of adipose tissue-derived mesenchymal stem cells (ADSCs) in the presence of EMF as a strategy in osteoporosis therapy. Alkaline phophatase (ALP) activity measurement, calcium assay and expression of several osteoblastic marker genes were examined to assess the effect of ZnSO4 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 ZnSO4, 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 ZnSO4, 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 ZnSO4, 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 μg/ml ZnSO4, 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):585-591, 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 μT) 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 observedrat 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 μT) 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.

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. 68(6):70-74, 2020. (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.

(E) Franczak A, Waszkiewicz EM, Kozlowska W, Zmijewska A, Koziorowska A. Consequences of electromagnetic field (EMF) radiation during early pregnancy - androgen synthesis and release from the myometrium of pigs in vitro. Anim Reprod Sci 218:106465, 2020. (VT, AE, GE)

An electromagnetic field (EMF) has been found to affect reproductive processes in females. The aim of this study was to determine the effect of low, non-ionizing EMF radiation on the steroidogenic activity of myometrium collected from pigs during the fetal peri-implantation period. Myometrial slices were treated with an EMF (50 and 120 Hz, 2 and 4 h of incubation) and examined for the aromatase cytochrome P450 17α-hydroxylase/C17-20lyase (CYP17A1) and 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase (HSD3B1) mRNA transcript abundance, cytochrome P450c17 and 3βHSD protein abundance and the secretion of androstenedione (A4) and testosterone (T). To determine whether progesterone (P4) functions as a protectant from EMF radiation, the selected slices were treated with P4. In slices incubated without P4, EMF at 50 Hz altered cytochrome P450c17 protein abundance (4 h), HSD3B1 mRNA transcript abundance (4 h) and A4 release (2 h) as well as T release (2 h) in P4-treated slices. The EMF at 120 Hz in non P4-treated slices altered A4 release (2 and 4 h) whereas in P4-treated slices altered CYP17A1 mRNA transcript abundance (4 h), 3βHSD protein abundance (4 h), A4 (4 h) and T release (2 h). In conclusion, EMF radiation in the myometrium collected during the peri-implantation period alters the CYP17A1 and HSD3B1 mRNA transcript and encoded protein abundance, and androgen release due to the time of treatment and P4 presence or absence. The P4 did not function directly as an obvious protector against EMF radiation in the myometrium of pigs during the fetal peri-implantation period.

(E)

Franczak A, Drzewiecka EM, Kozlowska W, Zmijewska A, Wydorski PJ, Koziorowska A. The effect of electromagnetic field (EMF) exposure on synthesis and release of steroid hormones by the porcine conceptuses during the peri-implantation period. Reprod Fertil Dev 34(10):722-735, 2022. (VT, AE, GE)

Context: Electrical devices and power systems are the sources of EM-waves which propagate everywhere in the environment. **Aims:** The study aimed to determine whether EMF induced changes in the steroidogenesis of conceptuses and whether progesterone (P4) may be a possible protectant against the effects of EMF radiation. **Methods:** The entire porcine conceptuses were collected during the peri-implantation period (days 15-16 of pregnancy), divided into fragments (100mg) and treated in vitro with EMF (50Hz or 120Hz, 2 or 4h exposure), and examined to determine of CYP17A1 , HSD3B1 , CYP19A3 , and HSD17B4 mRNA transcript and encoded protein abundance and the release of steroid hormones. Selected fragments of conceptuses were treated with P4 . **Key results:** In conceptuses incubated without P4 , EMF at 120Hz decreased androstenedione (A4) and testosterone (T) release after 2h and increased oestrone (E1) release at 50Hz and 120Hz after 4h exposure. In P4 -treated conceptuses, EMF (50 and 120Hz, 4h exposure) decreased CYP19A3 mRNA transcript abundance, and increased (120Hz, 2h exposure) oestradiol-17β (E2) release. **Conclusions:** The EMF radiation alters androgen and oestrogen synthesis and release from the conceptuses of pigs during the peri-implantation period. The P4 exerts protective effects on androgens and E1 release but it sensitises the conceptuses when comes to the mechanism of oestrogen synthesis and release during EMF radiation. **Implications:** The effect of EMF radiation on the steroidogenic pathway in conceptuses may induce disturbances in their proper development and implantation.

(E) Franczak A, Drzewiecka EM, Kozlowska W, Zmijewska A, Wydorski PJ.

Extremely low-frequency electromagnetic field (ELF-EMF) induces alterations in epigenetic regulation in the myometrium - An in vitro study. Theriogenology 200:136-146, 2023. (VT, AE, GE, EP)

Previous research by the authors indicated that an extremely low-frequency electromagnetic field (ELF-EMF) evokes molecular alterations in the porcine myometrium. It was hypothesized that the ELF-EMF could induce alterations in the epigenetic regulation of gene expression in the myometrium. In the current study, slices of the porcine myometrium during the peri-implantation period (n = 4) were used for further in vitro exposition to ELF-EMF (50 Hz, 8 mT, 2 h treatment duration). The study tested whether the ELF-EMF may affect: 1/the expression of DNA (cytosine-5)-methyltransferase 1 (DNMT1) and DNA (cytosine-5)-methyltransferase 3a (DNMT3a), 2/the level of genomic DNA methylation, and 3/the level of amplification of methylated and unmethylated variants of promoter regions of selected genes with altered expression in response to ELF-EMF. It was found that ELF-EMF treatment increased DNMT1, decreased DNMT3a mRNA transcript and protein abundance, and increased the level of genomic DNA methylation. The direction of alterations in the level of amplification of methylated and unmethylated variants of the promoter region of selected genes with altered expression, i.e. prodynorphin (PDYN), interleukin 15 (IL15) signal transducer and activator of transcription 5A (STAT5A), tumor necrosis factor (TNF), and between down-regulated genes were early growth response 2 (EGR2), hyaluronan and proteoglycan link protein 1 (HAPLN1), and uteroferrin associated basic protein-2 (UABP2), mostly involving the direction of changes in their transcriptional activity, which was evaluated in a previous study by the authors. Thus, ELF-EMF radiation disturbs epigenetic mechanisms, which may underlay ELF-EMF-related transcriptomic alterations in the myometrium.

(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 ^{60}Co 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) Gholamian-Hamadan M, Behzad M, Molaei S, Ghane ZZ, Talebi-Ghane E, Zamani A. Effect of 50-Hz magnetic fields on the expression of activation-induced deaminase, B-cell lymphoma 6 and serum levels of interleukin-6, interleukin-21. Int J Radiat Biol 99(9):1456-1462, 2023. (VO, LE, GE)

Background: Investigations showed different effects of magnetic fields (MFs) on the immune system. During humoral immune responses, genes of activation-induced deaminase (AID) and B-cell lymphoma-6 (Bcl-6) are expressed and interleukin (IL)-6 and IL-21 are produced. These factors play significant roles in class switching, affinity maturation of antibodies and activations of B cells germinal centers (GCs). Therefore, this study investigated the effect of 50-Hz MFs exposure with different densities on these factors. **Materials and methods:** Eighty rats were divided into four exposures and control groups. The treatment groups were exposed to magnetic flux densities of 1, 100, 500, and 2000 µT (50 Hz, 2 h/d for 60 d). To activation of the immune system, all the animals were immunized with human serum albumin on days 31, 44, and 58 of exposure. Reverse transcription-quantitative polymerase chain reaction was used to assay the expression levels of AID and Bcl-6 genes in the spleen. The serum levels of IL-6 and IL-21 were also detected by enzyme-linked immunosorbent assay at the pre-and post-immunization phases. **Results:** AID expression was significantly declined at 1µT magnetic flux density, while no change was observed in the expression of Bcl-6. Serum IL-6 was increased only in the 500 µT group at the post-immunization phase. **Conclusions:** It seems exposure to 50-Hz MFs at 1 µT density, suppresses AID and may cause a decline in class switching and affinity maturation of Abs. On the other hand, exposure to 500 µT, may activate them. These findings demonstrate the various potential effects of MFs on the humoral immune system.

(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 H_2O_2 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 386(1):1-15, 2021. (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) Guo, Y., FU, Y, Sun W. 50 Hz Magnetic Field Exposure Inhibited Spontaneous Movement of Zebrafish Larvae through ROS-Mediated syn2a Expression. Int. J. Mol. Sci. 24(8): 7576, 2023. (VO, LE, GE, OX)

Extremely low frequency electromagnetic field (ELF-EMF) exists widely in public and occupational environments. However, its potential adverse effects and the underlying mechanism on nervous system, especially behavior are still poorly understood. In this study, zebrafish embryos (including a transfected synapsin IIa (syn2a) overexpression plasmid) at 3 h post-fertilization (hpf) were exposed to a 50-Hz magnetic field (MF) with a series of intensities (100, 200, 400 and 800 μT, respectively) for 1 h or 24 h every day for 5 days. Results showed that, although MF exposure did not affect the basic development parameters including hatching rate, mortality and malformation rate, yet MF at 200 μT could significantly induce spontaneous movement (SM) hypoactivity in zebrafish larvae. Histological examination presented morphological abnormalities of the brain such as condensed cell nucleus and cytoplasm, increased intercellular space. Moreover, exposure to MF at 200 μT inhibited syn2a transcription

and expression, and increased reactive oxygen species (ROS) level as well. Overexpression of syn2a could effectively rescue MF-induced SM hypoactivity in zebrafish. Pretreatment with N-acetyl-L-cysteine (NAC) could not only recover syn2a protein expression which was weakened by MF exposure, but also abolish MF-induced SM hypoactivity. However, syn2a overexpression did not affect MF-increased ROS. Taken together, the findings suggested that exposure to a 50-Hz MF inhibited spontaneous movement of zebrafish larvae via ROS-mediated syn2a expression in a nonlinear manner.

(E)
Han Q, Chen R, Wang F, Chen S, Sun X, Guan X, Yang Y, Peng B, Pan X, Li J, Yi W, L i 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 106:194-203, 2018. (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) 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 μT 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) Heredia-Rojas, J.A., Beltcheva, M., Rodríguez de la Fuente, A.O., Gómez-Flores, R., Metcheva, R., Cantú-Martínez, P.C. & Heredia-Rodríguez, O. Evidence of Radioprotective Effect of Resveratrol against Clastogenic Effect of Extremely Low-Frequency Electromagnetic Fields. Acta Zoologica Bulgarica, Supplement 15, 49-54, 2020. (VO, AE, GT)

The living organisms have never before in its evolutionary history been exposed to electromagnetic radiation , especially to extremely low-frequency electromagnetic fields (ELF-EMFs) that are ubiquitous in the modern environment. There are some investigations that suggest that such fields have detrimental effects on cells. On the other hand, there have been many attempts to develop radioprotective agents. In the present study, BALB/c mice were exposed to 2.0 mT ELF-EMFs at 60 Hz frequency for 72 h, in the presence or absence of resveratrol (15 mg/kg), using sham-exposed mice and saline solution as negative controls, after which clastogenic effects were assessed by the micronucleus (MN) test. Resveratrol was shown to significantly ($p<0.05$) reduce ELF-EMFs-induced clastogenic effect on mice bone marrow MN. These findings suggest a potential use of resveratrol for radioprotection.

(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) Huang Z, Ito M, Zhang S, Toda T, Takeda J-I, Ogi T, Ohno K. Extremely low-frequency electromagnetic field induces acetylation of heat shock proteins and enhances protein folding. Ecotoxicol Environ Saf 264:115482, 2023. (VT, AE, GE)

The pervasive weak electromagnetic fields (EMF) inundate the industrialized society, but the biological effects of EMF as weak as 10 μT have been scarcely analyzed. Heat shock proteins (HSPs) are molecular chaperones that mediate a sequential stress response. HSP70 and HSP90 provide cells under undesirable situations with either assisting covalent folding of proteins or degrading improperly folded proteins in an ATP-dependent manner. Here we examined the effect of extremely low-frequency (ELF)-EMF on AML12 and HEK293 cells. Although the protein expression levels of HSP70 and HSP90 were reduced after an exposure to ELF-EMF for 3 h, acetylations of HSP70 and HSP90 were increased, which was followed by an enhanced binding affinities of HSP70 and HSP90 for HSP70/HSP90-organizing protein (HOP/STIP1). After 3 h exposure to ELF-EMF, the amount of mitochondria was reduced but the ATP level and the maximal mitochondrial oxygen consumption were increased, which was followed by the reduced protein aggregates and the increased cell viability. Thus, ELF-EMF exposure for 3 h activated acetylation of HSPs to enhance protein folding, which was returned to the basal level at 12 h. The proteostatic effects of ELF-EMF will be able to be applied to treat pathological states in humans.

(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 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) Hwang J, Jung H, Kim KM, Jeong D, Lee J, Hong J-H, Jang WY. Regulation of myogenesis and adipogenesis by the electromagnetic perceptive gene. Sci Rep 13(1):21167, 2023. (VT, AE, GE)

Obesity has been increasing in many regions of the world, including Europe, USA, and Korea. To manage obesity, we should consider it as a disease and apply therapeutic methods for its treatment. Molecular and therapeutic approaches for obesity management involve regulating biomolecules such as DNA, RNA, and protein in adipose-derived stem cells to prevent to be fat cells. Multiple factors are believed to play a role in fat differentiation, with one of the most effective factor is Ca2+. We recently reported that the electromagnetic perceptive gene (EPG) regulated intracellular Ca2+ levels under various electromagnetic fields. This study aimed to investigate whether EPG could serve as a therapeutic method against obesity. We confirmed that EPG serves as a modulator of Ca2+ levels in primary adipose cells, thereby regulating several genes such as CasR, PPARγ, GLU4, GAPDH during the adipogenesis. In addition, this study also identified EPG-mediated regulation of myogenesis that myocyte transcription factors (CasR, MyoG, MyoD, Myomaker) were changed in C2C12 cells and satellite cells. In vivo experiments carried out in this study confirmed that total weight/ fat/fat accumulation were decreased and lean mass was increased by EPG with magnetic field depending on age of mice. The EPG could serve as a potent therapeutic agent against obesity.

See also:

Krishnan V, Park SA, Shin SS, Alon L, Tressler CM, Stokes W, Banerjee J, Sorrell ME, Tian Y, Fridman GY, Celnik P, Pevsner J, Guggino WB, Gilad AA, Pelled G. Wireless control of cellular function by activation of a novel protein responsive to electromagnetic fields. Sci Rep 8(1):8764, 2018.

The Kryptopterus bicirrhis (glass catfish) is known to respond to electromagnetic fields (EMF). Here we tested its avoidance behavior in response to static and alternating magnetic fields stimulation. Using expression cloning we identified an electromagnetic perceptive gene (EPG) from the K. bicirrhis encoding a protein that responds to EMF. This EPG gene was cloned and expressed in mammalian cells, neuronal cultures and in rat's brain. Immunohistochemistry showed that the expression of EPG is confined to the mammalian cell membrane. Calcium imaging in mammalian cells and cultured neurons expressing EPG demonstrated that remote activation by EMF significantly increases intracellular calcium concentrations, indicative of cellular excitability. Moreover, wireless magnetic activation of EPG in rat motor cortex induced motor evoked responses of the contralateral forelimb in vivo. Here we report on the development of a new technology for remote, non-invasive modulation of cell function.

Hwang J, Choi Y, Lee K, Krishnan V, Pelled G, Gilad AA, Choi J. Regulation of Electromagnetic Perceptive Gene Using Ferromagnetic Particles for the External Control of Calcium Ion Transport. Biomolecules 10(2):308, 2022.

Developing synthetic biological devices to allow the noninvasive control of cell fate and function, in vivo can potentially revolutionize the field of regenerative medicine. To address this unmet need, we designed an artificial biological "switch" that consists of two parts: (1) the electromagnetic perceptive gene (EPG) and (2) magnetic particles. Our group has recently cloned the EPG from the Kryptopterus bicirrhis (glass catfish). The EPG gene encodes a putative membrane-associated protein that responds to electromagnetic fields (EMFs). This gene's primary mechanism of action is to raise the intracellular calcium levels or change in flux through EMF stimulation. Here, we developed a system for the remote regulation of [Ca2+]i (i.e., intracellular calcium ion concentration) using streptavidin-coated ferromagnetic particles (FMPs) under a magnetic field. The results demonstrated that the EPG-FMPs can be used as a molecular calcium switch to express target proteins. This technology has the potential for controlled gene expression, drug delivery, and drug developments.

(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, 2003. **(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 mu 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 mu 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, 2003. (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 ELFEMF.

(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 22(11):5785, 2021. (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 Ca^{2+} 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 Ca^{2+} 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 43(9):995-1001, 2021. (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, γH2AX 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), H(2)O(2) (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, H(2)O(2), and c-Myc activation. **CONCLUSIONS:** Our results demonstrate that ELF-MF did not enhance MN frequency by IR, H(2)O(2) 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), H_2O_2 (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, H_2O_2, 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 inhanced 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 untreatment 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) Karimi A, Ghadiri-Moghaddam F, Valipour M, Yahyavi Y. Effects of prolonged exposure to ELF-EMF on HERVs expression in human melanoma cells. Mol Biol Res Commun 11(2):67-71, 2022. (VT, AE, GE)

The Human endogenous retroviruses (HERVs) are ancient remnants of exogenous retroviral infections. Their abnormal activation is associated with several diseases, such as cancer and autoimmunity. Epigenetic and environmental factors are probably playing essential roles in the expression of these elements. This study aimed to examine the 96-hour effects of ELF-EMF on HERV-H, K, and W expression in human melanoma cells. SK-MEL-37 cells (the human skin malignant melanoma) were continuously exposed to ELF-EMF (50 Hz) at 1.5 and 3 mT intensity for 96 hours. Following mRNA extraction, the expression level of HERV-H, K, and W was assessed by qPCR. According to our results, exposure to ELF-EMF intensities for 96 hours could significantly downregulate HERV-H, K, and W env gene expression ($P<0.001$). Our obtained data suggest that low intensity and long-term exposure to ELF-EMF may pave using this type of radiation as a novel therapeutic approach by neutralizing the HERVs upregulated expression in melanoma cells.

(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 μT (magnitude) 4 h/d for 30 consecutive days. Before and after the exposure, VWM test was conducted using a coated devise on a movable stand. About 10 mL of the animals' blood was obtained from their femoral vain 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) Kazemi M, H. Aliyari, S. Golabi, E. Tekieh, H. Tavakoli, M. Saberi, H. Sahraei. Improvement of Cognitive Indicators in Male Monkeys Exposed to Extremely Low-Frequency Electromagnetic Fields. Arch Razi Inst 77(1):503-511, 2022. (VO, LE, LI, GE)

Today, the production of extremely low-frequency electromagnetic fields (ELF-EMFs) has significantly increased. This study aimed to investigate the effect of the ELF-EMFs on the structure and function of the brain in male rhesus monkeys in terms of visual learning (VL), visual memory (VM), and visual working memory (VWM). To conduct the study, four monkeys were selected, of whom two monkeys were irradiated by 12-Hz ELF-EMFs with a magnitude of 0.7 microtesla, and two monkeys were tested without irradiation (control group). A blood sample was taken in three stages, namely pre- and post-irradiated and the recovery phases. Changes in the plasma levels of sodium, potassium, and adrenocorticotropic hormone (ACTH) were evaluated. Moreover, gene expression of N-methyl-D-aspartate (NMDA) receptors was assessed. The anatomical change of the brain's prefrontal area was measured by magnetic resonance imaging and Digital Imaging and Communications in Medicine LiteBox file. The abilities of VL, VM, and VWM significantly improved after the irradiation. Furthermore, the expression of the NMDA receptors gene and the plasma levels of sodium, potassium, and ACTH significantly enhanced after the irradiation. However, the prefrontal area was not significantly affected by the irradiation. No significant differences were observed in any of the studied factors in the control group. Our findings suggested that ELF-EMFs irradiation at 12 Hz positively affected VL and VWM. Consequently, 12-Hz ELF-EMFs irradiations can be widely applied to improve cognitive abilities in monkeys.

(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, 100μT 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 µT) than those generally found in the environment. Effects of 10 and 30 µT 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 µT. 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 µT. The results indicate that the threshold for biological effects of ELF MFs is 10 µT 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-3β/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-3β/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 Wnt3α expression and GSK-3β/ERK/Akt phosphorylation. Our results confirmed that ELF-EMFs enhance hDPC activation and proliferation via the GSK-3β/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. 55(10):1141-1148, 2020. (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 23(7):3859, 2022. (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 ± 7.1 μT for 2 h or to a sham exposure (0.21 ± 0.05 μT) 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 ± 0.01 μT) 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) Kitaoka, K., M. Kitamura, S. Aoi, N. Shimizu, and K. Yoshizaki. Chronic exposure to an extremely low-frequency magnetic field induces depression-like behavior and corticosterone secretion without enhancement of the hypothalamic-pituitary-adrenal axis in mice. *Bioelectromagnetic* 34:43-51, 2013. (VO, LE, GE)

An extremely low-frequency magnetic field (ELF-MF) is generated by power lines and household electrical devices. Many studies have suggested an association between chronic ELF-MF exposure and anxiety and/or depression. The mechanism of these effects is assumed to be a stress response induced by ELF-MF exposure. However, this mechanism remains controversial. In the present study, we investigated whether chronic ELF-MF exposure (intensity, 1.5 mT; [corrected] total exposure, 200 h) affected emotional behavior and corticosterone synthesis in mice. ELF-MF-treated mice showed a significant increase in total immobility time in a forced swim test and showed latency to enter the light box in a light-dark transition test, compared with sham-treated (control) mice. Corticosterone secretion was significantly high in the ELF-MF-

exposed mice; however, no changes were observed in the amount of the adrenocorticotropic hormone and the expression of genes related to stress response. Quantification of the mRNA levels of adrenal corticosteroid synthesis enzymes revealed a significant reduction in Cyp17a1 mRNA in the ELF-MF-exposed mice. Our findings suggest the possibility that high intensity and chronic exposure to ELF-MF induces an increase in corticosterone secretion, along with depression- and/or anxiety-like behavior, without enhancement of the hypothalamic-pituitary-adrenal axis.

.

(E) Kostyn K, Boba A, Kozak B, Sztafrowski D, Widuła J, Szopa J, Preisner M. Transcriptome profiling of flax plants exposed to a low-frequency alternating electromagnetic field. Front Genet 14:1205469, 2023. (VO, AE, GE)

All living organisms on Earth evolved in the presence of an electromagnetic field (EMF), adapted to the environment of EMF, and even learned to utilize it for their purposes. However, during the last century, the Earth's core lost its exclusivity, and many EMF sources appeared due to the development of electricity and electronics. Previous research suggested that the EMF led to changes in intercellular free radical homeostasis and further altered the expression of genes involved in plant response to environmental stresses, inorganic ion transport, and cell wall constituent biosynthesis. Later, CTCT sequence motifs in gene promoters were proposed to be responsible for the response to EMF. How these motifs or different mechanisms are involved in the plant reaction to external EMF remains unknown. Moreover, as many genes activated under EMF treatment do not have the CTCT repeats in their promoters, we aimed to determine the transcription profile of a plant exposed to an EMF and identify the genes that are directly involved in response to the treatment to find the common denominator of the observed changes in the plant transcriptome.

(E) Koyama S, Sakurai T, Nakahara T, Miyakoshi J. Extremely low frequency (ELF) magnetic fields enhance chemically induced formation of apurinic/apyrimidinic (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/apyrimidinic (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) Koziorowska A, Depciuch J, Białek J, Woś I, Kozioł 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 23(4):537-544, 2020. (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)
Kozlowska W, Drzewiecka EM, Paukszto 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 72(6), 2021. (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) 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. 12(12):e0188880. 2017. (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 μT 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 μT 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 μT 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 μT 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.

(E)
Kwiatkowski P, Tabiś A, Fijałkowski K, Masiuk H, Łopusiewicz L, Pruss A, Sienkiewicz M, Wardach M, Kurzawski M, Guenther S, Bania J, Dołęgowska B, I. Regulatory and Enterotoxin Gene Expression and Enterotoxins Production in *Staphylococcus aureus* FRI913 Cultures Exposed to a Rotating Magnetic Field and *trans*-Anethole. Int J Mol Sci 23(11):6327, 2022. (VT, AE, GE)

The study aimed to examine the influence of a rotating magnetic field (RMF) of two different frequencies (5 and 50 Hz) on the expression of regulatory (*agrA*, *hld*, *rot*) and staphylococcal enterotoxin (SE-*sea*, *sec*, *sel*) genes as well as the production of SEs (SEA, SEC, SEL) by the *Staphylococcus aureus* FRI913 strain cultured on a medium supplemented with a subinhibitory concentration of *trans*-anethole (TA). Furthermore, a theoretical model of interactions between the bacterial medium and bacterial cells exposed to RMF was proposed.

Gene expression and SEs production were measured using quantitative real-time PCR and ELISA techniques, respectively. Based on the obtained results, it was found that there were no significant differences in the expression of regulatory and SE genes in bacteria simultaneously cultured on a medium supplemented with TA and exposed to RMF at the same time in comparison to the control (unexposed to TA and RMF). In contrast, when the bacteria were cultured on a medium supplemented with TA but were not exposed to RMF or when they were exposed to RMF of 50 Hz (but not to TA), a significant increase in *agrA* and *sea* transcripts as compared to the unexposed control was found. Moreover, the decreased level of *sec* transcripts in bacteria cultured without TA but exposed to RMF of 50 Hz was also revealed. In turn, a significant increase in SEA and decrease in SEC and SEL production was observed in bacteria cultured on a medium supplemented with TA and simultaneously exposed to RMFs. It can be concluded, that depending on SE and regulatory genes expression as well as production of SEs, the effect exerted by the RMF and TA may be positive (i.e., manifests as the increase in SEs and/or regulatory gene expression of SEs production) or negative (i.e., manifests as the reduction in both aforementioned features) or none.

(NE) Lacy-Hulbert, A, Wilkins, R.C., Hesketh, T.R., Metcalfe, J.C. No effect of 60 Hz electromagnetic fields on MYC or beta-actin expression in human leukemic cells. Rad. Res. 144:9-17, 1995. (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, 1997. (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-phenylnitrone block 60-Hz magnetic field-induced DNA single and double strand breaks in rat brain cells. J Pineal Res. 22(3):152-162, 1997. (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-phenylnitrone (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 singleand 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-phenylnitrone, 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) Lazzarini R, Eléxpuru-Zabaleta M, Piva F, Giulietti M, Fulgenzi G, Tartaglione MF, Laura Zingaretti L, Tagliabracci A, Valentino M, Santarelli L, Bracci M. Effects of extremely low-frequency magnetic fields on human MDA-MB-231 breast cancer cells: proteomic characterization. Ecotoxicol Environ Saf 253:114650, 2023. (VT, AE, GE, CS)

Extremely low-frequency electromagnetic fields (ELF-MF) can modify the cell viability and regulatory processes of some cell types, including breast cancer cells. Breast cancer is a multifactorial disease where a role for ELF-MF cannot be excluded. ELF-MF may influence the biological properties of breast cells through molecular mechanisms and signaling pathways that are still unclear. This study analyzed the changes in the cell viability, cellular morphology, oxidative stress response and alteration of proteomic profile in breast cancer cells (MDA-MB-231) exposed to ELF-MF (50 Hz, 1 mT for 4 h). Non-tumorigenic human breast cells (MCF-10A) were used as control cells. Exposed MDA-MB-231 breast cancer cells increased their viability and live cell number and showed a higher density and length of filopodia compared with the unexposed cells. In addition, ELF-MF induced an increase of the mitochondrial ROS levels and an alteration of mitochondrial morphology. Proteomic data analysis showed that ELF-MF altered the expression of 328 proteins in MDA-MB-231 cells and of 242 proteins in MCF-10A cells. Gene Ontology term enrichment analysis demonstrated that in both cell lines ELF-MF exposure up-regulated the genes enriched in "focal adhesion" and "mitochondrion". The ELF-MF exposure decreased the adhesive properties of MDA-MB-231 cells and increased the migration and invasion cell abilities. At the same time, proteomic analysis, confirmed by Real Time PCR, revealed that transcription factors associated with cellular reprogramming were upregulated in MDA-MB-231 cells and downregulated in MCF-10A cells after ELF-MF exposure. MDA-MB-231 breast cancer cells exposed to 1 mT 50 Hz ELF-MF showed modifications in proteomic profile together with changes in cell viability, cellular morphology, oxidative stress response, adhesion, migration and invasion cell abilities. The main signaling pathways involved were relative to focal adhesion, mitochondrion and cellular reprogramming.

(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 μT 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. Directional migration and transcriptional analysis of oligodendrocyte precursors subjected to stimulation of electrical signal. Am J Physiol Cell Physiol 309(8):C532-540, 2015. (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) Li Y, Sun C, Zhou H, Huang H, Chen Y, Duan X, Huang S, Li J. Extremely Low-Frequency Electromagnetic Field Impairs the Development of Honeybee (*Apis cerana*). Animals (Basel) 12(18):2420, 2022. (VO, LE, GE)

Increasing ELF-EMF pollution in the surrounding environment could impair the cognition and learning ability of honeybees, posing a threat to the honeybee population and its pollination ability. In a social honeybee colony, the numbers of adult bees rely on the successful large-scale rearing of larvae and continuous eclosion of new adult bees. However, no studies exist on the influence of ELF-EMFs on honeybee larvae. Therefore, we investigated the survival rate, body weight, and developmental duration of first instar larvae continuously subjected to ELF-EMF exposure. Moreover, the transcriptome of fifth instar larvae were sequenced for analyzing the difference in expressed genes. The results showed that ELF-EMF exposure decreases the survival rate and body weight of both white-eye pupae and newly emerged adults, extends the duration of development time and seriously interferes with the process of metamorphosis and pupation. The transcriptome sequencing showed that ELF-EMF exposure decreases the nutrient and energy metabolism and impedes the degradation of larvae tissues and rebuilding of pupae tissues in the metamorphosis process. The results provide an experimental basis and a new perspective for the protection of honeybee populations from ELF-EMF pollution.

(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 (8microT 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 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, Lu S, Liu S, Dong C, Chen Y, Xiao L, Zong Y, Zhang H, Liao A. 11.4 T ultra-high static magnetic field has no effect on morphology but induces upregulation of TNF signaling pathway based on transcriptome analysis in zebrafish embryos. Ecotoxicol Environ Saf 255:114754, 2023. (VO, AE, GE)

As magnetic resonance imaging (MRI) scanners with ultra-high field (UHF) have optimal performance, scientists have been working to develop high-performance devices with strong magnetic fields to improve their diagnostic potential. However, whether an MRI scanner with UHF poses a risk to the safety of the organism require further evaluation. This study evaluated the effects of 11.4 Tesla (T) UHF on embryonic development using a zebrafish model. Multiple approaches, including morphological parameters, physiological behaviors, and analyses of the transcriptome at the molecular level, were determined during 5 days after laboratory-controlled exposure from 6 hour post fertilization (hpf) to 24 hpf. No significant effects were observed in embryo mortality, hatching rate, body length, Left-Right patterning, locomotor behavior, etc. RNA-sequencing analysis revealed up-regulated tumor necrosis factor (TNF) inflammatory factors and activated TNF signaling pathways in the 11.4 T exposure group. The results were further validated using qPCR. Our findings indicate that although UHF exposure under 11.4 T has no effect on the development of zebrafish embryos, it has specific effects on the immune response that require further investigation.

(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.

(E) Liu Y, Liu W-B, Liu K-J, Ao L, Cao J, Zhong JL, Liu J-Y. Extremely Low-Frequency Electromagnetic Fields Affect the miRNA-Mediated Regulation of Signaling Pathways in the GC-2 Cell Line. PLoS One10(10):e0139949, 2015. **(VT, AE, GE)**

Extremely low-frequency electromagnetic fields (ELF-EMFs) can affect male reproductive function, but the underlying mechanism of this effect remains unknown. miRNA-mediated regulation has been implicated as an important epigenetic mechanism for regulatory pathways. Herein, we profiled miRNA expression in response to ELF-EMFs in vitro. Mouse spermatocyte-derived GC-2 cells were intermittently exposed to a 50 Hz ELF-EMF for 72 h (5 min on/10 min off) at magnetic field intensities of 1 mT, 2 mT and 3 mT. Cell viability was assessed using the CCK-8 assay. Apoptosis and the cell cycle were analyzed with flow cytometry. miRNA expression was profiled using Affymetrix Mouse Genechip miRNA 3.0 arrays. Our data showed that the growth, apoptosis or cell cycle arrest of GC-2 cells exposed to the 50 Hz ELF-EMF did not significantly change. However, we identified a total of 55 miRNAs whose expression significantly changed compared with the sham group, including 19 differentially expressed miRNAs (7 miRNAs were upregulated, and 12 were downregulated) in the 1 mT exposure group and 36 (9 miRNAs were upregulated, and 27 were downregulated) in the 3 mT exposure group. The changes in the expression of 15 selected miRNAs measured by real-time PCR were consistent with the microarray results. A network analysis was used to predict core miRNAs and target genes, including miR-30e-5p, miR-210-5p, miR-196b-5p, miR-504-3p, miR-669c-5p and miR-455-3p. We found that these miRNAs were differentially expressed in response to different magnetic field intensities of ELF-EMFs. GO term and KEGG pathway annotation based on the miRNA expression profiling results showed that miRNAs may regulate circadian rhythms, cytokine-cytokine receptor interactions and the p53 signaling pathway. These results suggested that miRNAs could serve as potential biomarkers, and the miRNA-mediated regulation of signaling pathways might play significant roles in the biological effects of ELF-EMFs.

(E) Liu Y, Liu W-B, Liu K-J, Ao L, Cao J, Zhong JL, Liu J-Y. Overexpression of miR-26b-5p regulates the cell cycle by targeting CCND2 in GC-2 cells under exposure to

extremely low frequency electromagnetic fields. Cell Cycle 15(3):357-367, 2016. (VT, AE, GE)

The increasing prevalence of extremely low frequency electromagnetic fields (ELF-EMFs) exposure has raised considerable public concern regarding the potential hazardous effects of ELF-EMFs on male reproductive function. Increasing evidence indicates that miRNAs are necessary for spermatogenesis and male fertility. However, the regulation of miRNA expression and the roles of miRNAs in response to ELF-EMFs remain unclear. In our study, mouse spermatocyte-derived GC-2 cells were intermittently exposed to a 50 Hz ELF-EMF for 72 h (5 min on/10 min off) at magnetic field intensities of 1 mT, 2 mT and 3 mT. MiR-26b-5p was differentially expressed in response to different magnetic field intensities of ELF-EMFs. The host gene CTDSP1 showed an unmethylation status in GC-2 cells at different magnetic field intensities of ELF-EMF exposure. MiR-26b-5p had no significant, obvious influence on the cell viability, apoptosis or cell cycle of GC-2 cells. However, the overexpression of miR-26b-5p significantly decreased the percentage of G0/G1 phase cells and slightly increased the percentage of S phase cells compared to the sham group that was exposed to a 50 Hz ELF-EMF. Computational algorithms identified Cyclin D2 (CCND2) as a direct target of miR-26b-5p. MiR-26b-5p and a 50 Hz ELF-EMF altered the expression of CCND2 at both the mRNA and protein levels. Overexpressed miR-26b-5p in GC-2 cells can change the mRNA expression of CCND2 following 50 Hz ELF-EMF at 3 mT. These findings demonstrate that miR-26b-5p could serve as a potential biomarker following 50 Hz ELF-EMF exposure, and miR-26b-5p-CCND2-mediated cell cycle regulation might play a pivotal role in the biological effects of ELF-EMFs.

(E) López-Díaz B, Mercado-Sáenz S, Burgos-Molina AM, González-Vidal A, Sendra-Portero F, Ruiz-Gómez MJ. Genomic DNA damage induced by co-exposure to DNA damaging agents and pulsed magnetic field. Int J Radiat Biol 99(5):853-865, 2023. (VT, AE, GT, IX)

Purpose: Many articles describe the effects of extremely low-frequency magnetic fields (MF) on DNA damage induction. However, the mechanism of MF interaction with living matter is not yet known with certainty. Some works suggest that MF could induce an increase in the efficacy of Reactive Oxygen Species (ROS) production. This work investigates whether pulsed MF exposure produces alterations in genomic DNA damage induced by co-exposure to DNA damaging agents (bleomycin and methyl methanesulfonate (MMS)). Materials and methods: Genomic DNA, prepared from *S. cerevisiae* cultures, was exposed to pulsed MF (1.5 mT peak, 25 Hz) and MMS (0-1%) (15-60 minutes), and to MF and bleomycin (0-0.6 IU/ml) (24-72 hours). The damage induced to DNA was evaluated by electrophoresis and image analysis. Results: Pulsed MF induced an increment in the level of DNA damage produced by MMS and bleomycin in all groups at the exposure conditions assayed. Conclusions: Pulsed MF could modulate the cytotoxic action of MMS and bleomycin. The observed effect could be the result of a multifactorial process influenced by the type of agent that damages DNA, the dose, and the duration of the exposure to the pulsed MF.

(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 mumol 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 micromol/mol dG, 62.80+/-31.91 mumol/mol dG, and 27.49+/-14.23 micromol/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):402-412, 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 μT 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 μT 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-μT 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 µT 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 µM. 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. 2021:8510315, 2021. (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 γH2AX 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 γH2AX immunofluorescence and western blot. The results showed no significant difference in the average number of foci per cell, the percentage of γH2AX foci-positive cells, or the expression of γH2AX 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)
Ma Q, Chen C, Deng P, Zhu G, Lin M, Zhang L, Xu S, He M, Lu Y, Duan W, Pi H, Cao Z, Liping Pei L, Li M, Liu C, Zhang Y, Zhong M, Zhou Z, Yu Z. Extremely Low-Frequency Electromagnetic Fields Promote In Vitro Neuronal Differentiation and Neurite Outgrowth of Embryonic Neural Stem Cells via Up-Regulating TRPC1. PLoS One 11(3):e0150923, 2016. (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 astrocyes 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 RORα 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 (RORα) 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 μT 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 RORα in the thymus were not significantly changed at different magnetic flux densities. The expression levels of RORα and c-Maf were significantly downregulated at the densities of 1 and 100 μT, while the expression of STAT6 was only significantly decreased at the density of 100 μT. In conclusion, low magnetic flux densities of ELF-EMFs may reduce the expression levels of c-Maf, STAT6, and RORα 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 38(10):457-462, 2018. (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 γT 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 μT 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 μT. At the preimmunization phase, the serum levels of IL-17 and TGF-β were significantly decreased at intensities of 1 and 100 μT. The expression of Foxp3 was also downregulated at intensities of 1 and 100 μT. 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 filed-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 filed-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 filed-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, 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 filed-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) 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 filed-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) 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, 2018. (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 μM), and EMF (50 Hz, 0.50 mT, "15 min field-on/15 min field-off") were used for estimation of relative IC_{50} values. The mRNA expression levels of antioxidant genes were determined by real-time PCR. The IC_{50} 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, 2018.(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. IC_{50} 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 IC_{50} values of β-Lap in designed treatments. There is a negative association between mRNA levels of NQO1 and IC_{50} values of β-Lap but not NQO2.

(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) 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. 40(3):375-383, 2021. (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 fallowing 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 NOTCH1and 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) Mansoury F, Babaei N, Abdi S, Entezari M, Doosti A. Extremely Low Frequency Magnetic Fields Induce mTOR and Hsa_Circ_100338 Expression Changes in Gastric Cancer and Normal Fibroblast Cell Lines. Cell J 24(7):364-369, 2022. (VT, AE, GE, CS)

Objective: Extremely low-frequency magnetic field (ELF-MF) exposure, as a targeted tumor therapy, presents several potential advantages. In this research, we investigated effects of different ELF-MF intensities on cell viability and expression levels of the mammalian target of rapamycin (mTOR) and hsa_circ_100338 in the normal fibroblast (Hu02) and human gastric adenocarcinoma (AGS) cell lines. **Materials and methods:** In this experimental study, cell lines of AGS and Hu02, were cultured under the exposure of ELFMF with magnetic flux densities (MFDs) of 0.25, 0.5, 1 and 2 millitesla (mT) for 18 hours. The 3-(4, 5-dimethylthiazoyl-2- yl)-2, 5-diphenyltetrazolium bromide (MTT) assay was used to evaluate the cell viability. Relative expression of mTOR and hsa_circ_100338 RNAs was estimated by quantitative real-time polymerase chain reaction (qRT-PCR) technique. **Results:** Viability of the normal cells was significantly increased at MFDs of 0.5, 1 and 2 mT, while viability of the tumor cells was significantly decreased at MFD of 0.25 and increased at MFD of 2 mT. Expression level of mTOR was significantly increased at the all applied MFDs in the normal cells, while it was

significantly decreased at MFDs of 0.25 and 0.5mT in the tumor cells. MFDs of 1 and 2 mT in tumor cells inversely led to the increase in mTOR expression. hsa_circ_100338 was downregulated in MFD of 0.25 mT and then it was increased parallel to the increase of MFD in the normal and tumor cells. **Conclusion:** Results of the present study indicated that ELF-MF at MFDs of 0.25 and 0.5 mT can lead to decrease in the both mTOR and hsa_circ_100338 expression levels. Given the role of mTOR in cell growth, proliferation and differentiation, in addition to the potential role of hsa_circ_100338 in metastasis, expression inhibition of these two genes could be a therapeutic target 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/m(2)) 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) Martínez, M.A., A. Úbeda, J. Martínez-Botas, M.Á. Trillo. 2022. Field exposure to 50 Hz significantly affects wild-type and unfolded p53 expression in NB69 neuroblastoma cells. Oncol. Lett 24:295, 2022. (VT, AE, GE)

Previous studies have shown that intermittent exposure to a 50 Hz, 100 µT sinusoidal magnetic field (MF) promotes proliferation of human neuroblastoma cells, NB69. This effect is mediated by activation of the epidermal growth factor receptor through a free radical-dependent activation of the p38 pathway. The present study investigated the possibility that the oxidative stress-sensitive protein p53 is a potential target of the MF, and that field exposure can affect the protein expression. To that end, NB69 cells were exposed to short intervals of 30 to 120 min to the aforementioned MF parameters. Two specific anti-p53 antibodies that allow discrimination between the wild and unfolded forms of p53 were used to study the expression and cellular distribution of both isoforms of the protein. The expression of the antiapoptotic protein Bcl-2, whose regulation is mediated by p53, was also analyzed. The obtained results revealed that MF exposure induced increases in p53 gene expression and in protein expression of the wild-type form of p53. Field exposure also caused overexpression of the unfolded form of p53, together with changes in the nuclear/cytoplasmic distribution of both forms of the protein. The expression of protein Bcl-2 was also significantly increased in response to the MF. As a whole, these results indicated that the MF is capable of interacting with the function, distribution and conformation of protein p53. Such interactions could be involved in previously reported MF effects on NB69 proliferation promotion.

(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, D enny **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.

(NE) 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 14(3):608-616, 2018. (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 (±TGF-β3) 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)Mehdizadeh R, Ansari AM, Forouzesh F, Shahriari F, Shariatpanahi SP, Salaritabar A, Javidi MA. P53 status, and G2/M cell cycle arrest, are determining factors in cell-death induction mediated by ELF-EMF in glioblastoma. Sci Rep 13(1):10845, 2023. (VT, LE. GE, CS)

The average survival of patients with glioblastoma is 12-15 months. Therefore, finding a new treatment method is important, especially in cases that show resistance to treatment. Extremely low-frequency electromagnetic fields (ELF-EMF) have characteristics and capabilities that can be proposed as a new cancer treatment method with low side effects. This research examines the antitumor effect of ELF-EMF on U87 and U251 glioblastoma cell lines. Flowcytometry determined the viability/apoptosis and distribution of cells in different phases of the cell cycle. The size of cells was assessed by TEM. Important cell cycle regulation genes mRNA expression levels were investigated by real-time PCR. ELF-EMF induced apoptosis in U87cells much more than U251 (15% against 2.43%) and increased G2/M cell population in U87 (2.56%, p value < 0.05), and S phase in U251 (2.4%) (data are normalized to their sham exposure). The size of U87 cells increased significantly after ELF-EMF exposure (overexpressing P53 in U251 cells increased the apoptosis induction by ELF-EMF). The expression level of P53, P21, and MDM2 increased and CCNB1 decreased in U87. Among the studied genes, MCM6 expression decreased in U251. Increasing expression of P53, P21 and decreasing CCNB1, induction of cell G2/M cycle arrest, and consequently increase in the cell size can be suggested as one of the main mechanisms of apoptosis induction by ELF-EMF; furthermore, our results demonstrate the possible footprint of P53 in the apoptosis induction by ELF-EMF, as U87 carry the wild type of P53 and U251 has the mutated form of this gene.

(E) Mercado-Sáenz S, Burgos-Molina AM, López-Díaz B, Sendra-Portero F, Ruiz-Gómez MJ[1]. 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.

(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) 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 16(6):770-6, 2016. (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 1α (IL-1α) (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-1α- and PEMF-induced changes in gene expression. Results: Although PEMF treatment did not completely inhibit the effects of IL-1α, PEMF treatment lessened the IL-1α-induced upregulation of genes expressed in degenerated IVDs. Consistent with our previous results, after 4 days, PEMF tended to reduce IL-1α-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-1α-induced gene expression of IL-17A (33%, p=.01) and MMP2 (24%, p=.006) in NP cells and NFκB (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.

(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, 1996.(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, 1996. (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):579-584, 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. 87(2-3):213-223, 2005. (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/m(2) , 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/m(2) 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. pneumonia 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 µM/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-kB 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) Molina-Montenegro MA, Acuña-Rodríguez IS, Ballesteros GI, Baldelomar M, Torres-Díaz C, Broitman BR, Vázquez DP. Electromagnetic fields disrupt the pollination service by honeybees. Sci Adv 9(19):eadh1455, 2023. (VO, AE., GE)

We assessed the effect that electromagnetic field (EMF) exerts on honeybees' pollination efficiency using field and laboratory experiments. First, we measured levels of gene and protein expression in metabolic pathways involved in stress and behavioral responses elicited by EMF. Second, we assessed the effect of EMF on honeybee behavior and seed production by the honeybee-pollinated California poppy and, lastly, by measuring the consequences of pollination failure on plants' community richness and abundance. EMF exposure exerted strong physiological stress on honeybees as shown by the enhanced expression of heat-shock proteins and genes involved in antioxidant activity and affected the expression levels of behavior-related genes. Moreover, California poppy individuals growing near EMF received fewer honeybee visits and produced fewer seeds than plants growing far from EMF. Last, we found a hump-shaped relationship between EMF and plant species richness and plant abundance. Our study provides conclusive evidence of detrimental impacts of EMF on honeybee's pollination behavior, leading to negative effects on plant community.

(E) Monirul Islam M, Gianpiero Vigani G, Massimo E Maffei ME. The Geomagnetic Field (GMF) Modulates Nutrient Status and Lipid Metabolism during *Arabidopsis thaliana* Plant Development. Plants (Basel) 9(12):1729, 2020. (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 was 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 μT) 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-CoAsynthase (*KCS*) genes, including *KCS1*, *KCS5*, *KCS6*, *KCS8,* and *KCS12*, a lipid transfer protein (*LTPG1*) and a lipase (*LIP1*). Ionomic 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 111:414-421, 2017. (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 823:11756, 2021. (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 μT 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 μg/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 98(10):1581-1591, 2022. (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 μT_{RMS} MFs for 24 h, then assayed or further treated with 100 µM menadione for 1 h before the assay. The levels of ROS and cytosolic superoxide anion ($O_2^{\bullet-}$) 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 $O_2^{\bullet-}$ 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, Ahmadieh 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.

(NE)Nguyen H, Segers S, Ledent M, Anthonissen R, Verschaeve L, Hinsenkamp M, Collard J-F, Feipel V, Mertens B. Effects of long-term exposure to 50 Hz magnetic fields on cell viability, genetic damage, and sensitivity to mutagen-induced damage. Heliyon 9(3):e14097, 2023. (VT, LE, GT)

Until today, it remains controversial whether long-term exposure to extremely low-frequency magnetic fields (ELF-MF) below the legislative exposure limits could result in adverse human health effects. In the present study, the effects of long-term *in vitro* MF exposure on three different study endpoints (cell viability, genetic damage, and sensitivity to damage induced by known mutagens) were investigated in the human B lymphoblastoid (TK6) cell line. Cells were exposed to 50 Hz MF at three selected magnetic flux densities (i.e., 10, 100, and 500 μT) for different exposure periods ranging from 96h up to 6 weeks. Cell viability following MF exposure was assessed using the ATP-based cell viability assay. Effects of MF exposure on cell genetic damage and cell sensitivity to mutagen-induced damage were evaluated using the *in vitro* alkaline comet assay and the *in vitro* cytokinesis block micronucleus assay. The results showed that long-term exposure up to 96h to 50 Hz MF at all tested flux densities could significantly increase TK6 cell viability. In contrast, long-term MF exposure did not affect cell genetic damage, and long-term pre-exposure to MF did not change cell sensitivity to damage induced by known mutagens. At certain time points, statistically significant difference in genotoxicity test results were observed between the MF-exposed cells and the control cells. However, these observations could not be confirmed in the repeat experiments, indicating that they are probably not biologically significant.

(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 22(2):128-133, 2019. (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-1α), 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-1α, 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.

(NE)Oliva M, De Marchi L, Cuccaro A, Fumagalli G, Freitas R, Fontana N, Raugi M, Barmada S, Pretti C. Introducing energy into marine environments: A lab-scale static magnetic field submarine cable simulation and its effects on sperm and larval development on a reef forming serpulid. Environ Pollut. 328:121625, 2023. (VT, AE, GT)

Non-chemical sources of anthropogenic environmental stress, such as artificial lights, noise and magnetic fields, are still an underestimate factor that may affect the wildlife. Marine environments are constantly subjected to these kinds of stress, especially nearby to urbanized coastal areas. In the present work, the effect of static magnetic fields, associated with submerged electric cables, was evaluated in gametes and early life stages of a serpulid polychaete, namely Ficopomatus enigmaticus. Specifically, biochemical/physiological impairments of sperm, fertilization rate inhibition and incorrect larval development were assessed. We evaluated differences between two selected magnetic field induction values (0.5 and 1 mT) along a range of exposure times (30 min-48 h), for a sound evaluation on this species. We found that a magnetic induction of 1 mT, a typical value that can be found at distance of tens of cm from a submerged cable, may be considered a biologically and ecologically relevant for sessile organisms and for coastal environments more generally. This value exerted statistically significant effects on membranes, DNA integrity, kinetic parameters and mitochondrial activity of sperm cells. Moreover, a significant reduction in fertilization rate was observed in sperm exposed to the same magnetic induction level (1 mT) for 3 h, compared to controls. Regarding early larval stages, 48-h exposure did not affect the correct development. Our results represent a starting point for a future focus of research on magnetic field effects on early life stages of aquatic invertebrates, using model species as representative for reef-forming/encrusting organisms and ecological indicators of soft sediment quality.

(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 41(2):163-173, 2022. (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, Lioliousis 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, Seo 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 23(3):1177, 2022. (VT, VO, LE, GE)

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 84(2):186-193, 2020. (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-1α) 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 β1 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 7(1):10948, 2017. (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 Ca^{2+} 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-1β 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 (DeltaPsi(m)). [Ca2+]i was determined by the ratiometric fura-2 technique. Changes in DeltaPsi(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 fiboblasts 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 [Ca2+]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 [Ca2+]i and/or DeltaPsi(m).

(E) Piszczek P, Wójcik-Piotrowicz K, Guzdek P, Gil K, Kaszuba-Zwoińska J Protein expression changes during phagocytosis influenced by low-frequency electromagnetic field exposure. Int J Biol Macromol S0141-8130(22)01510-0, 2022. (VT, AE, GE, OX)

The aim of our studies was to determine the influence of a low-frequency electromagnetic field (EMF) on the phagocytosis of latex beads (LBs) and the expression level of proteins/genes in the human monocytic macrophage Mono Mac 6 (MM6) cell line in in vitro conditions. Before phagocytosis assay cells were pre-stimulated with infectious agents such as lipopolysaccharide (LPS), Staphylococcal enterotoxin B (SEB), or the proliferatory agent phytohaemagglutinin (PHA), and then exposed to EMF (30 mT, 7 Hz, 3 h). The expression of cytoplasmic proteins like iPLA, cPLa, iNOS, NLR3/4, and Hsp70 involved in the immune response pathways to phagocytosed particles, were evaluated with the usage of the Western Blot analysis. mRNA encoding the iNOS protein was detected by reverse transcription PCR method. The most meaningful changes were observed for PLA2 and NLC4 proteins level and between iNOS protein expression and mRNA encoding iNOS protein amounts. The EMF exposure exerted the strongest effect on iNOS encoding mRNAs in cells prestimulated with LPS or SEB and phagocytosing LBs. The influence of EMF on phagocytosis was experimentally proved for the first time and there is a need for further investigations in terms of the usage of EMF as a prospect, supportive therapy.

(NE) Porcher A, Wilmot N, Bonnet P, Procaccio V, Vian A. Changes in Gene Expression After Exposing Arabidopsis thaliana Plants to Nanosecond High Amplitude

Electromagnetic Field Pulses. Bioelectromagnetics 2023 Jul 6. doi: 10.1002/bem.22475. Online ahead of print. (VO, AE, GE)

The biological effects of exposure to electromagnetic fields due to wireless technologies and connected devices are a subject of particular research interest. Ultrashort high-amplitude electromagnetic field pulses delivered to biological samples using immersed electrodes in a dedicated cuvette have widely demonstrated their effectiveness in triggering several cell responses including increased cytosolic calcium concentration and reactive oxygen species (ROS) production. In contrast, the effects of these pulses are poorly documented when electromagnetic pulses are delivered through an antenna. Here we exposed Arabidopsis thaliana plants to 30,000 pulses (237 kV m-1 , 280 ps rise-time, duration of 500 ps) emitted through a Koshelev antenna and monitored the consequences of electromagnetic fields exposure on the expression levels of several key genes involved in calcium metabolism, signal transduction, ROS, and energy status. We found that this treatment was mostly unable to trigger significant changes in the messenger RNA accumulation of calmodulin, Zinc-Finger protein ZAT12, NADPH oxidase/respiratory burst oxidase homolog (RBOH) isoforms D and F, Catalase (CAT2), glutamate-cystein ligase (GSH1), glutathione synthetase (GSH2), Sucrose non-fermenting-related Kinase 1 (SnRK1) and Target of rapamycin (TOR). In contrast, Ascorbate peroxidases APX-1 and APX-6 were significantly induced 3 h after the exposure. These results suggest that this treatment, although quite strong in amplitude, is mostly ineffective in inducing biological effects at the transcriptional level when delivered by an antenna.

(E) Potenza L, Ubaldi L, De Sanctis R, De Bellis R, Cucchiarini 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, 2004.(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, Cucchiarini L, Piatti E, Angelini U, Dachà M. Effects of high static magnetic field exposure on different DNAs. Bioelectromagnetics. 25(5):352-355, 2004. (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)Rahimi S, Ahrabi M, Samiei M, Roshangar L, Ahrabi B, Hashemi B, Shahi S, Dareh chi NR. The Effect of Low-Frequency Pulsed Electromagnetic Fields on the Differentiation of Permanent Dental Pulp Stem Cells into Odontoblasts. Iran Endod J 18(4):218-223, 2023.(VT, LE, GE)

Introduction: Exposure to pulsed electromagnetic field (PEMF) has been revealed to affect the differentiation and proliferation of human mesenchymal stem cells derived from dental pulp multipotent stromal stem cells (DP-MSCs). This study aimed to investigate the differentiation effect of electromagnetic fields (EMFs) on the DP-MSC. **Materials and methods:** PEMF was produced by a system comprising a multi-meter autotransformer, solenoid coils, and teslameter. This study included 10 groups of DP-MSCs which underwent different electromagnetic radiation time and beam intensity. Three samples tested for each group. The effect of PEMF with the intensity of 0.5 and 1 mT (mili Tesla) and 50 Hz on the proliferation rate of DP-MSC was evaluated at 20 and 40 minutes per day for seven days. MTT assay was applied to determine the growth and proliferation of DP-MSC. Gene expression of DMP1 for differentiation of DPSCs to odontoblasts was confirmed by Real Time PCR., ANOVA statistical analysis and Kruskal-Wallis test were used to analyze the data. **Results:** The survival in all exposure groups was significantly

higher than that in control except in the group of 40 minutes, 1 mT (P<0.05). In 20 minutes, 0.5 mT exposure, the survival intensity is significantly more than others (P<0.05). In general, the intensity of survival was recorded, 20, 0.5 mT≥20, 1 mT≥40, 0.5 mT≥40, 1 mT respectively. Therefore, according to the obtained results, ELF-EMF increases the survival of cells except for one case (40 minutes, 1 mT), even though the effective underlying mechanisms in this process are still unclear. **Conclusions:** The results obtained promise that in the future, by placing an important part of the pulp next to the electromagnetic field, the lost part of the pulp can be reconstructed and the dentin barrier can be created.

(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. 57(1):9-15, 2002. (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. Digoxygenin-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)Rasaeifar K, Zavareh S, Hajighasem-Kashani M, Nasiri M. Effects of pulsed electromagnetic fields and N-acetylcysteine on transplantation of vitrified mouse ovarian tissue. Electromagn Biol Med 42(2):67-80, 2023. (VO, LE, GE, RP)

In this experimental study, adult female NMRI mice were randomly assigned to five groups: control; (fresh ovarian transplantation, OT); sham ;(vitrified OT); NAC ;(vitrified OT treated with N-acetyl cysteine, NAC); EMF ;(vitrified OT treated with pulsed electromagnetic fields, PEMF); and NAC+EMF ;(vitrified OT combined with NAC and PEMF). We conducted histological assessments to evaluate follicle reservation and vascularization. Furthermore, we examined the relative expression of *Fgf-2, Vegf, Tnf-α, Il-6, Il-1*, and *Cd31* genes on days 2 and 7 after OT. Additionally, we measured total antioxidant capacity (TAC), malondialdehyde (MDA) levels, as well as the activity of superoxide dismutase (SOD) and glutathione peroxidase (GPX). Our results demonstrated that NAC, PEMF, and NAC+PEMF treatments significantly increased the number of follicles. Moreover, we observed a more pronounced development of vascularization in the NAC, PEMF, and PEMF+NAC groups. The relative expression levels of *Fgf-2, Vegf, Tnf-α, Il-1β*, and *Il-6* were significantly elevated in the NAC, PEMF, and NAC+PEMF groups. Notably, TAC levels decreased significantly in the NAC group compared to the control group. Additionally, the MDA level showed a significant decrease in the PEMF+NAC group when compared to the other groups. Overall, the combination of NAC and PEMF exhibited a synergistic effect in promoting angiogenesis and protecting against oxidative stress and inflammation during OT.

(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 chemotactic 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) 19(1):e00014, 2012. (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 μT 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, Nanong, 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, Nanong, 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, Nanong, and Oct-4 genes, therefore can be considered as a new differentiation inducer in addition to the other stimulators.

(E) Salari M, Eftekhar-Vaghefi SH, Asadi-Shekaari M, Esmaeilpour K, Solhjou S, Amiri M, Ahmadi-Zeidabadi M. Impact of ketamine administration on chronic unpredictable stress-induced rat model of depression during extremely low-frequency electromagnetic field exposure: Behavioral, histological and molecular study. Brain Behav 13(5):e2986, 2023. (AS, CE, GE, IX)

Objectives: In the study, we examined the effects of ketamine and extremely low-frequency electromagnetic fields (ELF-EMF) on depression-like behavior, learning and memory, expression of GFAP, caspase-3, p53, BDNF, and NMDA receptor in animals subjected to chronic unpredictable stress (CUS). **Methods:** After applying 21 days of chronic unpredictable stress, male rats received intraperitoneal (IP) of ketamine (5 mg/kg) and then were exposed to ELF-EMF (10-Hz, 10-mT exposure conditions) for 3 days (3 h per day) and behavioral assessments were performed 24 h after the treatments. Instantly after the last behavioral test, the brain was extracted for Nissl staining, immunohistochemistry, and real-time PCR analyses. Immunohistochemistry (IHC) was conducted to assess the effect of ketamine and ELF-EMF on the expression of astrocyte marker (glial fibrillary acidic protein, GFAP) in the CA1 area of the hippocampus and medial prefrontal cortex (mPFC). Also, real-time PCR analyses were used to investigate the impacts of the combination of ketamine and ELF-EMF on the expression of caspase3, p53, BDNF, and NMDA receptors in the hippocampus in rats submitted to the CUS procedure. Results were considered statistically significant when $p < .05$. **Results:** Our results revealed that the combination of ketamine and ELF-EMF increased depression-like behavior, increased degenerated neurons and decreased the number of GFAP (+) cells in the CA1 area and mPFC, incremented the expression of caspase-3, and reduced the expression of BDNF in the hippocampus but showed no effect on the expression of p53 and NMDA-R. **Conclusions:** These

results reveal that combining ketamine and ELF-EMF has adverse effects on animals under chronic unpredictable stress (CUS).

(E) Salek F, Baharara J, Shahrokhabadi KN, Amini E. The guardians of germ cells; Sertoli-derived exosomes against electromagnetic field-induced oxidative stress in mouse spermatogonial stem cells. Theriogenology 173:112-122, 2021. (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 (GFRα1). 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 (χ^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 μM 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 μT 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 μT 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, Kwok S, Vasilov A, Jefcoat SC, Partridge NC. Effects of BMP-2 and pulsed electromagnetic field (PEMF) on rat primary osteoblastic cell proliferation and gene expression. J Orthop Res 25(9):1213-1220, 2007. (VT, LE, GE)

Bone morphogenetic proteins (BMPs) strongly promote osteoblast differentiation. Pulsed electromagnetic fields (PEMFs) promote fracture healing in non-union fractures. In this study, we hypothesized that a combined BMP-2 and PEMF stimulation would augment bone formation to a greater degree than treatment with either single stimulus. BMP-2 maximally increased the proliferative activity of rat primary osteoblastic cells at 25 ng/ml concentration. Real-time reverse transcription-polymerase chain reaction (RT-PCR) showed that BMP-2 stimulated mRNA levels of alkaline phosphatase (ALP), alpha(1) (I) procollagen, and osteocalcin (OC) in the differentiation phase and only OC mRNA expression in the mineralization phase after 24-h treatment. Both BMP-2 and PEMF (Spinal-Stim) increased cell proliferation, which was additive when both agents were combined. PEMF alone or together with BMP-2 increased only ALP mRNA expression and only during the differentiation phase 24 h after one 4-h treatment. This effect was additive when both agents were combined. Continuous daily 4-h treatment with PEMF alone or together with BMP-2 increased expression of all three osteoblast marker genes during the differentiation phase and increased the mineralized matrix. This effect was additive when both agents were combined, suggesting that the two interventions may be working on different cellular pathways. Thus, a combined effect of BMP-2 and PEMF in vitro could be considered as groundwork for in vivo bone development that may support skeletal therapy.

(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 2017:2450327, 2017. (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) Sendera A, Adamczyk-Grochala J, Pikuła B, Cholewa M, Banaś-Ząbczyk A. Electromagnetic field (50 Hz) enhance metabolic potential and induce adaptive/reprogramming response mediated by the increase of N6-methyladenosine RNA methylation in adipose-derived mesenchymal stem cells in vitro. Toxicol In Vitro 95:105743, 2024. (VT, AE, GE)

Background: Electromagnetic fields (EMF) have an impact on numerous cellular processes. It can positively and negatively affect adipose-derived stem cells (ASCs) thus their fate through the influence of specific factors and protein secretion. EMF can be a great factor for preconditioning ASCs for regenerative medicine purposes, however, understanding the cell's biological response to its effects in vitro is essential. **Methods:** ASCs were exposed to the EMF (50 Hz; 1.5 mT) for 24 and 48 h, and then cell biological response was analyzed. **Results:** 24 h exposure of ASCs to EMF, significantly increased N6-methyladenosine (m^6A) RNA methylation, indicating epitranscriptomic changes as an important factor in ASCs preconditioning. Furthermore, the expression of stem cell markers such as Nanog, Oct-4, Sox-2, CD44, and CD105 increased after 24 h of EMF exposure. Besides, western blot analysis showed upregulation of p21 and DNMT2/TRDMT1 protein levels compared to control cells with no differences in the p53 profile. Moreover, after 24 h of exposure to EMF, cell membrane flexibility, the metabolic potential of cells as well as the distribution, morphology, and metabolism of mitochondria were altered. **Conclusion:** ASCs undergo a process of mobilization and adaptation under the EMF influence through the increased m^6A RNA modifications. These conditions may "force" ASCs to redefine their stem cell fate mediated by RNA-modifying enzymes and alter their reprogramming decision of as differentiation begins.

(E) Şenol N, Kaya E, Coşkun Ö, Aslankoç R, Çömlekçi S. Evaluation of the Effects of a 50 Hz Electric Field on Brain Tissue by Immunohistochemical Method, and on Blood Tissue by Biochemical, Physiological and Comet Method. Applied Sciences. 13(5):3276, 2023. (VO, LE, GT)

The aim of this study was to evaluate the possible effects of a 50 Hz electric field on brain tissue and the positive effects of juglone (5-hydroxy-1,4-naphthoquinone) antioxidant activity, using the immunohistochemical technique on male Wistar-Albino rats. The effects on blood tissue were also examined using biochemical, physiological and comet methods. Animals were randomly divided into three groups (eight in each group): group I: control, group II: electric

field, group III: 50 Hz electric field + juglone (5-hydroxy-1,4-naphthoquinone)/300 ppm. Juglone was applied per day by gavage over 30 days. At the end of the experimental procedure, animals were sacrificed and brain tissue was subjected to routine histologic and immunohistochemical processes. As a result of histopathological examination, the brain tissue of rats with 50 Hz electric field exposure showed severe histopathological changes. The differences between groups were statistically significant according to total comet score ($p = 0.001$). For the antioxidant parameters on the blood, SOD activity in the electric field group was significantly higher among the other groups, although we did not find significant differences in MDA, CAT activity level.

(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 μT 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% ± 1.36% vs. 42.8% ± 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:trophectoderm 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 38(10):125, 2021.

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 γH2AX 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 H_2O_2 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-rayinduced 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 μT magnetic flux densities for 60 days. To activate the immune system, 100 μg 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 μT. Serum IFN-γ and IL-4 levels were also significantly decreased in 100 μT-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, Koziorowski 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, Sauliutė 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 γH2AX foci per cell (9.37 ± 0.44 vs. 9.08 ± 0.28, P = 0.58) or percentage of γH2AX foci positive cells (49.22 ± 1.86% vs. 49.74 ± 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 γH2AX foci formation in $Atm^{+/+}$ MEFs. Similarly, the exposure did not significantly affect γH2AX 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):209-219, 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 (BMMSCs) during osteogenic differentiation. Exposure of BMMSCs 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 BMMSC-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.

(NE) 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 31(7):566-572, 2010. (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 16(6):499-501, 2001.(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.

(E) Szemerszky R, Zelena D, Barna I, Bárdos G. Stress-related endocrinological and psychopathological effects of short- and long-term 50Hz electromagnetic field exposure in rats. Brain Res Bull 81(1):92-99, 2010. (VO, LE, GE)

It is believed that different electromagnetic fields do have beneficial and harmful biological effects. The aim of the present work was to study the long-term consequences of 50 Hz electromagnetic field (ELF-EMF) exposure with special focus on the development of chronic stress and stress-induced psychopathology. Adult male Sprague-Dawley rats were exposed to ELF-EMF (50 Hz, 0.5 mT) for 5 days, 8h daily (short) or for 4-6 weeks, 24h daily (long). Anxiety was studied in elevated plus maze test, whereas depression-like behavior of the long-treated group was examined in the forced swim test. Some days after behavioral examination, the animals were decapitated among resting conditions and organ weights, blood hormone levels as well as proopiomelanocortin mRNA level from the anterior lobe of the pituitary gland were measured. Both treatments were ineffective on somatic parameters, namely none of the changes characteristic to chronic stress (body weight reduction, thymus involution and adrenal gland hypertrophy) were present. An enhanced blood glucose level was found after prolonged ELF-EMF exposure ($p=0.013$). The hormonal stress reaction was similar in control and short-term exposed rats, but significant proopiomelanocortin elevation ($p<0.000$) and depressive-like behavior (enhanced floating time; $p=0.006$) were found following long-term ELF-EMF exposure. Taken together, long and continuous exposure to relatively high intensity electromagnetic field may count as a mild stress situation and could be a factor in the development of depressive state or metabolic disturbances. Although we should stress that the average intensity of the human exposure is normally much smaller than in the present experiment.

(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)Co-γ). 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.

(NE) Takahashi M, Furuya N. Evaluation of the Effects of Power-Frequency Magnetic Field Exposure on B-Cell Differentiation From Human Hematopoietic Stem/Progenitor Cells. Bioelectromagnetics 44(5-6):119-128, 2023. (VT, LE, GE)

The causal relationship between exposure to power-frequency magnetic fields (MFs) and childhood leukemia has long been controversial. The most common type of childhood leukemia is acute B-lymphoblastic leukemia caused by abnormal proliferation of B cells in the early differentiation process. Here, we focused on B-cell early differentiation and aimed to evaluate the effects of exposing cells to power-frequency MF. First, we optimized an in vitro differentiation protocol of human hematopoietic stem/progenitor cells (HSPCs) to B-cell lineages. Following validation of the responsiveness of the protocol to additional stimulations and the uniformity of the experimental conditions, human HSPCs were continuously exposed to 300 mT of 50 Hz MF for 35 days of the differentiation process. These experiments were performed in a blinded manner. The percentages of myeloid or lymphoid cells and their degree of differentiation from pro-B to immature-B cells in the MF-exposed group showed no significant changes compared with those in the control group. Furthermore, the expression levels of recombination-activating gene (RAG)1 and RAG2 in the B cells were also similar to those in the control group. These results indicate that exposure to 50 Hz MF at 300 mT does not affect the human B-cell early differentiation from HSPCs.

(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 transcriptor 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) 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) Tian L, Y. Luo, A. Zhan, J. Ren, H. Qin, and Y. Pan. Hypomagnetic Field Induces the Production of Reactive Oxygen Species and Cognitive Deficits in Mice Hippocampus. *Int. J. Mol. Sci* 23:3622, 2022. (VO, LE, GE)

Previous studies have found that hypomagnetic field (HMF) exposure impairs cognition behaviors in animals; however, the underlying neural mechanisms of cognitive dysfunction are unclear. The hippocampus plays important roles in magnetoreception, memory, and spatial navigation in mammals. Therefore, the hippocampus may be the key region in the brain to reveal its neural mechanisms. We recently reported that long-term HMF exposure impairs adult hippocampal neurogenesis and cognition through reducing endogenous reactive oxygen species (ROS) levels in adult neural stem cells that are confined in the subgranular zone (SGZ) of the hippocampus. In addition to adult neural stem cells, the redox state of other cells in the hippocampus is also an important factor affecting the functions of the hippocampus. However, it is unclear whether and how long-term HMF exposure affects ROS levels in the entire hippocampus (i.e., the dentate gyrus (DG) and ammonia horn (CA) regions). Here, we demonstrate that male C57BL/6J mice exposed to 8-week HMF exhibit cognitive impairments. We then found that the ROS levels of the hippocampus were significantly higher in these HMF-exposed mice than in the geomagnetic field (GMF) group. PCR array analysis revealed that the elevated ROS levels were due to HMF-regulating genes that maintain the redox balance in vivo, such as *Nox4*, *Gpx3*. Since high levels of ROS may cause hippocampal oxidative stress, we suggest that this is another reason why HMF exposure induces cognitive impairment, besides the hippocampal neurogenesis impairments. Our study further demonstrates that GMF plays an important role in maintaining hippocampal function by regulating the appropriate endogenous ROS levels.

(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) Udroiu 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 muT magnetic field. **MATERIALS AND METHODS:** The micronucleus test with CREST (Calcinosis, Raynaud's phenomenon, Esophageal dismotility, Sclerodactility, Telangectasia) 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) Udroiu 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) 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 μT, 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 μT. 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 μT 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)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 4NQOinduced

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-SY5Y5 and SK-N-BE-2 cells underwent exposure to 50Hz ELF-MF (0.01, 0.1 or 1mT) or $AlCl_3$ (4 or 40μM) or co-exposure to 50Hz ELF-MF and $AlCl_3$ 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 $AlCl_3$ 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 $AlCl_3$ 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 7(6):e39317, 2012. (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 14:22-28, 2020. (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 TGFβ2, b) positive control contained medium with TGFβ2, 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, TGFβ1, TGFβ2, and TGFβ3. Alcian blue staining to detect glycosaminoglycan deposition was also performed. Medium supernatant was used to detect endogenous latent TGF-β1 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-β1 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) 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-β2 (TGF-β2) 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 42(6):441-454, 2021. (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-1β. 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-1β 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 integrinβ1 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 μT 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 μT 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 μT 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 96(12):1633-1640, 2020. (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 Y, Chen L, Wang L, Pei G, Cheng H, Zhang Q, Wang S, Hu D, He Y, He C, Fu C, Wei Q. Pulsed Electromagnetic Fields Combined with Adipose-derived Stem Cells Protect Ischemic Myocardium by Regulating miR-20a-5p/E2F1/p73 Signalling. Stem Cells 41(7):724-737, 2023. (VO, LE, GE)

Myocardial infarction (MI) is a serious threat to human health. Although monotherapy with pulsed electromagnetic fields (PEMFs) or adipose-derived stem cells (ADSCs) has been reported

to have positive effect on the treatment of MI, a satisfactory outcome has not yet been achieved. In recent years, combination therapy has attracted widespread interest. Herein, we explored the synergistic therapeutic effect of combination therapy with PEMFs and ADSCs on MI and found that the combination of PEMFs and ADSCs effectively reduced infarct size, inhibited cardiomyocyte apoptosis and protected the cardiac function in mice with MI. In addition, bioinformatics analysis and RT–qPCR showed that the combination therapy could affect apoptosis by regulating the expression of miR-20a-5p. A dual-luciferase reporter gene assay also confirmed that the miR-20a-5p could target E2F transcription factor 1 (E2F1) and inhibit cardiomyocyte apoptosis by regulating the E2F1/p73 signalling pathway. Therefore, our study systematically demonstrated the effectiveness of combination therapy on the inhibition of cardiomyocyte apoptosis by regulating the miR-20a-5p/E2F1/p73 signalling pathway in mice with MI. Thus, our study underscored the effectiveness of the combination of PEMFs and ADSCs and identified miR-20a-5p as a promising therapeutic target for the treatment of MI in the future.

(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..

(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 Brelated 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 JPulsed electromagnetic field induces Ca^{2+}-dependent osteoblastogenesis in C3H10T1/2 mesenchymal cells through the Wnt-Ca^{2+}/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 [Ca^{2+}]i in C3H10T1/2 cells can be upregulated upon exposure to PEMF and that PEMF-induced C3H10T1/2 cell differentiation was Ca^{2+}-dependent. The pro-osteogenic effect of PEMF on Ca^{2+}-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/Ca2+ 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-Ca2+/Wnt-β-catenin signaling pathway in C3H10T1/2 mesenchymal cells.

(E) Wydorski PJ, Kozlowska W, Drzewiecka, EM, Zmijewska A, Franczak A. Extremely low-frequency electromagnetic field exposure alters DNA methylation levels in the endometrium of pigs during the peri-implantation period. Reprod Fertil Dev 35(12):601-613, 2023. (VT, AE, GE, EP, RP)

Context: Extremely low-frequency electromagnetic field (ELF-EMF) emission is increasing due to substantial technological progress. The results of previous research provided evidence that ELF-EMF may exert changes in molecular mechanisms that control female reproduction. **Aims:** We hypothesised that short-term ELF-EMF treatment alters the DNA methylation level of genes in the endometrium. Hence, the research aimed to determine the methylation level of selected genes whose expression was altered in response to ELF-EMF radiation in the endometrium of pigs during the peri-implantation period (days 15-16 of pregnancy). **Methods:** Porcine endometrial slices (100±5mg) were collected during the peri-implantation period and exposed to ELF-EMF at a frequency of 50Hz for 2h in vitro. The control endometrium was not exposed to ELF-EMF. The level of DNA methylation in the promoter regions of EGR2, HSD17B2, ID2, IL1RAP, MRAP2, NOS3, PTGER4, SERPINE1, VDR and ZFP57 was tested using qMS-PCR. **Key results:** In the endometrium exposed to ELF-EMF, the level of methylation of HSD17B2, MRAP2, SERPINE1, VDR and ZFP57 was not altered; the level of methylation of EGR2, ID2 and PTGER4 increased, and the level of methylation of IL1RAP and NOS3 decreased. **Conclusions:** ELF-EMF may alter the level of DNA methylation in the endometrium during the peri-implantation period. **Implications:** Changes in the DNA methylation induced by ELF-EMF may affect the transcriptomic profile of the endometrium and disturb physiological processes accompanying implantation and embryo development.

(E) Wydorski PJ, Kozlowska W, Zmijewska A, Franczak A. Exposure to the extremely low-frequency electromagnetic field induces changes in the epigenetic regulation of gene expression in the endometrium. Theriogenology 217:72-82, 2024. (VT, AE, GE, RP, FP)

Increasing technological development results in more sources of the extremely low-frequency electromagnetic field (ELF-EMF), which is recognized as an environmental risk factor. The results of the past study indicate that the ELF-EMF can affect the level of DNA methylation. The study aimed to determine whether the ELF-EMF induces changes in epigenetic regulation of gene expression in the endometrium of pigs during the peri-implantation period. Endometrial

slices (100 ± 5 mg) collected on days 15-16 of pregnancy were exposed in vitro to the ELF-EMF at a frequency of 50 Hz for 2 h of treatment duration. To determine the impact of the ELF-EMF on elements of epigenetic regulations involved in DNA methylation, histone modification, and microRNA biogenesis in the endometrium, the DNMT1 and DNMT3a; EZH2, UHRF1, and MBD1; DICER1 and DGCR8 mRNA transcript and protein abundance were analyzed using Real-Time PCR and Western blot, respectively. Moreover, EED and SUZ12 mRNA transcript, global DNA methylation, and the activity of histone deacetylase (HDAC) were analyzed. The changes in the abundance of DNMT1 and DNMT3a, EZH2 mRNA transcript and protein, EED and SUZ12 mRNA transcript, global DNA methylation level, HDAC activity, and the abundance of proteins involved in microRNA biogenesis evoked by the ELF-EMF in the endometrium were observed. The ELF-EMF possesses the potential to alter epigenetic regulation of gene expression in the porcine endometrium. Observed alterations may be the reason for changes in the transcriptomic profile of the endometrium exposed to the ELF-EMF which in turn may disrupt biological processes in the uterus during peri-implantation.

(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 49(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)Yahyapour R, Khoei S, Kordestani Z, Larizadeh MH, Jomehzadeh A, Amirinejad M, Ahmadi-Zeidabadi M. Comparative study of extremely low-frequency electromagnetic field, radiation, and temozolomide administration in spheroid and monolayer forms of the glioblastoma cell line (T98). Curr Radiopharm 16(2):123-132, 2023. (VT, AE, GE, IX)

Background: Glioblastoma is the most common primary malignant tumor of the central nervous system. The patient's median survival rate is 13.5 months, so it is necessary to explore new therapeutic approaches. **Objective:** Extremely low-frequency electromagnetic field (EMF) has been explored as a noninvasive cancer treatment. This study applied the EMF with previous conventional chemoradiotherapy for glioblastoma. **Methods:** In this study, we evaluated the cytotoxic effects of EMF (50 Hz, 100 G), temozolomide (TMZ), and radiation (Rad) on gene expression of T98 glioma cell lines in monolayer and spheroid cell cultures. **Results:** Treatment with Rad and EMF significantly increased apoptosis-related gene expression compared to the control group in monolayers and spheroids ($p<0.001$). The expression of apoptotic-related genes in monolayers was higher than the similar spheroid groups ($p<0.001$). We found that treatment with TMZ and EMF could increase the gene expression of the autophagy cascade markers compared to the control group ($p<0.001$). Autophagy-related gene expression in spheroids was

higher than in the similar monolayer group ($p<0.001$). We demonstrated that co-administration of EMF, TMZ, and Rad significantly reduced cell cycle and drug resistance gene expression in monolayers and spheroids ($p<0.001$) compared to the control group. **Conclusion:** The combinational use of TMZ, Rad and, EMF showed the highest antitumor activity by inducing apoptosis and autophagy signaling pathways and inhibiting cell cycle and drug resistance gene expression. Furthermore, EMF increased TMZ or radiation efficiency.

(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 Ca(2+) 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 asses 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.10(5), respectively) were significantly higher than sham group at 50 and 100 days (3.02+/-0.63 and 3.46+/-0.38 8OHdG/dG.10(5)) ($p<0.001$, $p<0.001$). 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) Yokus 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 H_2O_2 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 H_2O_2. 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). 12(4):3662-3681, 2020. (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 nephroblastoma. 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.

(E) Yun J, Jin X, Sun Q, Xu L, Gao J, Wang X, Zhao S. Transcriptional Analysis of Mice Melanoma B16-F10 Cells in Response to Directed Current Electric Fields. Bioelectromagnetics 43(5):297-308, 2022. (VT, AE, GE)

The endogenous electric field (EF) is widely observed among tissues. It is supposed to be an important environmental factor in tumor metastasis. To explore the role of endogenous EFs in tumor metastasis, the migration of mouse melanoma B16-F10 cells in directed current EFs (dcEFs) was investigated. The transcriptome of melanoma B16-F10 cells in response to EF stimulation was analyzed using RNA sequencing. The results demonstrated that the mouse melanoma B16-F10 cells migrated toward the cathode in applied dcEFs. Directional migration occurred in a voltage-dependent manner. Approximately 3000 upregulated and 2613 downregulated genes were identified under dcEF. Some genes correlated with cell migration, such as Serpine1, Ctgf, Fosb, and Fos, were upregulated. The signaling pathways involved in cell motility were significantly altered. Some genes, highly related to tumorigenesis, invasion, and metastasis, are upregulated in response to EF stimulation. Endogenous EFs may play a role in tumorigenesis and metastasis in vivo.

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 97(9) 1324-1332, 2021. (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 μT. 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, effective protect the damage induced by the ELF-EMF radiation. In addition, LSPCs prevented the decline of DNA content caused by ELF-EMF. Western blot determinated 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 98:574-584, 2020.(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 μT) exposed E1 group, and ELF-EMF (50 Hz, 500 μT) 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 μT) 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 X-J, Xiao Z-B, Gu J-X, Chen K, Wang J, Xu S-L, Xing K-K, Chen T. Investigating the molecular mechanisms of delirium-like neuropsychiatric disorder induced by electromagnetic pulse based on bioinformatics analysis. Mol Brain 16(1):21, 2023. (VO, LE, GE)

Electromagnetic pulse (EMP), a unique type of electromagnetic radiation, may induce diverse neuropsychiatric disorders, such as irritability, hyperkinesis, retardation of learning and memory. However, the underlying mechanism of EMP exposure on neuronal injury has not been elucidated. Here, we aimed to delineate the regulatory expression networks based on high-throughput sequencing data to explore the possible molecular mechanisms related to EMP-induced delirium-like neuropsychiatric disorder in rats. It's shown that EMP exposure induced anxiety, cognitive decline and short-term memory impairment. The expression profiles of the long noncoding RNAs (lncRNAs) and mRNAs, along with their biological function and regulatory network, were explored in rats after EMP exposure. We identified 41 differentially expressed lncRNAs (DELs) and 266 differentially expressed mRNAs (DEMs) between EMP and sham groups. Sixty-one co-expression relationships between 18 DELs and 56 DEMs were mostly associated with synapse- and metabolic-related pathways. We predicted 51 DEL-miRNA pairs and 290 miRNA-mRNA pairs using the miRanda database to constructed a DEL-miRNA-DEM network. LncRNA AABR07042999.1 and mRNA Tph2, Slc6a4, Dbh and Th were

upregulated, and the contents of serotonin, dopamine and norepinephrine were increased in both PFC and HIP after EMP exposure. The current study provided a better understanding of the ceRNA network, which might reveal the pathological mechanism and provide more treatment options for the EMP-induced neurobehavioral disorder.

(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 Y, Zeng L, Wei Y, Zhang M, Pan W, Sword GA, Yang F, Chen F, Wan G. Reliable reference genes for gene expression analyses under the hypomagnetic field in a migratory insect. Front Physiol 13:954228, 2022. (VO, LE, GE, DE)

Manipulating the hypomagnetic field (HMF), which is the absence or significant weakening (<5 μT) of the geomagnetic field (GMF), offers a unique tool to investigate magnetic field effects on organismal physiology, development, behavior and life history. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) has been utilized to study changes in gene expression associated with exposure to the HMF. However, selecting appropriate reference genes (RGs) with confirmed stable expression across environments for RT-qPCR is often underappreciated. Using three algorithms (BestKeeper, NormFinder, and GeNorm), we investigated the expression stability of eight candidate RGs when exposed to the HMF condition versus local GMF during developmental from juveniles to adults in the migratory insect pest, the brown planthopper *Nilaparvata lugens*. During the nymphal stage, *RPL5* & *α-TUB1, EF1-α* & *ARF1*, *RPL5* & *AK*, *EF1-α* & *RPL5*, and *ARF1* & *AK* were suggested as the most stable RG sets in the 1st to 5th instars, respectively. For 1- to 3-day-old adults, *AK* & *ARF1*, *AK* & *α-TUB1, AK* & *ARF1* and *EF1-α* & *RPL5*, *AK* & *α-TUB1*, *AK* & *EF1-α* were the optimal RG sets for macropterous and brachypterous females, respectively. *ACT1* & *RPL5, RPL5* & *EF1-α, α-TUB1* & *ACT1* and *EF1-α* & *RPL5, ARF1* & *ACT1, ACT1* & *ARF1* were the optimal RG sets for macropterous and brachypterous males, respectively. These results will facilitate accurate gene expression analyses under the HMF in *N. lugens*. The verification approach illustrated in this

study highlights the importance of identifying reliable RGs for future empirical studies of magnetobiology (including magnetoreception) that involve magnetic field intensity as a factor.

(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) Zhou H, Xuanyuan X, Lv X, Wang J, Feng K, Chen C, Ma J, Xing D. Mechanisms of magnetic sensing and regulating extracellular electron transfer of electroactive bacteria under magnetic fields. Sci Total Environ 895:165104, 2023. (VO, AE, GE)

Electroactive bacteria can display notable plasticity in their response to magnetic field (MF), which prompted bioelectrochemical system as promising candidates for magnetic sensor applications. In this study, we explored the sensing and stimulatory effect of MF on current generation by Geobacter sulfurreducens, and elucidated the related molecular mechanism at the transcriptomic level. MF treatment significantly enhanced electricity generation and overall energy efficiency of G. sulfurreducens by 50 % and 22 %, respectively. The response of current to MFs was instantaneous and reversible. Cyclic voltammetry analysis of the anode biofilm revealed that the redox couples changed from -0.31 to -0.39 V (vs. Ag/AgCl), suggesting that MFs could alter electron transfer related components. Differential gene expression analysis further verified this hypothesis, genes associated with electron transfer were upregulated in G. sulfurreducens under MF treatment relative to the control group, specifically, genes encoding periplasmic c-type cytochromes (ppcA and ppcD), outer membrane cytochrome (omcF, omcZ, omcB), pili (pilA-C, pilM, and pilV2), and ribosome. The enhanced bacterial extracellular electron transfer process was also linked to the overexpression of the NADH dehydrogenase I subunit, the ABC transporter, transcriptional regulation, and ATP synthase. Overall, our findings shed light on the molecular mechanism underlying the effects of magnetic field stimuli on EAB and provide a theoretical basis for its further application in magnetic sensors and other biological system.

(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 play 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 (γH2AX) 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 γH2AX foci formation in human LECs after 2, 6, 12, 24, or 48 hr exposure. No significant differences had been detected in γH2AX 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(2)O(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.

Genetic effects of static and ELF EMF (*study with no significant effect observed; Author in red = gene expression study).

	Exposure conditions	Results
Abdelhaliem et al. (2023)	Leaves of corn (Zea mays) seedlings exposed to 60-Hz, 10 mT EMF for 1, 3, or 5 days	Changes in pattern of gene expression and increased DNA damage (Comet assay)
Agliassa et al. (2018)	Arabidopsis thaliana (thale cress) exposed to 0.00004 mT static magnetic field for 38 days after sowing.	Changes in gene expression in leaf and floral meristem (cryptochrome-related gene involved); delayed flowering time and a significant reduction of leaf area index and flowering stem length, with respect to controls under geomagnetic field.
Ahmadi-Zeidabadi et al. (2019)	Human glioblastoma U87 cells exposed to 100-Hz 10 mT MF for 120-144 h	Increased expression of Nestin, CD133, Notch4 and GFAP genes, synergistic with temozolmide. Oxidative processes involved.
Ahuja et al. (1999)	Human peripheral blood samples exposed to 50 Hz EMF at 2, 3, 5, 7, or 10 mT	Increased DNA single strand breaks (Comet assay) in lymphocytes. (Damage levels higher in female than in male subjects.)
Akbarnejad et al. (2017)	Human U87 and T98G glioma cells exposed to 100-Hz 10 mT MF, 144 h	Increased expression of P53, Bax, Caspase-3, and oxygenase-1, and decreasing that of Bcl-2 and Cyclin-D1. Oxidative processes involved.
Albaqami et al. (2020)	Arabidopsis (rockcress) seedlings exposed to 0.0002 mT static MF; 3 h under blue light activation	Increased expression of cryptochrome-regulated genes (IAA, PIN1, and PIN3)
*Albert et al. (2009)	Human subjects exposed to exposed to 60-Hz	No significant effect on DNA single strand breaks (Comet assay) and micronucleus frequency in lymphocytes.

	magnetic field at 0.2 mT for 4 h	
Alcaraz et al. (2014)	Swiss mice exposed to 50-Hz magnetic field at 0.2 mT for 7, 14, 21, or 28 days	Increased micronucleus frequency in bone marrow. Effect not affected by antioxidants.
Al-Huqail and Abdelhaliem (2015)	Maize seedlings exposed to 50-Hz electric field at 6 kV/m for 1, 3, or 5 days	Increased DNA single strand breaks (comet assay)
Amara et al. (2006)	Male rats exposed to a static magnetic field at 128 mT, I h/day for 30 days	Increased 8-oxo-dG concentration and oxidative damage in testis.
Amara et al. (2007a)	Human monocytic leukemia THP-1 cells exposed to static magnetic field at 250 mT for 1, 2, or 3 h	Lower level of DNA single strand breaks (Comet assay) at 3 h of exposure, no effect on oxidative damages and enzymes and oxidative DNA damage.
Amara et al. (2007b)	Rats exposed to a static magnetic field at 128 mT, 1 h/day for 30 days	Increased 8-oxo-7,8-dihydro-2'-desoxyguanosine in kidney but not in liver. Also decreased anti-oxidative enzymes and increased lipid peroxidation. Zinc supplementation attenuated DNA oxidation induced by static magnetic field in kidney to the control level.
*Amara et al. (2009)	Rats exposed to a static magnetic field at 128 mT, 1 h/day for 30 days	No significant effect on 8-oxo-7,8-dihydro-2'-deoxyguanosine in frontal cortex and oxidative stress induced. However, there was an increase in metallothioneins level which might have protected DNA from oxidative damage.

*Amara et al. (2011)	Rats exposed to a static magnetic field at 128 mT, 1 h/day for 30 days, also treated with cadmium (Cd)	Magnetic field had no interaction on Cd-induced increase in 8-oxo-7,8-dihydro-2-desoxyguanosine in the frontal cortex and hippocampus. However, static magnetic field enhanced Cd-induced increase in oxidative damage in the rat brain.
Arruda-Neto et al. (2009)	*Microcystis panniformis*, the eukaryote *Candida albicans* and human MRC5 lung cells exposed to gamma radiation and then to static electric field for 2-20 h at 20- 1250 V/cm	Static electric field caused suppression of DNA repair in *C. albicans*. It decreased cell growth in *M. panniformis* when compared with gamma radiation alone. The electric field increased number of nuclei with γ-H2AX foci in the irradiated MRC5 cells. Electric field interferes mostly in the DNA repair mechanisms.
Ashta et al. (2020)	Human glioblastoma cells (A172) exposed to 10 Hz or static magnetic field at 5 mT, up to 96 h	Increased p52 gene expression, cytotoxicty and free radical formation; effects enhanced by Temozolomide.
Back et al. (2019)	Mouse embryonic stem cells exposed to hypomagnetic field (<0.005 mT) up to 12 days	Induced abnormal DNA methylation through the dysregulation of DNA methyltransferase3b (Dnmt3b) expression, eventually resulting in incomplete DNA methylation during differentiation.
Bagheri Hosseinabadi et al. (2019)	Blood samples from 102 thermal power plant workers as the exposure group and 136 subjects as the unexposed group.	Increased DNA single strand breaks (Comet assay) in lymphocytes of exposed subjects.
Bagheri Hosseinabadi et al. (2020)	Blood samples from thermal power plant workers; mean levels of exposure to ELF magnetic and electric fields were .0165 mT	DNA single strand breaks (Comet assay) in lymphocytes decreased by antioxidants.

	(±6.46) and 22.5 V/m (±5.38), respectively,	
Bai et al. (2021)	Neural stem cells exposed to 50-Hz 5 mT EMF 30 min/day for 3 days	Increased expression of Tuj-1 gene; accelerated stem cell differentiation into neurons.
Balamuralikrishnan et al. (2012)	Blood from electrical workers exposed to ELF EMF occupationally	Increased chromosome aberrations and micronucleus in lymphocytes.
Barati et al. (2021)	Mouse MC4-L2 breast cancer cells exposed to 1 Hz 100 mT EMF. 2 h/day for 28 days	Suppressed expression of Ki-67, CD31, VEGFR2 and MMP-9; involved free radicals and calcium
Baraúna et al. (2015)	Chromobacterium violaceum bacteria cultures exposed to ELF-EMF for 7 h at 0.00066 mT	Five differentially expressed proteins detected including the DNA-binding stress protein, which may help to prevent physical damage to DNA.
Belyaev et al. (2005)	Human lymphocytes exposed to 50 Hz magnetic field at 0.015 mT (peak) for 2 h (measurements made at 24 and 48 h after exposure).	Induced chromatin conformation changes and decreased background 53BP1 (protein co-localized with DNA double strand breaks and involves in DNA damage signaling pathway.)
Bernardini et al. (2007)	porcine aortic endothelial cells exposed to 50-Hz 1 mT MF for 4 h	Increased mRNA for Hsp70.
Bertea et al. (2015)	Arabidopsis thaliana (thale cress) exposed to artificially reversed geomagnetic field	Significant effects on plant growth and gene expression observed. This supports the hypothesis that GMF reversal contributes to inducing changes in plant development that

	conditions for 10 days at .0419 mT	might justify a higher selective pressure, eventually leading to plant evolution.
Borhani et al. (2011)	Female NMRI mice exposed to a 50-Hz EMF at 0.5 mT for 4 h/day, 6 days/week for 2 weeks. Mated on day 8 after exposure, on day 4, blastocysts were obtained by flushing the uterus horns.	DNA fragmentation index increased and decrease in blastocytes in exposed group.
*Brix t al. (2020)	Young volunteers allocated to three study arms were exposed to [^{18}F] fluoro-D-glucose alone, to a 3-T SMF alone or to both combined over 60 min at a PET/CT or a PET/MRI system.	No significant change in lymphocyte DNA double strand breaks (γH2AX) to static magnetic field or interaction with [^{18}F] fluoro-D-glucose.
Buddak et al. (2012)	Murine AT478 carcinoma cells cultured with cisplatin exposed to 50-Hz EMF for 16 min at 1 mT	Exposure to ELF-EMF alone resulted in an increase in DNA single strand breaks (Comet assay) 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. The addition of ELF-EMF exposure to cisplatin treatment resulted in decreased ROS levels and antioxidant enzyme activity.
Burgos-Molina et al (2020)	DNA double strand breaks were induced in Saccharomyces cerevisiae yeast and exposed to a 50-Hz	Long-term magnetic field exposure increased the DNA repair activity.

	magnetic field for 21 days at 2.45 mT	
Calabrò et al. (2011)	Human neuronal-like cells exposed to static (2 mT) and 50 Hz (1 mT) for 3 h.	Fourier self deconvolution spectroscopic analysis showed alteration in DNA/RNA and increased beta-sheet.
Calabrò et al. (2020)	Human Neuronal-like cells and roots of Allium sativum and Vicia faba exposed to a static and 50 Hz magnetic fields at intensities ranging from 1 mT to 0.8 T	Exposure to both low- and high-intensity magnetic fields 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.
*Cantoni et al.(1996)	Cultured mammalian cells exposed to 50 Hz electric (0.2 - 20 kV/m), magnetic (0.0002-0.2 mT), or combined electric and magnetic fields.	Repair of DNA single strand breaks (Comet assay) induced by the carcinogens methylmethane sulphonate (MMS), chromate, and 254 U.V. radiation not affected by ELF EMF exposure.
Celikler et al. (2009)	Workers from transfrormer and distribution line stations. The electric field was in the range from 130–8310 V/m and from 300–15,000 V/m, the magnetic field was between 0.5 and 1.7 A/m and 0.25–17 A/m around and inside transformer buildings. Average time of exposure was 19 years.	Increased chromosomal aberrations and micronucleus in peripheral lymphocytes. The frequency of chromosomal aberration in exposed groups correlated with the years of exposure.

*Cellini et al. (2008)	Escherichia coli ATCC 700926 exposed to 50-Hz EMF (0.1, 0.5, 1.0 mT); 20-120 min	No changes among DNA finger-printings. Other measurements indicates 50 Hz EMF acts as a stressing factor on bacteria
*Chahal et al. (1993)	Escherichia coli strain AB1157 exposed to a frequency of 1 Hz with field strengths of 1 or 3 kV m-1	Low frequency electromagnetic fields do not increase spontaneous mutation, induce DNA repair or increase the mutagenic effects of UV or mitomycin C.
Chang et al. (2004)	Neonatal mouse calvarial bone cells exposed to a pulsed EMF (15 Hz, 0.1 mT) for 14 days	Increased osteoprotegerin (OPG) mRNA expression and decreased the receptor activator of NF-kappaB ligand (RANKL) mRNA expression
Chen GD et al. (2008)	Human MCF-7 breast cancer cells exposed to a 50-Hz magnetic fields for 24 h at 0.4 mT	Identified three 50 Hz MF responsive genes in MCF-7 cells.
*Chen G et al. (2012)	*Saccharomyces cerevisiae* yeast cells exposed to a 50-Hz magnetic field at 0.4 mT for 6 h	Yeast cells did not alter gene expression in response to 50 Hz magnetic field.
Chan J. et al. (2020)	Human choriocarcinoma cells exposed to DC electric field (150 mV/mm) for 8 h	Increased gene expressions of ErbB and HIF-1 signaling pathways involved in cell migration/motility, cell cycle progression and proliferation.
Chen WF et al. (2010)	Human myelogenous leukemia K562 cells exposed to static magnetic field at 8.8 mT with or without cisplatin	Static magnetic field exposure induced DNA to become thicker than controls, and enhanced DNA breakage (Comet assay) induced by cisplatin.
Chen Y et al. (2021)	Human mesenchymal stem/stromal cells exposed to pulsed EMF	Increased expression of extracellular matrix genes *COL1A1*, *FN1*, and *BGN* depending on waveform and frequency.

	(51.8 and 52.3 Hz); 6-0.282 mT, 7 or 30 min	
Cheng L et al. (2022)	Caenorhabditis elegans Exposed to 0.67 T static MF or 50-Hz 0.7 mT MF; 30 min every10 h for a total of 300 min	Affected expression of serotonin-related genes.
Cheng Y et al. (2017)	Human umbilical vein endothelial cells exposed to 50-Hz 2.25 mT pulsed EMF for 15 min	Increased expression of mTOR gene
Cho S et al. (2014)	Human lymphocytes exposed to 60-Hz EMF at 0.8 mT for 12-72 h with or without gadolinium.	ELF-EMF increased cell death, micronucleus frequency, DNA single strand break (Comet assay), and apoptosis induced by gadolinium.
Cho YH et al. (2007)	Human fibroblasts exposed to 60-HZ EMF at 0.8 mT plus bleomycin for 28, 88, and 240 h	The co-exposure of cells to bleomycin and EMF led to a significant increase in the frequencies of micronucleus and aneuploidy compared to the cells treated with bleomycin alone.
Chow and Tung (2000a)	Escherichia coli strain XL-1 Blue exposed a 50-HZ magnetic field at 0.1-1.2 mT for 1 h	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).
Chow and Tung (2000b)	Escherichia coli strain XL-1 Blue (transformed by plasmid pUC8 that had been mutagenized by hydroxylamine exposed a 50-HZ	Improved efficiency of DNA repair mediated by the induced overproduction of heat shock proteins DnaK/J (Hsp70/40).

	magnetic field at 0.1-1.2 mT for 1 h	
Cichoń et al. (2018)	Human patients after stroke; 40-Hz, 5 mT EMF (rectangular bipolar waveform); ten 15-min sessions	Increased gene expression of brain-derived neurotrophic factor. (level measured in blood)
Cichoń et al. (2019)	Human patients after stroke; 40-Hz, 5 mT EMF (rectangular bipolar waveform); ten 15-min sessions	Increased expression of interleukin 1β mRNA
Cichoń et al. (2020)	Human patients after stroke; 40-Hz, 5 mT EMF (rectangular bipolar waveform); ten 15-min sessions	Increased apoptotic gene expressions (*BAX*, *CASP8*, *TNFα*, and *TP53)*
Ciombor et al. (2002)	Model of demineralized bone matrix (DBM)-induced bone formation in implanted rats;4.5 ms pulses, 15 pulses/second 1.6 mT EMF, ,75 Hz; 8 h/day for 6-8 days	Increased expression of PG and type II collagen mRNA
Colciago et al. (2021)	Human vestibular schwannoma HEI-193 cells expsoed to 50-Hz 0.1 T MF for 10 min or 10 min/day for 5 days	Hearing loss-related genes (NEFL, TPRN, OTOGL, GJB2, and REST) deregulated in chronic exposed cells.
Collard et al. (2013)	Epidermis cultures harvested from human abdominoplasty exposed to ELF electric fields (a biphasic, asymmetric, charge-balanced current	Observed a significant change in genes expression after 4 days and change in expression in another group of genes at day 4 and 7. Genes are involved in cell proliferation or differentiation, mitosis, cell

	stimuli, with a repetition frequency of 4 0 Hz modulated by a fundamental frequency of 0.125 Hz. The exposure was repeated during 4 s followed by a 4 s break for 40 min/day for 11 days	cycle or in the DNA replication transcription and translation.
Consales et al. (2018)	Human SH-SY5Y neuroblastoma cells and mouse primary cortical neurons exposed to a 50-Hz magnetic field at 1 mT for 4-72 h	Expressions of microRNA miR-34b/c that caused mitochondrial oxidative stress, also altered α-synuclein expression involved in synaptic functions. These effects may be related to neuro-degeneration.
Consales et al. (2022)	Neuroblastoma SH-SY5Y cells exposed to 50 Hz, 1 mT EMF for 24 h	Increased *egr-1* and *c-fos* expression
Coskun et al. (2021)	Rat pain -model; 10 or 30 Hz pulsed MF 1.5 mT, 1 h/day for 4 weeks	Changes in expression of gene related to voltage-gated sodium channels in dorsal root ganglion tissue
Costantini et al. (2019)	Human gingival fibroblast model for wound healing; 1 mT sinusoidal (50 Hz) or pulsed (0.3 ms, 12 Hz) EMF for 6 or 18 h	Increased in an early expression of IL-6, TGF-β, and iNOS genes and a later induction of MMP-2, MCP-1, and HO-1 genes
*Coulton et al. (2004)	Human blood exposed to 50 Hz EMF; 0-100 μT, 4 h	No detectable effect on expression of the genes encoding HSP27, HSP70A and HSP70B,
Cuccurazzu et al. (2010)	Mice exposed to 50 Hz EMF at 1 mT for 1-7 h/day for 7 days	Induced increases in the transcription of pro-neuronal genes (Mash1, NeuroD2, Hes1) and genes encoding Ca(v)1.2 channel α(1C)

		subunits in the hippocampus. Generation of new granule cells in the dentate gyrus.
Dehghani-Soltani et al (2021)	Temozolomide-treated T98 and A172 cells exposed to 50-Hz 7 mT EMF	overexpression of the p53 gene and downregulation of cyclin-D1 protein.
de Kleijn et al. (2016)	Hypothalamic paraventricular nucleus, pituitary, and adrenal glands from mice exposed to 20-500 Hz 0.01 mT EMF 1, 4, or 24 h/day for 1 or 15 weeks	Decreased proopiomelanocortin (POMC) gene expression; hypothalamic-pituitary-adrenal axis affected.
De Mattei et al. (2005)	MG-63 human osteoblast-like cell exposed to pulsed EMF (1.3 ms, 75 Hz, 1:10 duty cycle, 2.3 mT) for 0.5-24 h	Increased then decreased with longer exposure in c-myc and c-fos gene expression (involved in cell differentiation)
Del Re et al. (2006)	Escherichia coli exposed to sinusoidal or pulsed square wave 50-Hz magnetic field at 1 mT for 40 min	Sinusoidal magnetic field exposure induced a significantly higher level of DnaK and GroEL, whereas a lower level was observed after pulsed magnetic field exposure. When bacterial cells were exposed to heat shock (HS) after ELF-magnetic field exposure: again sinusoidal and pulsed fields resulted in an increase and in a reduction of HSP amount.
Delimaris et al. (2006)	Human lymphocytes exposed to 50-Hz pulsed electric fields (10-Hz carrier frequency) at 4 x 10^5 V/m for 120 min	Increased in DNA single strand breaks (Comet assay).
Di et al. (2021)	Mice exposed to static electric field (56.3 kV/m) 24h/day for 28 days	Decreased mRNA of testicular StAR, PBR, CYP11A1 (involved the mitochondria and free radicals)

Di Campli et al. (2010)	Helicobacter pylori biofilm exposed to 50-Hz EMF at 1 mT for 2 days	No changes in DNA patterns were recorded, whereas a modulation in amiA gene expression was detected; phenotypic changes induced.
Dominici et al. (2011)	Lymphocytes from welders (average magnetic field exposure from personal dosimeters 0.00781 mT (general environmental level 0.00003 mT)	Higher micronucleus frequency correlated with EMF exposure levels; decreased in sister chromatid exchange frequency.
Dong D et al. (2019)	Human pre-osteoclast RAW264.7 cells exposed to a 16 T static magnetic field for 2-4 days	HiSMF markedly blocked the expression of osteoclast-associated transcription factors and osteoclast marker genes and inhibited iron absorption and iron storage-related protein expression. Mitochondrial concentration and oxidative stress levels in osteoclasts were decreased under magnetic field exposure.
Dong L et al. (2022)	Mice exposed to static electric field of 56.3 kV/m for 21 days	Increased mRNA expression levels of tumor necrosis factor-α (TNF-α) and nuclear factor-κB (NF-κB) in the spleen; related to increase in free radicals
Dong Y et al. (2021)	Mouse MC3T3-E1 cells (can differentiate into osteoblasts and osteocytes) coated with piezoelectric scaffold (a property of bone) exposed to 50 Hz pulsed EMF (0.6 mT)	Increased expression of late osteogenic genes
Drzewiecka et al. (2021)	Myometrium of pigs exposed to 50 Hz 8 mT EMF for 2 h	Increased expression of genes involved in defense and immune responses
Du et al. (2008)	Cultured human lens epithelial cells exposed 50-Hz magnetic field at	Increased DNA doubled strand breaks (γ–H2AX foci) after 24 h exposure.

	0.4 mT for 2 h, 6 h, 12 h, 24 h and 48 h	
Duan et al. (2015)	A mouse spermatocyte-derived GC-2 cell line intermittently (5 min on and 10 min off) exposed to a 50 Hz EMF at 1, 2 or 3 mT for 24 h	Increased DNA strand breaks (Comet assay and γ–H2AX foci) at 3 mT exposure.
El-Bialy and Rageh (2013)	Mice with Ehrlich tumors exposed to a 50-Hz magnetic field 1 h/day for 2 weeks at 10 mT	Exposure cause DNA single strand breaks (Comet assay) in tumor cells and increased micronucleus frequency in bone marrow cells. ELF-MF enhanced the effects of cisplatin.
Erdal N et al. (2007)	Wistar rats exposed to 50 Hz magnetic field at 1 mT for 4 h or 4h/day for 45 days	Micronucleus frequency higher in bone marrow cells of long-term exposed rat. Mitotic index decreased in both exposed groups.
Erdal ME et al. (2018)	Mice (3- or 10-weeks old) exposed to 50 Hz 1 mT EMF. 4 h/day for 60 days	Age-dependent Increase or decrease in miRNA expression in blood and brain
Fadel et al (2017)	Agrobacterium tumefaciens exposed to square amplitude modulated wave at 10 Hz EMF (10 V Peak to peak), 90 min	Modified DNA structure (inhibited the growth and affected the microbe pathogenicity)
*Fairbairn and O'Neill (1994)	Human cells exposed to ELF-EMF	No significant effect on DNA single strand breaks (Comet assay)
Fan et al. (2015)	Rat bone marrow derived-mesenchymal stem cells exposed to a 50-Hz EMF at 1 mT for 4 h/day for 3 days	Increased cell viability, DNA synthesis and proportion of cells in S phase and up-regulated the expressions of hematopoietic growth factors.

Fan et al. (2018)	*Enterococcus faecalis* (isolated from dental infection) exposed to a static magnetic field at 170 mT for 24 or 72 h.	Static magnetic field up-regulated the expression of stress gene (dnaK) and virulence genes (efaA and ace). Synergistic with alkaline pH induced by calcium hydroxide (a major dental antimicrobial) in antimicrobial action and up-regulation of stress and virulence genes.
Fathi and Farahzadi (2017)	Rat adipose tissue-derived mesenchymal stem cells exposed to 50 Hz 20 mT EMF with Zn SO_4	Increased mRNA expressions of β-catenin, Wnt1, Wnt3a, LRP5 and DKK1; induced oeteogenic differentiation of the stem cells
Fatigoni et al. (2005)	Tradescantia (a perennial wildflower) exposed to a 50-Hz magnetic field at 1 mT for 6 or 24 h	Caused a time-dependent increase in micronucleus frequency.
Fedrowitz and Loscher (2012)	Female F344 and Lewis rats exposed to a 50-Hz magnetic field at 0.1 mT 24 h/day for two weeks	F344 breast tissue showed alterations in gene expression, which were absent in Lewis rats, particularly, α-amylase, a stress marker.
*Fiorani et al. (1992)	Human immortalized myelogenous leukemia K562 cells exposed to50-Hz electric (0.2-20 kV/m) or magnetic (0.0002-.2 mT) or combination of electric and magnetic fields, for 24 h	No detectable DNA lesions (measured by filter elution technique).
Focke et al. (2010)	Human fibroblasts exposed to intermittent (5 min ON/10 min OFF) 50-Hz EMF at 1 mT for 15 h	Increased DNA single strand breaks (Comet assay) caused by magnetic and not electric field, No oxidative DNA damage. Could be caused by minor disturbances in S-phase processes and occasional triggering of apoptosis rather than by the generation of DNA damage.

*Frahm et al. (2006)	Mouse macrophages exposed to a 50-Hz magnetic field for 45 min, 12, 24, or 48 h; 0.05 – 1 mT	No genotoxic effect (micronucleus formation); increased phagocytic activity, free radicals, and IL-1 beta production.
Franczak et al. (2020)	Procine myometrial slices expoed to 50 or 120 Hz EMF for 2 or 4 h	Altered CYP17A1 mRNA transcript abundance after 4 h exposure,
Franczak et al. (2022)	Porcine conceptuses during the peri-implantation period (days 15-16 of pregnancy) exposed to 50 and 120 Hz EMF for 2 or 4 h	Decreased CYP19A3 mRNA transcript in conceptuses treated with progesterone
Franczak et al. (2023)	Porcine myometrium during the peri-implantation period exposed to 50Hz EMF, 8 mT EMF for 2 h	Increased the level of genomic DNA methylation (epigenetic mechanisms)
*Frazier et al. (1990)	Human lymphocytes induced with DNA damage with ionizing radiation were exposed to 60-Hz magnetic field at 1 mT, electric field at 1 or 20 V/m, or combinations of magnetic and electric fields (0.2 V/m and 0.05 mT, 6 V/m and 0.6 mT, or 20 V/m and 1 mT) up to 180 min	EMF exposure did not affect repair of DNA single strand breaks (Comet assay).

Frisch et al. (2013)	Transfected rat primary fibroblast (RAT1) cells exposed to 10 Hz electric fields at 20-500 V/m for 2 h	Induced HSP70 heat shock expression, with peak responses obtained at 8 h following exposure.
Gholamian-Hamadan et al. (2023)	Rats exposed to 50 Hz EMF; 2 h/day for 60 days at 0.001, 0.1, 0.5 and 2 mT (with the immune system activated by human serum albumin)	Gene of activation-induced deaminase decreased by 0.0001 mT exposure.
Giorgi et al. (2011)	Two Escherichia coli model systems were exposed to sinusoidal or pulsed-square wave magnetic fields of various frequencies (20, 50, 75 Hz) and for different exposure times (15 and 90 min). at 1 mT	ELF-MF exposure affected transposition activity (transposon (Tn) mobility) and the effects critically depended on the wave shape of the field, but not on the frequency and the exposure time.
*Giorgi et al. (2014)	Human neuroblastoma BE(2)C cells treated with hydrogen peroxide exposed to 50-Hz pulsed magnetic field at 1 mT for 1-72 h	Pulsed magnetic field exposure did not interfere with genotoxicity (DNA double strand breaks measured by γ-H2AX foci) and cytotoxicity induced by oxidative stress.
Giorgi et al. (2017)	Human neural cells (BE(2)C) exposed to pulsed 50-Hz magnetic field at 1 mT for 24 and 48 h in combination with oxidative stress (hydrogen peroxide)	Pulsed magnetic field and oxidative stress induced weak decreases and increases of DNA methylation levels; combined exposure led to significant transient decrease of DNA methylation levels at different genome loci.

Guo et al. (2023)	Zebrafish larvae exposed to 50 Hz MF at 100-800 μT for 1 or 24 h for 5 days	Increased expression of the neurodevelopment-related gene syn2a. Attenuated by ROS scavenger.
Han et al. (2018)	Human MCF-7 breast cancer cells and MCF10A normal breast cells exposed to 50 Hz 1 mT EMF for 2-12 h, with 5-fluorouracil	Promoted DNA synthesis in MCF-7 cells affected cell cycle. EMF increased mRNA of cell cycle-related proteins Cyclin D1 and Cyclin E.
He et al. (2018)	Human bone marrow macrophages exposed to pulsed EMF (from a commercial machine)	Upregulation of genes of mesenchymal or osteoblastic cells and included members of the TGF-β signaling pathway and many extracellular matrix proteins,
Heidari et al. (2021)	Human adenocarcinoma gastric cancer cells exposed to 0.2 and 2 mT 50-Hz EMF continuously and discontinuously (1.5 h on/1.5 h off) for 18 h	Decreased expression of B-cell lymphoma 2 gene, and increased expression of miR-15-b and miR-16
Heredia-Rojas et al. (2010)	Human non-small cell lung cancer cells (INER-37) and mouse lymphoma cells (RMA E7) (transfected with a plasmid with hsp70 expression when exposed to magnetic field and contains the reporter for the luciferases gene) exposed to a 60-Hz magnetic field at 0.008 and 0.00008 mT for 20 min.	An increased in luciferase gene expression was observed in INER-37 cells exposed to magnetic field, but similar exposure had no effect on the RMA E7 cell line.

Heredia-Rojas et al. (2020)	Bone marrow micronucleated erythrocytes frequency of mice exposed to 60 Hz EMF at 2 mT for 72 h	Increased micronuclear formation; effect attenuated by resveratrol
Hirai et al. (2006)	Hippocampal neurons from embryonic rats exposed to static MF at 100 mT for 15 min	Induction of amidohydrolase for N-terminal asparagine gene (leading to MAP2 protein degradation through ubiquitin-proteasome pathway)
Hong et al. (2005)	Mice exposed to a 50-Hz EMF at 0.2 or 6.4 mT for 4 weeks	EMF induced DNA single strand breaks (Comet assay) in testicular cells and chromatin condensation in spermatozoa.
*Huang et al. (2023)	AML12 and HEK293 cells exposed to increasing frequency EMF 1-8 Hz in 8 sec of 4 msec pulses at 10 μT for 3 h	No effect on HSP80 and HSP90 gene expression. (Decreased protein expression and increased acetylation of HSP80 and HSP90, and enhanced protein folding.)
*Huwiter et al. (2012)	Escherichia coli K-12 MG1655 exposed at 50-Hz magnetic fields generated by three signal types (sinusoidal continuous, sinusoidal intermittent, and power line intermittent) at 1 mT for 8 min, 2.5 h, or 15 h	No effect on transcription of 4358 gene studied.
Hwang et al. (2023)	Immortalized mouse myoblast C2C12 cells transfected with electromagnetic perceptive gene and exposed to 196 mT static	Decreased gene expressions of Glucose transporter type 4 (GLUT4) and peroxisome proliferator-activated receptors (PPARg2), leading to decreased triglyceride synthesis via a calcium-dependent reaction.

	magnetic field for 12 or 48 h	
Ivancsits et al. (2002)	Human diploid fibroblasts exposed to continuous or intermittent (5 min ON/10 min OFF) 50-Hz EMF at 1 mT for 24 h	Intermittent exposure induced DNA single and double strand breaks (Comet assay).
Ivancsits et al. (2003a)	Human diploid fibroblasts exposed to intermittent (5 min ON/10 min OFF)50-Hz EMF at 0.02- 1 mT for 1-24 h	DNA Single and double strand breaks (Comet assay) observed at 0.035 mT at 15 h; recovered within 9 h.
Ivancsits et al.(2003b)	Fibroblasts from human subjects of different ages exposed to intermittent (5 min ON/10 min OFF) 50-Hz EMF at 1 mT for 1-24 h	Increased DNA Single and double strand breaks (Comet assay) at 15 h; more pronounced in cells from older donors
Ivancsits et al. (2005)	Various cell types exposed to intermittent (5 min ON/10 min OFF) 50-Hz EMF at 1 mT for 1-24 h	Effects on DNA Single and double strand breaks (Comet assay) showed three responder (human fibroblasts, human melanocytes, rat granulosa cells) and three non-responder cell types (human lymphocytes, human monocytes, human skeletal muscle cells).
Jajte et al. (2001)	Rat peripheral blood lymphocytes exposed to a 50-Hz magnetic field at 7 mT for 3 h	Increased DNA single strand breaks (Comet assay) in cells treated with ferrous chloride; melatonin attenuated the effect.
Jedrzejczak-Silicka et al. (2021)	Fibroblast L929 and HaCaT cells exposed to rotating MF of different frequencies (30 and 50	Exposure parameter-dependent up and down regulations of genes (including wound healing genes) (free radicals and calcium involved)

	Hz) and intensities (up to 28.4 mT) for 4 h	
Jeong et al. (2021)	Human glioblastoma cells (U373) exposed to 'tumor-treating field' (120 kHz, 1.2 V/cm electric field)	Decreased DNA damage and gene expression levels of BRCA1, PCNA, CDC25C, and MAD2
*Jin H. et al. (2015)	Non-tumorigenic human lung epithelial L132 cells exposed to a 60-Hz magnetic field at 1 or 2 mT for 9 h	No G2/M arrest or aneuploidy nor interaction with gamma radiation and H_2O_2
*Jin YB et al, (2012)	Mouse embryonic fibroblast NIH3T3 cells and human lung fibroblast WI-38 cells exposed to a 60 Hz magnetic field at 1 mT for 4 h	No significant effect on micronucleus frequency and interaction with ionizing radiation, H_2O_2, or c-Myc activation.
*Jin YB et al, (2014)	NIH3T3 mouse fibroblast cells, WI-38 human lung fibroblast cells, L132 human lung epithelial cells, and MCF10A human mammary gland epithelial cells exposed to a 60-Hz magnetic field at 1 mT for 4 or 16 h	No significant effect on DMA single strand breaks (Comet assay), and interaction with ionizing radiation, H_2O_2, or c-Myc activation.
Jin Y et al. (2019)	Arabidopsis young seedlings exposed to a static magnetic field at 600 mT	Increased auxin (a plant growth hormone) from expression of PIN3 and AUX1 genes in root tips; cryptochromes (cry1 and cry 2) are also involved. Root growth enhanced. Effects occurred when static magnetic field was

		parallel and perpendicular not opposite, to geomagnetic field.
Jouni et al. (2012)	*Vicia faba* (broad bean) culture in soil with high background radioactivity and exposed to static magnetic field at 15 mT for 8h/day for 8 days	Increased chromosomal aberration and DNA damage in root tip cells with lowering of antioxidant defense; soil radioactivity enhanced the effects.
Karimi et al. (2022)	Human endogenous retroviruses in human skin malignant melanoma SK-MEL-37 cells exposed to 50-Hz EMF at 1.5 and 3 mT for 96 hours.	Downregulated HERV gene expression
Kazemi et al. (2018)	Male rhesus macaques exposed to1 or 12 Hz 0.0007 mT EMF 4h/day for 30 days	Increased NMDA receptor gene expression with 12 Hz exposure (enhanced visual working memory function)
Kazemi et al. (2022)	Male rhesus macaques exposed to 12 Hz 0.0007 mT EMF 4h/day for 30 days	Increased NMDA receptor gene expression (enhanced visual working and visual workin memory function; increased plasma adrenocorticotropic hormone)
Kesari et al. (2015)	Human neuroblastoma SH-SY5Y cells exposed to a 50-Hz 100 μT magnetic field for 24 h.	Micronucleus formation was observed at 15 and 30 days postexposure. Effect not related to oxidative changes.
Kesari et al. (2016)	Human glioblastoma SH-SY5Y and rat glioma C6 cells exposed to a 50-Hz magnetic field at 0.01 and 0.03 mT for 24 h with menadione as a cofactor	Micronuclei were significantly increased in SH-SY5Y cells at 0.03 mT Increased cytosolic and mitochondrial superoxide levels were observed in C6 cells. The results indicate that the threshold for biological effects of ELF magnetic field is 0.01 mT or less.

Khalil and Qassem (1991)	Human lymphocytes exposed to a pulsing 50-Hz EMF at 1.05 mT for 24, 48 and 72 h	Suppression of mitotic activity and a higher incidence of chromosomal aberrations. Delay in cell proliferation index and an increase in the baseline frequency of sister-chromatid exchanges occurred only after 72 h f exposure.
Ki et al. (2020)	Human hair follicle dermal papilla cells, a type of cells involved in hair growth, exposed to a 70 Hz EMF at intensities ranging from 0.5 to 10 mT over four days	Increased the expression of anagen-related molecules, including collagen IV, laminin, ALP, and versican, and increased β-catenin and Wnt3α expression and GSK-3β/ERK/Akt phosphorylation. Cell proliferation enhanced.
Kim HJ. et al. (2013)	Bone marrow derived mesenchymal stem cells (BM-MSCs) were subjected to a 50-Hz EMF	Increased levels of neuronal differentiation marker (MAP2), while early neuronal marker (Nestin) was down-regulated; increased differentially expression of 8 proteins; notably, a significantly increased expression of the ferritin light chain.
Kim J. et al. (2010)	IMR90 (human lung fibroblast) primary cells and HeLa (human cervical carcinoma) cells exposed to a time-varying (rotating) 60-Hz magnetic field at 6 mT for 60 min or 30 min/day for 3 days	Repeated exposure showed DNA double strand breaks (γ-H2AX foci) and decreased cell viability and increased apoptosis through p38 activation.
Kim J. et al. (2012)	Human primary fibroblast and cervical cancer cells exposed to a time-varying 60-Hz magnetic field at 7 mT for 10-60 min	DNA double strand breaks (γ-H2AX foci and Comet assay) detected (intracellular reactive oxygen species not affected).

Kimsa-Dudek et al. (2018)	Normal human dermal fibroblasts exposed to static magnetic field at 0.65 T for 24 h and sodium fluoride	Static magnetic field attenuated expression of antioxidant defense genes (SOD1, PLK3, CLN8, XPA, HAO1) induced by sodium fluoride.
Kimsa-Dudek et al. (2020)	Normal human dermal fibroblasts exposed to static magnetic field at 0.45, 0.55 and 0.5 T for 24 h and sodium fluoride	The field reduced fluoride-induced apoptosis and affected apoptosis gene expression; reduced fluoride-induced increases in reactive oxygen species and lipid peroxidation and decrease in antioxidant enzymes.
Kimsa-Dudek et al. (2022)	Human skin malignant melanoma C32 cells treated with phenolic acid and exposed to 0.7 T Static MF for 24 h	Static MF interacted with phenolic acid affected expression of apoptosis (Bax, BC12 and BclX1)and Caspase 3 and 9 genes.
Kimura et al. (2008)	*Caenorhabditis elegans* exposed to 2, 3, or 5 T static magnetic field for 4-24 h	Genes involved in motor activity, actin binding, cell adhesion, and cuticles are transiently and specifically induced; also hsp (heat shock protein) 12 and 16 family genes.
Kindzelskii and Petty (2000)	Human neutrophils exposed to pulsed square-wave (20 msec) DC electric field at 0.2 V/m for 30, 45, 60 min	Increased DNA single strand breaks (Comet assay).
*Kirschenlohr et al. (2012)	Male human subjects exposed to 50-Hz EMF at 0.062 mT for 2 h (Exposure repeated two more times.)	No genes or gene sets in blood samples showed consistent response profiles to repeated ELF-EMF exposures (including immediate early genes, stress response, cell proliferation and apoptotic genes).
Kitaoka et al. (2013)	Mice exposed to 60-Hz 1.5 mT EMF for 200 h	Reduction in Cyp17a1 mRNA (adrenal corticosteroid synthesis enzymes); animals showed depression-like behavior

Kostyn et al. (2023)	Flax seedlings exposed to 50 Hz EMF at 0.5 mT for 30 min	Increased expressions of different genes including those involved in stress and ROS processing.
Koyama et al. (2008)	Human glioma A172 cells exposed to a 60-Hz magnetic field at 5 mT for 2, 4, 8, 16, 24 h	The number of apurinic/apyrimidinic sites induced by gentoxic agents methyl methane sulfonate and H_2O_2 was enhanced by exposure to ELF magnetic fields. (Apurinic/apyrimidinic sites are common DNA lesions arise from spontaneous depurination or by base excision repair of oxidized, deaminated or alkylated bases.)
Koziorowska et al. (2020)	Honeybee (Apis mellifera L.) exposed to 50 Hz 1.6 mT EMF 2-48 h	Changes in structure in DNA and RNA
Kozlowska et al. (2021)	Pig endometrium exposed to 50 Hz 8 mT EMF for 2 h	Affected 1561 transcriptionally active regions in the genome including genes encoding proteins that are involved in proliferation and metabolism in endometrial tissue.
Kubinyi et al. (2010)	Human lymphocytes exposed to an inhomogeneous static magnetic field with a lateral magnetic flux density gradient of 47.7, 1.2, or 0.3 T/m by 10 mm lateral periodicity, or a homogeneous SMF of 159.2 mT magnetic flux density for a time period of 0.5 min, 1, 2, 4, 6, 18, 20, or 24 h.	Increased DNA single strand breaks (Comet assay); affected DNA repair induced by gamma ray when exposure occurred after ionizing radiation treatment.
Kumari et al. (2017)	Mice exposed continuously for 5 weeks	Expression of the pro-inflammatory cytokine tumor necrosis factor alpha mRNA was

	to 7.5 KHz MF at 0.12 mT	significantly increased in the hippocampal region; impairment of memory observed.
Kwiatkowski et al. (2022)	Staphylococcus aureus FRI913 strain exposed to 5 or 50 Hz rotating magnetic field for 12 h	50 Hz increased *agrA* and *sea* transcripts (regulatory genes)
*Lacy-Hulbert et al. (1995)	Human leukemic cells (HL60) exposed to a 60-Hz EMF for 20 min at 0.00057, 0.0057, or 0.057 mT	No change in MYC and beta-actin gene expression observed.
Lagroye and Poncy (1997)	Rat tracheal epithelial cell lines were first exposed to gamma rays and then cultured in a 50-Hz magnetic field at 0.1 mT for 24 h.	Increased binucleated cells with micronuclei in cells exposed to gamma rays and magnetic field, compared with gamma irradiation alone. Magnetic field alone had no significant effect on micronucleus frequency.
Lai and Singh (1997a)	Male Sprague-Dawley rats exposed to a 60-Hz magnetic field at 0.1, 0.25, or 0.5 mT for 2 h	Increased DNA single and double strand break (Comet assay) in brain cells.
Lai and Singh (1997b)	Male Sprague-Dawley rats exposed to a 60-Hz magnetic field at 0.5 mT for 2 h	Increased DNA single and double strand break (Comet assay) in brain cells. Effects blocked by melatonin and a spin-trap compound.
Lai and Singh (2004)	Male Sprague-Dawley rats exposed to a 60-Hz magnetic field at 0.01 mT for 24 or 48 h	Increased DNA single and double strand break (Comet assay) in brain cells. More effect with 48-h than 24-h exposure. Effects blocked by Trolox (a vitamin E analog) and 7-nitroindazole (a nitric oxide synthase inhibitor).
Laramee et al. (2014)	Transfected rat primary fibroblast (RAT1) cells exposed to static	Induction of heat shock protein (HSP70) expression showed a dependency on flux

	magnetic fields of 1 to 440 mT for 16, 24, or 48 h starting at 24 and 48 h post transfection	density, exposure duration, and start time post transfection.
Lazzarini et al. (2023)	Human breast cancer MDA-MB-231 and normal MCF-10A breast cells exposed to 50 Hz 1 mT EMF for 4 h	Up-regulated the genes enriched in "focal adhesion" and "mitochondrion in both cell lines; transcription factors associated with cellular reprogramming were upregulated in MDA-MB-231 cells and downregulated in MCF-10A cells. (Involved free radicals)
Lee C-H et al. (2010)	*Caenorhabditis elegans* exposed to exposed to a static magnetic field at 200 mT	Expression of genes involved in development and aging. Accelerated development and shorten lifespan.
Lee HC et al. (2016)	MCF10A, MCF7, Jurkat, and NIH3T3 cells exposed to a 60 Hz magnetic field at 1 mT for 4 or 16 h	MCF10A and MCF7 cells showed consistent and significant decreases in cell number, cell viability, and DNA synthesis rates (cell cycle delay), whereas Jurkat and NIH3T3 cells showed no effect. MCF7 cells (2 mT for 16 h) showed up-regulation of PMAIP1 gene (involved in apoptosis).
Lee JW et al. (2011)	Human lymphocytes exposed to EMF generated during MRI scanning (clinical routine brain examination protocols: three-channel head coil) for 22, 45, 67, and 89 min	Significant increases in DNA single-strand breaks (Comet assay), and frequencies of both chromosome aberrations and micronuclei in a time-dependent manner.
Leone et al. (2014)	Neural stem cells isolated from hippocampi of newborn mice exposed to a 50-Hz EMF at 1 mT for 10 days	Histone acetylation-related chromatin remodeling leading to enhanced proliferation and neuronal differentiation.

Li and Chow (2001)	E. coli XL-1 Blue transformed with plasmid pUC18 and DNA samples exposed to a 50-Hz magnetic field at 1.2 mT for 1-5 h, with heat shock response suppressed	Without the protection of the heat shock response, magnetic field exposure induced DNA degradation, which could be attenuated by the presence of an antioxidant,
*Li L. et al (2015)	Workers from a power supply bureau (inspection workers vs. logistic staff); The average time-weighted average was 0.0073 mT (0.00156-0.02633 mT) and the subjects were subgrouped by cumulative ELF-magnetic field exposure dose: low (<0.0156 mT), middle (0.0156-0.073 mT) and high (> 0.073 mT)	No significant effect on the frequency of micronucleus lymphocytes or micronuclei frequency; no changes in antioxidant enzymes and cellular oxidative damage.
Li SS et al. (2013)	Male *Drosophila melanogaster* fruit flies exposed to a 50-HZ EMF at 3 mT for 72 or 312 h	Different sets of genes were up- and down-regulated after short- or long-term exposure. Short-term exposure may decrease the reproductive ability of males, whereas long-term exposures had no effect on reproductive ability.
Li Y. et al. (2014)	Fertilized embryos of zebra fish (*Danio rerio*) exposed to a 50-Hz magnetic field at 0.1 - 0.8 mT for 96 h	The transcription of apoptosis-related genes (caspase-3, caspase-9) was significantly up-regulated in exposed embryos. Delayed hatching and apoptosis observed.
Li, Y. et al. (2015)	Rat oligodendrocyte precursor cells exposed	Mitogen-activated protein kinase pathway that signals cell migration was significantly

	to DC electric field at 50, 100. Or 200 mV/mm for 1.5 h	upregulated in cells treated with an EF of 200 mV/mm compared with control cells and downregulation of differentially expressed genes in chemotaxis.
Li Y. et al. (2019)	Dementia rats induced by streptozotocin (STZ) intracerebroventricular injection exposed to a 10 mT 20-Hz pulsed EMF, 2 h/day, 10 days	Pulsed EMF increased expression of insulin growth factor 2 (IFG-2) in the hippocampus and improved the ability of learning and memory in STZ-treated rats.
Li Y. et al. (2022)	Honeybee (*Apis cerana*) larvae exposed to 50Hz 3 mT EMF from second day of life to end of development	Altered expression of 422 genes, with six genes related to energy production and nutrient u[take and utilization downregulated (decreased survival rate and extended development time)
Lin H et al. (1998)	60 Hz EMF at 0.008 mT	Induction of HSP70 gene expression by c-myc protein
Lin KW et al. (2016)	Budding yeast exposed to a 50-Hz EMF at 6 mT for 96 h	The transcription levels of 28 genes were upregulated and those of four genes were downregulated. Exposure can upregulate the expression of genes involved in glucose transportation and the tricarboxylic acid (TCA) cycle, but not the glycolysis pathway.
Liu C et al. (2023)	Zebrafish embryos exposed to 11.4 T magnetic field from 6-24 hours post fertilization	up-regulation of tumor necrosis factor genes
Liu Y et al. (2015a)	Mouse spermatocyte-derived GC-2 cell line exposed to an intermittent (5 min ON/10 min OFF) 50-Hz EMF at 1, 2, or 3 mT for 72 h	Exposure decreased genome-wide methylation at 1 mT, but global methylation was higher at 3 mT. Expression of DNMT1 and DNMT3b (DNA methyltransferases) was decreased at 1 mT, and increased at 3 mT.

Liu Y et al. (2015b)	Mouse spermatocyte-derived GC-2 ells exposed to 50-Hz EMF for 72 h (5 min on/10 min off) at 1, 2, or 3 mT	Up- and down-regulation of 15 miRNA depending on field intensity (These miRNAs may regulate circadian rhythms, cytokine-cytokine receptor interactions and the p53 signaling pathway).
Liu Y et al. (2016)	Mouse spermatocyte-derived GC-2 ells exposed to 50-Hz EMF for 72 h (5 min on/10 min off) at 1, 2, or 3 mT	Increased expression of Cyckin D2 (CCND2) gene and miR-26b-5p
López-Díaz et al. (2022)	DNA prepared from *S. cerevisiae* cultures exposed to 25 Hz 1.5 mT EMF up to 72 h	EMF enhanced DNA damage induced by bleomycin and methyl methanesulfonate.
*Lopucki et al. (2005)	Cotyledons dissected from placentas obtained immediately after physiological labors exposed to a 50-Hz magnetic field at 2 or 5 mT for 3 h	No significant effect on level of 8-hydroxy-2'-deoxyguanosine in DNA (oxidative DNA damage).
Lourencini da Silva et al. (2000)	SnCl2-treated pBR322 plasmids exposed to a 3400 Hz square-wave EMF with peak power of 4V for 2 h	An EMF-dependent potentiation of DNA scission (i.e. the appearance of relaxed plasmids) was observed. The results indicate that the EMF, in the presence of a transition metal, is capable of causing DNA damage.
*Luceri et al. (2005)	Human peripheral blood lymphocytes and DBY747 *Saccharomyces cerevisiae* exposed to a50-Hz magnetic field at 0.001, 0.01or 0.1 mT for 18 h	No significant effects on DNA single strand breaks (Comet assay), oxidated DNA base, and gene expression.
Luo et al. (2006)	Preimplantation mouse embryos in vitro exposed	Induction of gammaH2AX foci (double strand break) formation

	to 50 Hz 0.3 or 0.5 Mt EMF for 24 or 48 h	
Lupke et al (2006)	Human umbilical cord blood-derived monocytes exposed to a 50-Hz magnetic field at 1 mT for 45 min	Alteration of 986 genes involved in metabolism, cellular physiological processes, signal transduction and immune response.
Luukkonen et al. (2011)	Human SH-SY5Y neuroblastoma cells. Exposed to a 50-Hz magnetic field at 0.1 mT for 24 hours, followed by chemical (menadione) exposure for 3 h	Magnetic field enhanced menadione-induced DNA damage, DNA repair rate, and micronucleus formation. No effects were observed after magnetic field exposure alone.
Luukkonen et al. (2014)	Human SH-SY5Y neuroblastoma cells. Exposed to a 50-Hz magnetic field at 0.1 mT for 24 hours, followed by menadione exposure for 3 h	Persistently elevated levels of micronuclei were found in the progeny of magnetic field (alone)-exposed cells at 8 and 15 days after exposure, indicating induction of genomic instability. (No magnetic field x menadione interaction effect). Magnetic field disturbed oxidative balance immediately after the exposure, which might explain the previous findings on MF altered cellular responses to menadione-induced DNA damage.
Luukkonen et al. (2017)	Human SH-SY5Y neuroblastoma cells. Exposed to a 50-Hz magnetic field at 0.1 mT for 24 hours, followed by menadione exposure for 1 or 3 h	Decreased p21 protein (a DNA damage response-related proteins) level after 1-h menadione treatment, as well as increased proportion of cells in the G1 phase and decreased proportion of S phase cells after 3-h menadione treatment. Magnetic field exposure decreased DNA single strand breaks (Comet assay) caused by I h treatment with menadione.
*Lv et al. (2021)	Human amnion epithelial cells (FLs), human skin fibroblast cells (HSFs),	No effects on γH2AX foci (double strand break) in cells

	and human umbilical vein endothelial cells (HUVECs) were exposed to 50 Hz ELF-MF at 0.4, 1, or 2 mT for 15 min, 1 h, or24 h,	
Ma et al. (2014)	Mouse embryonic neural stem cells exposed to a 50-Hz EMF at 2 mT for 3 days	Expression of genes regulating neuronal differentiation was altered.
Ma et al. (2016)	Embryonic neural stem cells exposed to 50-Hz 1 mT EMF for 4 h/day for 1, 2, or 3 days	Up-regulated the expression of transient receptor potential canonical 1 and proneural genes (NeuroD and Ngn1) (gene responsible for neuronal differentiation and neurite outgrowth, which were observed after EMF exposure)
Mahaki et al. (2019)	Rats exposed to a 50-Hz EMF at 0.001-2 mT for 2 h/day for 60 days	In the spleen, gene expression levels of RORα (retinoid-related orphan receptor alpha) and c-Maf (transcription factor Maf) were significantly down-regulated at 0.001 and 0.1 mT, while the expression of STAT6 (signal transducer and activator of transcription 6) was only significantly decreased at the density of 0.1 mT. No effect on thymus.
Mahdavinejad et al. (2018)	Spleen Th17 and regulatory T (Treg) cells of rats exposed to 50 Hz EMF at 0.001, 0.1, 0.5 and 2 mT for 2 h/day for 2 months	expression of transcription factor forkhead box P3 (Foxp3) was downregulated at intensities of 0.001 and 0.1 mT
Mahmoudinasab and Saadat (2016a)	Human MCF-7 cells exposed to a 50-Hz magnetic field at 0.25 and 0.5 mT (5 min ON/5 min OFF, 15 min ON/15	Alterations in the *NQO1* and *NQO2* (NAD(P)H: quinone oxidoreductase) mRNA levels seen at the "5 min ON/5 min OFF" condition.

	min OFF, or 30 min field-on continuously) for 30 min	
Mahmoudinasab and Saadat (2016b)	Human MCF-7 cells exposed to 50 Hz 0.25 or 0.5 mT EMF (5 min field-on/5 min-off; 15 min field-on/15 min field-off; or continuos field) for total exposure time of 30 min	Up and down regulations of the antioxidant genes (*CAT*, *SOD1*, *SOD2*, *GSTO1*, *GSTM3*, *MSGT1*, and *MSGT3*) depending on exposure pattern and intensity.
Mahmoudinasab et al. (2016c)	Human MCF-7 cells exposed to a 50-Hz magnetic field at 0.25 and 0.5 mT (5 min ON/5 min OFF, 15 min ON/15 min OFF, or 30 min field-on continuously) for 30 min	Significant changes in mRNA levels of seven antioxidant genes for "the 15 min field-on/15 min field-off condition".
Mahmoudinasab and Saadat (2018a)	MCF-7 and SH-SY5Y cells exposed to 50-Hz EMF at 0.5 mT (15 min ON/ 15 min OFF), and treated with morphine and cisplatin.	EMF exposure could protect SH-SY5Y cells from the cytotoxicity of cisplatin and morphine, whereas it has no significant change in MCF-7 cells. Expression patterns of antioxidant genes are different in both cell lines.
Mahmoudinasab and Saadat (2018b)	SH-SY5Y cells exposed to 50-Hz EMF at 0.5 mT (“15 min ON/ 15 min OFF” and “30 min ON”) for 30 min, and treated with morphine and beta-lapachone	NQO1 mRNA level decreased in the "15 min field-on/15 min field-off" condition, the expression level of NQO2 was increased. Morphine and EMF reduced the cytotoxicity of beta-lapachone.
Mairs et al. (2007)	UVW human glioma cells to a 50-Hz EMF at 1 mT for 12 h	Induced 0.011 mutations/locus/cell, which was equivalent to a 3.75-fold increase in mutation induction compared with unexposed controls. The field also

		potentiated the mutagenic capacity of gamma-irradiation.
Mansoury et al. (2021)	Human gastric adenocarcinoma cell line (AGS) and human normal fibroblast (Hu02) cells exposed to 50-Hz EMF of 0.25, 0.5 , 1, or 2 mT for 18 h (1 h ON/1 h OFF)	Downregulation of NOTCH1 and its regulatory circular RNA (circ-RNA) in cancer cells and upregulated in normal cells.
Mansoury et al. (2022)	Human gastric adenocarcinoma cell line (AGS) and human normal fibroblast (Hu02) cells exposed to 50-Hz EMF of 0.25, 0.5 ,1, or 2 mT for 18 h continuously.	Decreased rapamycin (mTOR) and hsa_circ_100338 in cancer cells at 0.25 and 0.5 mT . mTOR and hsa_circ_100338 are involved in cell growth, proliferation, differentiation, and metastasis). mTOR expression increased in normal cells.
Manzella et al. (2015)	Human dermal fibroblasts exposed to a 50 Hz magnetic field at 0.1 mT for 1 h	Changes in expression of clock genes.
Mariucci et al. (2010)	CD1 mice exposed to a 50-Hz magnetic field at 1 mT for 1 or 7 days (15 h/day)	Increased DNA single strand breaks (Comet assay) in brain areas detected immediately after 7-day exposure. No effect on HSP-70 expression.
Markkanen et al. (2008)	Murine L929 fibroblasts exposed to a 50-Hz magnetic field at 0.1 or 0.3 mT for 24 h, with or without ultraviolet B (UVB, wavelength 280-320 nm) radiation or menadione (MQ)	Pre-exposure to magnetic field can alter cellular responses to other agents, and indicate that magnetic field as low as 0.1 mT has measurable impacts on cancer-relevant cellular processes such as DNA-damage.
Martinez et al. (2022)	Human NB69 neuroblastoma cells exposed to 50 Hz 0.1 mT MF for 30 or 120 min	Increased p53 gene expression

Martini et al. (2020)	Human mesenchymal stem cells exposed to pulsed 75 Hz 1.5 mT EMF in combination with bone morphogenetic protein-2 (BMP2) for 28 days	increased BMP2, BMP6, and BMP type I receptor gene expression, involved in mesenchymal stem cells osteogenic differentiation
Mastrodonato et al. (2018)	Mice exposed to a 50 Hz, 1 mT EMF 3.5 h/day for 12 days	Increased Wnt3 (neurogenesis gene) mRNA expression and nuclear localization of its downstream target β-catenin in subventricular zone of the lateral ventricle. Mice showed enhanced olfactory memory at 30 days post-exposure.
*Mayer-Wagner et al. (2018)	human mesenchymal stem cells exposed to 15 Hz 5 mT EMF for 3 weeke	No effect on COLXA1 and COL2A1 gene expression. MF interacts with simulated microgravity on expression of these gene.
*McNamee et al. (2002)	10-day-old mice exposed to a 60-Hz magnetic field at 1 mT for 2 h, cerebellum assayed at 0, 2, 4, and 24 h after exposure	DNA single strand breaks (Comet assay): "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." "Taken together, these results do not support the hypothesis that acute MF exposure causes DNA damage in the cerebellums of immature mice." No change in apoptosis.
*McNamee et al. (2005)	Rodents (adult rats, adult mice, and immature mice) exposed to a 60-Hz magnetic field at 0.1, 1 or 2 mT for 2 h. Assayed at 0, 2 and 4 h after exposure	This study provided no evidence of magnetic-field-induced DNA single strand breaks (Comet assay) in the brain.

Mehdizadeh et al. (2023)	Uman glioblastoma U87 and U251 cells exposed to 1 Hz 100 mT EMF , 2 h/day for 5 days	Expression level of P53, P21, and MDM2 increased and CCNB1 decreased in U87, MCM6 expression decreased in U251. (Changes may ber elated to apoptosis induced by the field.)
Mercado-Sáenz et al. (2019)	*Saccharomyces cerevisiae* wild type strain (WS8105-1C) exposed to sinusoidal magnetic field (2.45 mT, 50 Hz, continuous) or pulsed magnetic field (1.5 mT, 25 Hz, 8 h/day). Chronological aging was evaluated during 40 days	Decreased spontaneous frequency of mitochondrial mutation during aging was observed in pulsed magnetic field-treated samples.
*Miller et al. (1999)	Human promonocytic U937 leukemia cells exposed to 60 Hz EMF of 0.08, 0.1, 1.0 or 1.3 mT	No effect on NF-kappaB or AP-1-dependent reporter gene expression
Miller et al. (2016)	Human annulus fibrosus (AF) and nucleus pulposus (NP) cells exposed to pulsed EMF (15 Hz burst frequency square wave), 4 h/day for 7 days; cells were exposed to interleukin 1α (IL-1α) to stimulate the inflammatory environment	EMF reduced IL-1α-associated gene expression of IL-6 in NP cells and MMP13 in AF cells
Miyakawa et al. (2001)	Transgenic Caenorhabditis elegans exposed to 60 Hz 0.5 T EMF	Expression of the hsp-16-lacZ gene was enhanced.

*Miyakoshi et al. (1996a)	Chinese hamster ovary (CHO) cells exposed to a 60-Hz magnetic field at 5 mT for 130 h	No significant effect on c-myc expression and cell growth rate.
Miyakoshi et al. (1996b)	Human melanoma MeWo cells exposed to a 50-Hz magnetic field at 400 mT up to 20 h	Induced mutations in the hypoxanthine-guanine phosphoribosyl transferase gene, synergistic with X-ray. No significant increase in mutant frequency occurred when DNA replication was inhibited during magnetic field exposure. DNA replication error is suspected of causing the mutations produced by ELFMF exposure.
Miyakoshi et al. (1997)	Human melanoma MeWo cells exposed to a 50-Hz magnetic field at 400 mT for 2 h	Induced mutations in the hypoxanthine-guanine phosphoribosyl transferase gene, DNA replication errors and/or disturbance of the mismatch repair systems caused by exposure to ELF-MF may be involved in the mutagenic effect.
Miyakoshi et al. (1998)	Human osteosarcoma cells (Saos-LP-12), with deleted 53 gene, exposed to a 50-Hz magnetic field at 400 mT for 4 h	Induced mutations in the hypoxanthine-guanine phosphoribosyl transferase gene. Introduction of the wild-type (wt) p53 expression plasmid (pOPRSVp53) suppressed the magnetic induced mutation. The findings suggest that wt p53 has a function in suppression of DNA replication errors and/or in maintenance of genomic stability after high-density magnetic field exposure.
Miyakoshi et al. (1999)	Chinese hamster ovary K1 (CHO-K1) cells exposed to a 60-Hz magnetic field at 5 mT for up to 6 weeks	No effect on mutant frequency of the hypoxanthine-guanine phosphoribosyl transferase but enhanced the effect of x-ray.
Miyakoshi et al. (2000)	Human glioma MO54 cells exposed to a 50-Hz magnetic field at 55, 50,	Exposure to magnetic field at more than 50 mT potentiated X-ray-induced DNA single strand breaks (Comet assay).

	or 400 mT at 4^0C or on ice, for 30 min	
*Mizuno et al. (2014)	Human fibroblast WI38VA13 subcloned 2RA and XP2OS(SV) cells exposed to a 60-Hz magnetic field at 5 mT for 24 h	Magnetic field exposure did not have modification effect on cell survival after UV-B irradiation and on repair process of DNA damage induced by UV-B irradiation.
Mohamed et al. (2022)	Human MCF-7 breast cancer cells exposed to 1 mT ELF-EMF for 1 or 2 h	Up-regulation in the expression of p53, iNOS and NF-kB genes, and down-regulation of Bcl-2 and miRNA-125b genes
Molina-Montenegro et al. (2023)	Honetbees exposed to ELF EMF at 7.8 μT for 5 min	Enhanced expression of heat-shock proteins and genes involved in antioxidant activity and affected the expression levels of behavior-related genes.
Monirul Islam et al. (2020)	Arabidopsis thaliana exposed to near null magnetic field (30 nT)	Genes involved in lipid metabolism affected.
Moraveji et al. (2016)	Dermal papilla mesenchymal cells exposed to 50-Hz EMF at 1 mT for 5-14 days	Increased expression of MAP gene with decreased cell proliferation (cell differentiation occurred.) (MAP2 protein involves in neuritogenesis to stabilize microtubules.)
Moretti M et al. (2005)	Jurkat cells exposed to a 50-Hz magnetic field at 1 mT for 1 h with added xenobiotics	Magnetic field exposure enhanced genotoxic effects (DNA single strand breaks (Comet assay) of xenobiotics.
Mouhoub et al. (2017)	Salmonella hadar grown under static magnetic field of 200 mT for 3, 6, or 9 h	Increased expression of gene involved in the production of acdiolipin and phosphatidylethanolamine (both components of bacteria cell membrane).

Mustafa et al. (2021)	Murine FDC-P1 hematopoietic cells exposed 50 Hz 0.2 mT MF for 15 min, 2 h, 12 h, or 24 h	Circadian rhythm-related genes were upregulated after 12 h of MF exposure and downregulated after 24 h of MF exposure
Mustafa et al. (2022)	Human SH-SY5Y neuroblastoma cells exposed to 0.1 mT 50-Hz or 60-Hz MF for 24 h	A general low-magnitude increase in the expression of reactive oxygen species-related genes
Nakayama et al. (2016)	Macrophages stimulated with the bacterial endotoxin, lipopolysaccharide and posed to a 50-Hz magnetic field at 0.5 mT for 24 h	Increased DNA single strand breaks (Comet assay) and decreased viability.
Nasrabadi et al. (2018)	Neonatal human retinal pigment epithelial cells exposed to pulsed 50-Hz EMF at 1 mT for 8 h daily for 3 days	Both gene and protein expressions of retinal progenitor cell markers were reduced.
*Nguyen et al. (2023)	Human B lymphoblastoid (TK6) cell exposed to 50 Hz MF at 10, 100, or 500 μT) for different exposure periods ranging from 96h up to 6 weeks.	No effect on cell genetic damage (Comet assay), and cell sensitivity to damage (micronucleus formation) induced by known mutagens
Nikolova et al. (2005)	Mouse embryonic stem (ES) cells exposed to an intermittent (5 min ON/30 min OFF) 50-Hz EMF at 2 mT for 6 or 48 h	Significantly affected transcript levels of the apoptosis-related bcl-2, bax, and cell cycle regulatory "growth arrest DNA damage inducible" GADD45 genes, No effect on DNA single and double strand breaks (Comet assay).

*Okudan et al. (2010)	Swiss mice exposed to a 50-Hz EMF at 0.001 - 0.005 mT for 40 days	The results suggest that </=0.005 mT intensities of 50 Hz EMFs did not cause genotoxic effect in the mouse.(However, The number of micronucleus per peripheral blood lymphocytes in the 0.004 and 0.005 mT-exposure groups were significantly higher than those of the lower intensity exposure groups. The males in 0.004 mT-exposure group displayed the highest micronucleus number per lymphocyte).
Oladnabi et al. (2019)	Retinal pigment epithelial cells exposed to 50 Hz 1 mT pulsed (20 ms pulses) EMF for 8 h/day for 3 days	Transcript levels of proangiogenic genes (HIF-1α, VEGFA, VEGFR-2, CTGF, cathepsin D, TIMP-1, E2F3, MMP-2, and MMP-9) increased significantly.
*Oliva et al. (2023)	Sperm from reef forming serpulid exposed to static MF at 0.5- 1 mT for 30 min - 48 h	No effect on single strand DNA break (Comet assay)
Ozturk et al. (2022)	Rat dams exposed to 50-Hz 0.5 mT EMF 24h/day during the gestational and lactational period, offspring studied	Exposure in the prenatal and postnatal period upregulates IL-17 gene expression in the spleen, resulting in $CD4^+$ cell proliferation and inflammation.
Panagopoulos et al. (2013)	Newly eclosed *Drosophila melanogaster* exposed to 50-Hz magnetic field (0.1, 1.1, and 2.1 mT) continuously during the first 5 days of their adult lives	Severe DNA damage (DNA fragmentation by TUNEL assay) and consequent cell death induction in the reproductive cells.
Park et al. (2022)	Human bone marrow mesenchymal stem cells exposed to 30. 45, 60, or	Increased expression of several neural development genes at 60 and 75 Hz

	75 HZ MF at 10 mT, 30 min/day for 3 days	
Patruno et al. (2015)	HaCaT keratinocytes exposed to 50 Hz 1 mT EMF for 1 or 24 h	EMF exposure modulated distinct patterns of gene expression involved in cell proliferation and cell cycle (mTOR)
Peng et al. (2020)	myocardial infarction mice exposed to 15 Hz 1.5 mT pulsed EMF or 30 Hz 3.0 mT pulsed EMF for 45 min/ day for 2 weeks	Increased the mRNA level of VEGF and hypoxia inducible factor 1-alpha (HIF-1α) in the infarct border zone. (Heart functions improved).
Pesqueira et al. (2017)	Human tendon-derived cells exposed to a 2 Hz magnetic field at 350 mT for 4 or 8 h, or 8 h every 24 or 48 h up to 14 days	8-h exposure significantly upregulated the expression of tendon-associated genes SCX, COL1A1, TNC and DCN. 8 h every 24 h exposure significantly upregulated COL1A1, COL3A1 and TNC at day 14.
Pilger et al. (2004)	Human fibroblasts exposed to an intermittent (5 min ON/10 min OFF) 50-Hz EMF at 1 mT for 15 h	Exposure resulted in an increase in DNA single strand breaks (Comet assay) unlikely to be caused by intracellular changes that affect intracellular [Ca2+] or mitochondrial membrane potential.
Piszczek et al. (2022)	Human monocytic macrophage Mono Mac 6 (MM6) cells exposed to 7 Hz 30 mT EMF or 3 h (Cells were phagocytosing.)	Upregulate mRNA encoding iNOS (nitric oxide synthase)
Porcher et al. (2023)	Arabidopsis thaliana plants exposed to 30,000 EM pulses (237 kV/m, 280 ps rise-time, duration of 500 ps)	No effect on expression of genes involved in calcium metabolism, signal transduction, ROS, and energy status. (Ascorbate peroxidases APX-1 and APX-6 were significantly induced 3 h after the exposure).
Potenza et al. (2004a)	*E. coli* XL-1Blue exposed to static	Increased cell proliferation and changes in gene expression observed. The field

	magnetic field at 300 mT up to 50 h	magnetic field may stimulate transposition activity.
Potenza et al. (2004b)	Escherichia coli DNA, plasmid, and amplification products of different lengths exposed to static magnetic field at 200-150 mT for 5 h	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.. This genotoxic effect of the magnetic field, however, is minimized in living organisms due to the presence of protective cellular responses.
Rageh et al. (2012)	Newborn rats (10 days after delivery) exposed continuously to a 50 Hz magnetic field at 0.5 mT for 30 days	Increased DNA single strand breaks (Comet assay) in brain cells and micronucleus frequency in bone cells. Changes in anti-oxidative enzymes and increased lipid peroxidation.
Rahimi e al. (2023)	Human mesenchymal stem cells derived from dental pulp multipotent stromal stem cells exposed to50 Hz EMF at 0.5 or 1 mT for 20 or 40 min/day for 7 days	Increased expression of dentin matrix acidic phosphoprotein 1 (involved in proper mineralization of bone and dentin) gene in differentiating cells.
Rao and Handerson (1996)	HeLa cells were transiently transfected with plasmids containing upstream regulating regions of c-fos coupled with the prokaryotic reporter gene CAT exposed to 60-Hz 0.006 mT EMF	CAT expression observed after 5 min and peaked at 20 min of exposure. Expression returned to normal at 40 min.
*Rao et al. (2002)	Human neuroblastoma IMR-32 cells exposed to 60 Hz 0.05, 0.1 or 0.2 mT EMF for 4 h	No effect on APP695 gene expression

Rasaeifar et al. (2023)	Transplanted mouse ovary tissues, 4.5 msec EM pulses, 15 Hz, 1.2 mT; 8 h/day from 2nd to 5th days after transplantation	Affected expression of genes involved in angiogenesis, cell migration, vascularization and inflammation.
Reale et al. (2006)	Human monocytes exposed to 50-Hz 1 mT EMFovernight	Down-regulation of nitric oxide synthase (iNOS) and up-regulation of monocyte chemotactic protein-1 (MCP-1) genes
*Reese et al. (1998)	Chinese hamster ovary (CHO) cells exposed to 60-Hz magnetic fields (0.1 or 2 mT), electric fields (1 or 38 V/m), or combined magnetic and electric fields (2 mT and 38 V/m, respectively) for 1 h	No significant effect on DNA single strand breaks (Comet assay) from exposures.
Renáta Szemerszky et al. (2010)	Rats exposed to 50-Hz 0.5 mT EMF 8 h/day for 5 days or 24 h/day for 4-6 weeks	Increased proopiomelanocortin mRNA level in anterior lobe of the pituitary gland following long-term exposure.
Reyes-Guerrero et al. (2010)	Adult male and female Wistar rats exposed to a 60-Hz magnetic field at 1 mT for 2 h/day for 9 days	ELF EMF modulates estrogen receptor- beta gene expression in the olfactory bulb of female adult rats but not in males.
Robison et al. (2002)	HL-60, HL-60R, and Raji cell lines exposed to a 60-Hz EMG at 0.15 mT for 24 h	EMF exposure offers significant protection from apoptosis (DNA double strand breaks (Comet assay) and significantly decreased DNA repair rates in HL-60 and HL-60R cell lines but not in the Raji cell line.
Rodríguez-De la Fuente et al. (2012)	Plasmid labelled as pEMF electrotransferred into quadriceps muscles	Increased luciferase gene expression.

	of mice that were later exposed to 60-Hz 0.08 mT EMF, 2 h/day for 7 days	
*Ross et al. (2018)	Human mesenchymal stromal cell exposed to a 5-Hz EMF at 0.4 mT for 20 min/day, 3 times a week for 2 weeks	No chromosome breaks, viability and proliferation rate detected.
*Ruiz-Gómez et al. (2010)	Wild type (wt) and radiation sensitive mutant yeast strains (Saccharomyces cerevisiae) exposed to a 50 Hz magnetic field at 2.45 mT for 96 h	The exposure did not induce alterations in cell cycle and cause DNA damage.
Sadri et al. (2017)	Human mesenchymal stem cells derived from human newborn cords exposed to a static magnetic field of 12, 18, or 24 mT for 2 h	Induced differentiation and decreased expression of Sox-2, Nanong, and Oct-4 genes (These genes are involved in embryonic orgen development, maintenance of multipentency and self-renewal of undifferentiated embryonic stem cell.)
Salek et al. (2021)	Mouse spermatogonial stem cells exposed to 50-Hz 2.5 mT EMF 1 h/day for 5 days	Up-regulation of apoptotic gene (Caspase-3) and down-regulation of spermatogonial stem cells specific gene (GFRα1)
Salari et al. (2023)	Brain of arts subjected to chronic unpredictable stress, injected with ketamine and exposed to 1a 10 Hz 10 mT EMF for 3 h/day for 3 days	Increased gene expression of caspase-3, and reduced expression of brain-derived neurotropic factor (related to memory, neural plasticity, and emotional behavior) in the hippocampus; showed no effect on the expression of p53 and NMDA-Receptor.
Sanie-Jahromi et al. (2016)	Human breast adenocarcinoma MCF-7 and neuroblastoma SH-	mRNA levels of seven genes involved in DNA repair pathways down regulated in

	SY5Y cells exposed to 50-Hz EMF at 0.25 and 0.5 mT (5 min ON/5min OFF; 15 min ON/15min OFF, or 30 ON continuously) for 30 min	MCF-7 cells. Synergistic with cisplatin in MCF-7 and SH-SY5Y cells.
Sanie-Jahromi and Saadat (2017)	MCF-7 and SH-SY5Y cells exposed to an intermittent (15 min OF/15-min OFF) 50-Hz EMF at 0.5 mT for 30 min. Cells were also treated with cisplastin and bleomycin	EMF exposed MCF-7 cells treated with cisplastin and bleomycin showed more effects on some DNA repair gene expression compared with "cisplastin and bleomycin" treatment alone, while SH-SY5Y susceptibility was not changed between the two treatments.
Sanie-Jahromi and Saadat (2018)	MCF-7 and SH-SY5Y cells were treated with 5.0 μM morphine and exposed to an intermittent (15 min ON/15 min OFF) 50-Hz EMF at 0.50 mT for 30 min	Morphine treatment showed significant down-regulation of expression of genes involved in DNA repair pathways, while in "Morphine + EMF" treatment, the genes were not significantly changed.
Sarimov et al. (2011)	Human lymphocytes exposed to 50-Hz magnetic field at 0.005-0.02 mT for 15-180 min	Magnetic field condensed relaxed chromatin and relaxed condensed chromatin.
*Scarfi et al (2005)	Human diploid fibroblasts exposed to an intermittent (5 min ON/10 min OFF) 50-Hz EMF or a 50-Hz field plus its harmonics for 24 h (1,2,4-BT) also studied	No significant effects on DNA single strand breaks (Comet assay) and micronucleus frequency.
Scassellati Sforzolini et al. (2004)	Cells exposed to a 50-Hz magnetic field at 5 mT; co-genotoxic effects with	Magnetic field showed genotoxic (micronucleus test) and co-genotoxic (comet assay) capabilities.

	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), 4-nitroquinoline N-oxide (4NQO), benzene, 1,4-benzenediol (1,4-BD), or 1,2,4-benzenetriol	
Schmitz et al. (2004)	Male adult mice exposed to a 50-Hz magnetic field at 1.5 mT for 8 weeks	A significant increase in both unscheduled DNA synthesis and in situ nick translation was only found for epithelial cells of the choroid plexus. Mitochondrial DNA synthesis was exclusively increased in renal epithelial cells of distal convoluted tubules.
Selvamurugan et al. (2007)	Rat primary osteoblastic cells exposed to pulsed EMF (triangular pulses, pulse frequency of 3.8 kHz, with a burst frequency of 1.5 Hz, a burst duration of 25.6 ms, and a burst period of 670 ms); 4 h/day during differentiation and mineralization phases	Increased alkaline phosphatase mRNA and three three osteoblast marker genes during differentiation phase.
Selvamurugan et al. (2017)	Human bone marrow stromal cells exposed to pulsed EMF (15 Hz burst frequency and burst period of 67 ms) up to 120 min	Affected expression of genes of cell cycle regulation, cell structure, and growth receptors or kinase pathways.
Sendera et al. (2024)	Adipose-derived stem cells exposed to 50 Hz MF at 1.5 mT for 24 h	Increased N6-methyladenosine (m^6A) RNA methylation. (Cell membrane flexibility, the metabolic potential of cells as well as the

		distribution, morphology, and metabolism of mitochondria were altered.)
Senol et al. (2023)	Brain from rats exposed to 50-Hz electric field, 21 h/day for 30 days	Increased DNA single strand breaks (Comet assay)
Seo et al. (2018)	Bone marrow-derived mesenchymal stem cells exposed to 50-Hz 1 mT EMF for 1 h	Increased mRNA expression of growth factors (S100 (Schwann cell marker), glial fibrillary acidic protein (astrocyte marker), and brain-derived neurotrophic factor and nerve growth factor (neurotrophic factors) involved in nerve regeneration
Seong et al. (2014)	Human bone marrow-mesenchymal stem cells exposed to a 50 Hz EMF at 1 mT for 8 days	Increased expression of early growth response protein 1 (Egr1).
Sharma et al. (2021)	Cumulus cells and buffalo somatic cell nuclear transfer (SCNT) embryos exposed to 0.03 mT pulsed EMF for 3 h	Altered the expression level of several important genes related to pluripotency, apoptosis, metabolism, and stress.
*Shen et al. (2016)	Chinese Hamster Lung cells exposed to a 50-Hz EMF at 0.4 mT for 30 min or 24 h	Increase in LC3-II expression and increased autophagosome formation; no significant effect on γH2AX foci.(EMF-induced autophagy may balance the cellular homeostasis to protect the cells from severe adverse biological consequences.)
Shokrollahi et al. (2018)	Soybean plants exposed to static magnetic field at 20 and 30 mT for 5 h/day for 5 days	Exposure to 20 mT decreased gene expression of Fe transporter, ferrous and H_2O_2 contents and gene expression, content and activity of ferritin and catalase. Opposite responses were observed at 30 mT exposure. Tertiary structures of ferritin, apoferritin and catalase altered by static magnetic field.

Singh and Lai (1998)	Rats exposed to a 60-Hz magnetic field at 0.5 mT for 2 h	Data suggested that both DNA-protein and DNA-DNA crosslinks (Comet assay) were formed in brain cells.
Skyberg et al. (2001)	Blood samples from high voltage laboratory workers exposed to electromagnetic fields and mineral oil	In inhibited (hydroxyurea-inhibits DNA synthese, and caffeine-inhibits DNA repair) lymphocyte cultures, there were indications that electromagnetic fields in combination with mineral oil exposure may produce chromosomal aberrations. No effect on un-inhibited cells.
Sobhanifard et al. (2019)	Spleen and thymus of rats injected with human serum albumin (HSA) and exposed to 50-Hz (0.001, 0.1, 0.5, or 2 mT) EMF; 2 h/day for 60 days	Expression of T-bet and GATA-3 mRNA (involved in T-helper cell functions) decreased in the spleen in rats exposed to 0.001 and 0.1 mT.
Solek et al. (2017)	Mouse spermatogenic cell lines (GC-1 spg and GC-2 spd) exposed to pulsed (1sec on/off) or continuous-wave 2, 50, 120 Hz EMF at 2.5- 8 mT for 2 h	EMF activated oxidative and nitrosative stress-mediated DNA damage pathways, resulting in p53/p21-dependent cell cycle arrest and apoptosis
*Song et al. (2018)	HeLa and primary IMR-90 fibroblasts exposed to a 60-Hz EMF at 1, 3, 6, or 10 or mT continuously for up to 168 h or 30 min every 24h for 3 days	No effect on DNA damage (gamma-H2AX foci).; promoted cell proliferation (probably due to decreased reactive oxygen species).
Stankevičiūtė et al. (2019)	Rainbow trout (*Oncorhynchus mykiss*) exposed to a 50-Hz EMF at 1 mT for 40 days; and	Trout and ragworm erythrocytes and clam gill cells showed elevated micronucleus frequency, nuclear buds, nuclear buds on filament cells, and cells with blebbed nuclei.

	the common ragworm (*Hediste diversicolor*) and the Baltic clam (*Limecola balthica*) for 12 days	
*Stronati et al. (2004)	Human whole blood exposed to a 50-Hz magnetic field at 1 mT for 2 h	No significant effects on DNA single strand breaks (Comet assay), sister chromatid exchanges, chromosome aberrations, and micronucleus frequency in lymphocytes. A slight decrease in cell proliferation observed.
*Sun C et al. (2018)	ATM-proficient ($Atm^{+/+}$) and ATM-deficient ($Atm^{-/-}$) mouse embryonic fibroblasts exposed to a 50-Hz magnetic field at 2 mT for 15 min.(Ataxia telangiectasia mutated (ATM) plays a central role in DNA damage repair.)	No effect on γ-H2AX foci in both types of cells.
Sun L et al. (2019)	Irpex lacteus, a white-rot fungus, exposed to a 50-Hz magnetic field ay 3.5 mT for 3 h/day for 4 days	Global gene expression changes were observed.
Sun L-Y et al. (2010)	Human bone marrow mesenchymal stem cells exposed pulsed EMF (15 Hz in 20 pulses at 4.5 ms burst); peak 1.8 mT, 8 h/day for 7 days	Increased expression of the key osteogenesis regulatory gene cbfa1.
Sun RG et al.(2012)	K562 human leukemia cells exposed to paclitaxel in the presence or absence of	The potency of the combination of SMF and paclitaxel was greater than that of SMF or paclitaxel alone on K562 cells, and these

	8.8 mT static magnetic field for 24 h	effects were correlated with DNA single strand breaks (Comet assay).
*Sun W. et al. (2010)	Trophoblasts from first trimester human chorionic villi at 8-10 weeks' gestation exposed to 50-Hz, 0.4 mT for 72 h	No effect on mRNA levels of apoptosis-related genes bcl-2, bax, caspase-3, p53, and fas.
Suryani et al. (2019)	Calvarial osteoblast precursor cells exposed to 50-Hz 0.6 mT EMF 15-60 min/day for 28 days	Up-regulation of osteogenic genes
Suzuki et al. (2001)	Mouse exposed to high intensity static magnetic fields (3.0 T for 48 and 72 h and 4.7 T for 24, 48 and 72 h).	Increased micronucleus frequency in bone marrow cells.
Svedenstal et al. (1999)	Brain cells of CBA mice exposed to a 50 Hz magnetic field at 0.5 mT 2 h, 5 days or 14 days	DNA single strand breaks (Comet assay) increased after 14 days of exposure,
*Szerencsi et al. (2013)	Peripheral blood samples from men exposed to EMF produced by 3T magnetic resonance imaging equipment for 0, 22, 45, 67, and 89 min during the scanning procedure	No significant effect on DNA single strand breaks (Comet assay) and DNA integrity in lymphocytes.
*Takahashi and Furuya (2023)	Human Hematopoietic Stem/Progenitor Cells exposed to 50-Hz MF at 300 mT for 35 days	No effect on expression levels of recombination-activating genes RAG1 and RAG2 in the B cells

Tasset et al. (2013)	Effect of transcranial magnetic stimulation on a Huntington's disease-like rat model induced by 3-nitropropionic acid (3-NP).	Modulated the Nrf2 transcriptor factor in the brain (increase in (cytoplasm and nucleus)
Teodori et al. (2014)	Human glioblastoma cells exposed to static magnetic field at 80 mT for 6,12, or 24 h, also in combination with X-ray	Increased in DNA single strand breaks (Comet assay) after 24 h of exposure; x-ray induced DNA strand breaks significantly reduced by post-irradiation exposure to static magnetic field. Further data suggested that static magnetic field modulated DNA damage and/or repair, possibly through a mechanism that affects mitochondria.
*Testa et al. (2004)	Human blood samples exposed to a 50-Hz magnetic field at 1 mT for 48 h	No significant effect on micronucleus frequency and proliferation of lymphocytes. No interaction with x-ray.
Tian F et al. (2002)	Ku80-deficient cells (xrs5) and Ku80-proficient cells (CHO-K1) irradiated by X-ray and then exposed to 60-Hz 5 mT EMF for 24 h	In xrs5 cells, EMF suppressed the apoptosis effect of X-ray by decreasing the levels of caspase-3, p21, p53 and phospho-p53 and by increasing bcl-2 gene expression (apoptosis related genes).
Tian L et al. (2022)	Male C57BL/6J mice exposed to hypomagnetic field (i.e., with the geomagnetic field eliminated) for 8 weeks	Increased reactive oxidative species in hippocampus by modulating hypomagnetic-regulating genes (Nox4, Gpx3). Mice showed cognitive deficits.
Tipping et al. (1999)	Drosophila larvae exposed to 50-Hz 0.008 mT MF for 20 min	Decreased transcript levels of HSP 70a, Histone 1.9, and Copia.

*Tiwari et al. (2015)	Blood samples of human subjects occupationally exposed to 132 kV high-voltage substations (mean duration on job 9.27 years, range 2-30 years).	No significant effect on DNA single strand breaks (Comet assay) in lymphocytes, increased oxidative stress observed.
Udroiu et al. (2006)	Liver and peripheral blood sampled from newborn mice exposed to a 50-Hz magnetic field of 0.65 mT during the whole intra-uterine life (21 days), and on bone marrow and peripheral blood from adult mice exposed to the same magnetic field for the same period	Data obtained in newborn mice showed a significant increase in micronuclei frequencies. No significant effect was recorded on exposed adults.
Udroiu et al. (2015)	Mice exposed to 50-Hz, 0.065 mT magnetic field, 24 hours/day, for a total of 30 days, starting from 12 days post-conception	Magnetic field induced a slight genotoxic damage (micronucleus formation) and no interaction with x ray in erythrocytes, but modulate the response of male germ cells to X-rays with an impact on proliferation/differentiation processes. Magnetic field exposure decreased DNA single and double strand breaks (Comet assay) in germ cells at 42 days after birth.
Vergallo et al. (2020)	Human peripheral blood lymphocytes exposed to 6 mT static MF	modulates gene expression of ATP-binding cassette transporter A1 (ABCA1) (promotes cellular phospholipid and cholesterol efflux)
*Verschaeve et al. (2011)	Salmonella typhimurium exposed to a 50-Hz magnetic field at 0.1 or 0.5 mT for 1 or 2 h	The magnetic field did not induce mutagenicity in S. typhimurium bacteria and did not show any synergetic effect when combined with chemical mutagens.

*Verschaeve et al. (2016)	Salmonella typhimurium exposed to 50 Hz magnetic field at 0.1 mT for 1 h	The magnetic field did not damage DNA and had no influence on the DNA damaging capacity of several mutagens.
Villarini et al. (2006)	Human leukocytes exposed to a 50-Hz magnetic field at 3 mT for 30, 60, or 120 min and treated with mutagens	Magnetic field exposure increased N-methyl-N'-nitro-N-nitrosoguanidine and decreased 4-nitroquinoline N-oxide-induced DNA single strand breaks (Comet assay).
Villarini et al. (2013)	Male CD1 mice exposed to a 50-Hz magnetic field at 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	Magnetic field exposure induced DNA single strand breaks (Comet assay) and did not affect hsp70 expression in the brain.
Villarini et al. (2015)	Blood leukocytes from electric arc welders presumably exposed to 50-Hz EMF(mean 0.0078 mT; range: 0.00003-0.171 mT)	Decreased DNA single strand beaks (Comet assay), may be caused by DNA-protein crosslinks by metal exposure.
*Villarini et al. (2017)	SH-SY5Y and SK-N-BE-2 human neuroblastoma cells exposed to a 50-Hz magnetic field at 0.01. 0.1, or 1 mT for 1 h continuously or 5 h intermittently (15 min ON/15 min OFF), and also aluminum	Neither exposure to ELF-MF or $AlCl_3$ alone induced DNA single strand breaks (Comet assay), changes in GSH/GSSG ratio or variations in Hsp70 expression. Co-exposure to ELF-MF and $AlCl_3$ did not have any synergic toxic effects.
Vincenzi et al. (2012)	PC12 rat adrenal pheochromocytoma and U87MG human	Increased A_{2a} and A_3 adenosine receptor mRNA (Inhibit cancer growth)

	glioblastoma cell lines exposed to pulsed EMF (75 Hz, 1.3 ms duration, 0.1 duty cycle) at 1.5 mT for 24 h	
*Vinod et al. (2020)	human articular cartilage derived chondroprogenitors exposed to pulsed EMF (15 Hz, 6 ms duration, 2 mT) 10 min or 10 min every 3 days for 21 days (7 exposures)	No effect on gene expression of ACAN, SOX9, COL2A1, TGFβ1, TGFβ2, and TGFβ3
Wahab et al. (2007)	Human peripheral blood lymphocytes exposed to 50 Hz sinusoidal (continuous or pulsed) or square (continuous or pulsed) magnetic fields at 0.001 or 1 mT for 72 h	A significant increase in the number of sister chromatid exchange /cell observed.
Wang J. et al. (2013)	Human fetal scleral fibroblasts exposed to 50-Hz 0.2 mT EMF for 24 h	Suppressed type I collagen (COL1A1) and fibroblast growth factor-2 (FGF2) mRNA expression; increased matrix metalloproteinases-2 (MMP-2) and transforming growth factor-β2 (TGFβ2) mRNA expression
Wang L et al. (2021)	Female mice exposed to 8, 50, or 75 Hz 1.6 mT pulsed EMF 1 h/day for 4 weeks	50 and 75 Hz promoted expression of the osteoblast-related genes (ALP, OCN, Runx2) and suppressed osteoclast-related mRNA expression (CTSK, NFATc1, TRAP)
Wang Q et al. (2014)	Human amniotic epithelial cells exposed to 50 Hz pulsed EMF (applied field increased from 0 to 1 mT in 1.5 ms and then decayed back to	Increased expression of osteoblast specific genes including alkaline phosphatase and osteocalcin; gene expression of BMP-2, Runx2, β-catenin, Nrf2, Keap1 and integrinβ1 were up-regulated in the

	0 in 5 ms) 30 min twice a day for 21 days	osteogenic differentiation of amniotic epithelial cells.
*Wang Y et al. (2019)	Human ventricular cardiomyocytes exposed to a 50-Hz magnetic field at 0.1 mT for 1 h continuously or 75 min intermittently (15 min ON/15 min OFF). Sprague-Dawley rats exposed to 50 Hz magnetic field at 0.1 mT for 15 h/day for 7 days	Magnetic field exposure did not cause DNA single strand breaks (Comet assay) in heart cells in both in vitro and in vivo experiments.
Wang Y. et al. (2020)	*Caenorhabditis elegans* exposed to 50-Hz, 3 mT EMF for 15 generations	Expression levels of the *r53.4, hpo-18, atp-5*, and *atp-3* genes encoding ATPase and *sod-1, sod-2,* and *sod-3* genes encoding superoxide dismutase (SOD) were significantly upregulated.
Wang Y et al. (2023)	Mouse model of myocardial infarction, 30 Hz, 3 mT, 45 min/day for 14 days	Down regulation of expression of miR-20a-5p; protected ischemia myocardium by regulating the miR-20a-5p/E2F1/p73 signaling pathway; synergistic with adipose-derived stem cell treatment.
Wang Z et al. (2009)	Human embryoid body derived (hEBD) LVEC cell line exposed to 0.23-0.28 T static magnetic field for 24 h	Gene expression in cells showed nine signaling networks responded to static magnetic field
*Williams et al. (2006)	Salmonella bacteria cultures exposed to a 60-Hz intermittent magnetic field (5 min ON/10 min OFF) at 14.6 mT for 4 h	No significant increase in recombination events and DNA single and double strand breaks (assayed using a recombination event counter).However, magnetic field exposure induced protection from heat stress.

Wilson et al. (2015)	BALB/c×CBA/Ca F1 hybrid males exposed to 50Hz magnetic fields at 0.01, 0.1 or 0.3 mT for 2 or 15 h	There was a marginally significant increase in a non-dose-dependent mutation frequency in sperm, and not in blood cells.
Winker et al. (2005)	Human fibroblasts exposed to a 50-Hz intermittent (5 min ON/10 min OFF) EMF at 1 mT for 2-24 h	Increased micronucleus frequency and chromosomal aberration.
Wolf et sl. (2005)	HL-60 leukemia cells, Rat-1 fibroblasts, and WI-38 diploid fibroblasts exposed to a 50-Hz EMF at 0.5-1 mT for 24-72 h	Dose-dependent increases in DNA single strand breaks (Comet assay) and formation of 8-hydroxy-2'-deoxyguanosine adducts were observed in all cell lines. There were increases in cell proliferation and reactive oxygen species.
Wu et al. (2018)	C3H10T1/2 mesenchymal cells were exposed to 30-Hz, 1 mT pulsed EMF, 2 h/day for 3 days	Promoted the gene expression of the Wnt/β-catenin pathway. Calcium involved.
Wydorski et al. (2023)	Porcine endometrial slices exposed to 50 Hz EMF at 8 mT for 2 h	Increase or decrease in DNA methylation depending on promoter regions.
Wydorski et al. (2024)	Porcine endometrial slices exposed to 50 Hz EMF at 8 mT for 2 h	Changes in expression of certain genes, with DNA methylation and micoRNA changes.
Xu C et al. (2021)	Arabidopsis (rockcrest) exposed (from seed to 5 day old fruit).to near-null magnetic field (hypomagnetic field) (compared with plant grew in geomagnetic field)	.Expressions of GA(gibberellin)20-oxidase (GA20ox) genes (GA20ox1 and GA20ox2) and GA3-oxidase (GA3ox) genes (GA3ox1 and GA3ox3) in fruits downregulated. Effects not found in cryptochrome double mutant, cry1/cry2 indicating that suppression of fruit growth by the near-null magnetic field is mediated by cryptochrome.

Yagci and Kesim (2016)	Human gingival fibroblasts exposed in vitro to static magnetic fields produced by dental magnetic attachments for 10-12 days. (The maximum magnetic flux densities measured at the magnet centers of 4 types of attachment were 95.6-148.1 mT and became almost zero at 10 mm away)	Increased micronucleus frequency.
Yaguchi et al. (1999)	Mouse embryonic skin m55 cells exposed to a 60-Hz magnetic field at 5, 50, or 400 mT for 42 h	Increase in sister chromatid exchanges after 400 mT exposure.
Yaguchi et al. (2000)	Mouse embryonic skin m55 cells exposed to 60-Hz (5 or 50 mT) or 50-Hz (400 mT) magnetic fields for 40 h. Some cells also treated with mitomycin C or X-ray	Increased chromosomal aberration, synergistic with mitomycin C and X-ray.
Yahyapour et al, (2022)	Human T98 glioma cells exposed to 50-Hz 10 mT EMF	Increased apoptosis-related gene and Autophagy-related gene expression. (Synergistic with ionizing radiation and temozolomide.)
Yao et al. (2015)	Rat Schwann cells exposed to DC electric field for 36-72 h at 50, 100, or 200 mV/mm	Differentially expression of 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 cell cycle regulation).

Yao et al. (2019)	Oligodendrocyte precursor cells (OPC) (that can differentiate into oligodendrocytes) exposed to 50-Hz 1.8 mT square-wave pulsed EMF, 2 h/day for 3, 7, 14, and 21 days	Up-regulated miR-219-5p (associated with OPC differentiation) and down-regulated Lingo1 (involved in myelination) expression. EMF promoted OPC differentiation.
Yin et al. (2016)	Primary cultured rat hippocampal neurons exposed to a 50-HZ EMF at 8 mT for 90 min	Increase in DNA single strand breaks (Comet assay); free radicals involved.
Yokus et al. (2005)	Female Wistar rats exposed to a 50-Hz magnetic field at 0.97 mT for 3 h/day for 50 and 100 days	Increased 8-hydroxy-2'-deoxyguanosine in blood cells.
Yokus et al. (2008)	Male Sprague-Dawley rats exposed to a 50-Hz magnetic field at 0.1 or 0.5 mT for 2 h/day for 10 months	Increased DNA base modifications in leucocytes [8-hydroxyguanine (8-OH-Gua), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua), and 4,6-diamino-5-formamidopyrimidine (FapyAde)]
Yoon et al. (2014)	Human lung fibroblast WI38 cells and human lung epithelial L132 cells exposed to a 60-Hz magnetic field at 2 mT for 6 h	2 mT field induced increased γ-H2AX expression, as well as γ-H2AX foci production. Interacted with gamma radiation but not H_2O_2.
Yuan et al. (2020)	Tumor cell lines including lung cancer, gastric cancer, pancreatic cancer and nephroblastoma exposed to a 50-Hz EMF modulated by static MF with time-average	Induced DNA single strand breaks (Comet assay), gamma-H2AX and activation of DNA repair pathways, increased reactive oxygen species and ferroptosis, and decreased proliferation.

	intensity of 5.1 mT, for 2 h/day for 3 days	
Yun et al. (2022)	Mouse melanoma B16-F10 cells in DC electric fields (0-500 mV/mm) for 3 h	cells migrated toward the cathode in a voltage-dependent manner; Approximately 3000 upregulated and 2613 downregulated genes were identified under DC electric field. Some genes correlated with cell migration.
Zavarch et al. (2021)	Human gastric adenocarcinoma cell line (AGS) exposed to 50-Hz EMF at 0.2 and 2 mT for 18 h, continuously and discontinuously (1.5 h on/1.5 h off).	Up-regulation of miR-144 and miR-375 (involved in cell proliferation and tumor suppressor).
Zendehdel et al. (2019)	Peripheral blood cells of male power line workers in a power plant. The median value of the magnetic field at the working sites was 0.00085 mT	Increased in DNA single strand breaks (Comet assay).
Zhang H et al. (2016)	ICR mice exposed to a 50-Hz EMF at 8 mT for 4 h/day for 28 days	Declined DNA content and increased expression of apoptosis genes in spleen. Free radical may be involved.
Zhang M et al. (2020)	Onchidium struma (a mollusk) exposed to 50-Hz 0.1 or 0.5 mT EMF for 24, 72, or 168 h	There were 341 differentially expressed genes (DGEs) between unexposed and exposed groups, including 209 up-regulated and 132 down-regulated genes with those associated with immune response.
Zhang X-J et al. (2023)	Rtas exposed to electromagnetic pulses (1 Hz pulse, 700 kV/m, 400 pulses)	Found 41 differentially expressed long non-coding RNA and 266 differentially expressed mRNAs (with genes associated with synapse- and metabolic-related pathways) between EMP and sham groups

Zhang Y et al. (2016)	Workers with or without exposure to ELF-EMF (50 Hz) of 110-420kV power lines	Increased urinary 8-isoprostane and 8-OHdG were observed in workers with EMF exposure. Free radical may be involved.
Zhang Y et al. (2022)	Nilaparvata lugens (brown planthopper insect) exposed to hypomagnetic field (<0.005 mT) during developmental from juveniles to adults	Different reference gene expression were observed at different stages of development.
Zheng et al. (2018)	dental pulp stem cells exposed to a static magnetic field of 1,2, 4 mT for 15 min, 30 min, 1 h or 24 h	Increased expression of several growth factors (FGF-2, TGF-β, and VEGF), migration genes (MMP-1 and MMP-2), and upregulated the two YAP/TAZ-regulated genes, CTGF and ANKRD1. (YAP/TAZ are transcriptional activators particularly involved in cancer cell proliferation, therapy resistance and metastasis. Increased cell proliferation, osteo/odontogenesis and mineralization observed in the stem cells.
Zhou et al. (2023)	Geobacter sulfurreducens exposed to static MF at 100 mT for 16 h or 8 h ON/8 h OFF	Increased expression of genes involved in electron transfer.
Zmyslony et al. (2000)	Rat exposed to a static or 50-Hz magnetic field at 7 mT for 3 h	In combination with $FeCl_2$, increases in DNA single strand breaks (Comet assay) observed for both static and 50-Hz field exposure in lymphocytes.
Zmyslony et al. (2004)	Rat lymphocytes exposed first to ultraviolet radiation and then to a 50-Hz magnetic	60-min magnetic field exposure (plus UVA) caused an increase in DNA single strand breaks (Comet assay). MF may affect the

	field at 0.04 mT for 5 or 60 min	radical pairs generated during the oxidative or enzymatic processes of DNA repair.

Studies that reported genetic effects at low flux densities (≤ 0.01 mT (10 μT))

Agliassa et al. (2018)	Arabidopsis thaliana (thale cress) exposed to 0.00004 mT static magnetic field for 38 days after sowing	Changes in gene expression in leaf and floral meristem.
Back et al. (2019)	Mouse embryonic stem cells exposed to hypomagnetic field (<0.005 mT) up to 12 days	Induced abnormal DNA methylation.
Baraúna et al. (2015)	Chromobacterium violaceum bacteria cultures exposed to ELF-EMFfor 7 h at 0.00066 mT	Five differentially expressed proteins detected including the DNA-binding stress protein.
Belyaev et al. (2005)	Human lymphocytes exposed to 50 Hz magnetic field at 0.015 mT (peak) for 2 h (measurements made at 24 and 48 h after exposure).	Induced chromatin conformation changes.
de Kleijn et al. (2016)	Hypothalamic paraventricular nucleus, pituitary, and adrenal glands from mice exposed to 20-500 Hz 0.01 mT EMF 1, 4, or 24 h/day for 1 or 15 weeks	Decreased proopiomelanocortin (POMC) gene expression; hypothalamic-pituitary-adrenal axis affected
Dominici et al. (2011)	Lymphocytes from welders (average magnetic field exposure from personal dosimeters 0.00781 mT (general environmental level 0.00003 mT)	Higher micronucleus frequency correlated with EMF exposure levels; decreased in sister chromatid exchange frequency.

Gholamian-Hamadan et al. (2023)	Rats exposed to 50 Hz EMF; 2 h/day for 60 days at 0.001, 0.1, 0.5 and 2 mT (with the immune system activated by human serum albumin)	Gene of activation-induced deaminase decreased by 0.0001 mT exposure.
Heredia-Rojas et al. (2010)	Human non-small cell lung cancer cells (INER-37) and mouse lymphoma cells (RMA E7) (transfected with a plasmid with hsp70 expression when exposed to magnetic field and contains the reporter for the luciferases gene) exposed to a 60-Hz magnetic field at 0.008 and 0.00008 mT for 20 min.	An increased in luciferase gene expression was observed in INER-37 cells.
Kazemi et al. (2018)	Male rhesus macaques exposed to1 or 12 Hz 0.0007 mT EMF 4 h/day	Increased NMDA receptor gene expression with 12 Hz exposure (enhanced visual working memory function).
Kazemi et al. (2022)	Male rhesus macaques exposed to 12 Hz 0.0007 mT EMF 4h/day for 30 days	Increased NMDA receptor gene expression (enhanced visual working and visual workin memory function; increased plasma adrenocorticotropic hormone)
Lai and Singh (2004)	Male Sprague-Dawley rats exposed to a 60-Hz magnetic field at 0.01 mT for 24 or 48 h	Increased DNA single and double strand break (Comet assay) in brain cells. More effect with 48-h than 24-h exposure.
Lin H et al. (1998)	60 Hz EMF at 0.008 mT	Induction of HSP70 gene expression by c-myc protein

Mahaki et al. (2019)	Rats exposed to a 50-Hz EMF at 0.001-2 mT for 2 h/day for 60 days	In the spleen, gene expression levels of RORα (retinoid-related orphan receptor alpha) and c-Maf (transcription factor Maf) were significantly down-regulated at 0.001 and 0.1 mT, while the expression of STAT6 (signal transducer and activator of transcription 6) was only significantly decreased at the density of 0.1 mT. No effect on thymus.
Mahdavinejad et al. (2018)	Spleen Th17 and regulatory T (Treg) cells of rats exposed to 50 Hz EMF at 0.001, 0.1, 0.5 and 2 mT for 2 h/day for 2 months	expression of transcription factor forkhead box P3 (Foxp3) was downregulated at intensities of 0.001 and 0.1 mT
Monirul Islam et al. (2020)	Arabidopsis thaliana exposed to near null magnetic field (30 nT)	Genes involved in lipid metabolism affected.
Rao and Handerson (1996)	HeLa cells were transiently transfected with plasmids containing upstream regulating regions of c-fos coupled with the prokaryotic reporter gene CAT exposed to 60-Hz 0.006 mT EMF	CAT expression observed after 5 min and peaked at 20 min of exposure. Expression returned to normal at 40 min.
Sarimov et al. (2011)	Human lymphocytes exposed to 50-Hz magnetic field at 0.005-0.02 mT for 15-180 min	Magnetic field condensed relaxed chromatin and relaxed condensed chromatin.
Sobhanifard et al. (2019)	Spleen and thymus of rats injected with human serum albumin (HSA) and exposed to 50-Hz (0.001, 0.1, 0.5, or 2 mT) EMF; 2 h/day for 60 days	Expression of T-bet and GATA-3 mRNA (involved in T-helper cell functions) decreased in the spleen in rats exposed to 0.001 and 0.1 mT.

Tian L et al. (2022)	Male C57BL/6J mice exposed to hypomagnetic field (i.e., with the geomagnetic field eliminated) for 8 weeks	Increased reactive oxidative species in hippocampus by modulating hypomagnetic-regulating genes (Nox4, Gpx3). Mice showed cognitive deficits.
Tipping et al. (1999)	Drosophila larvae exposed to 50-Hz 0.008 mT MF for 20 min	Decreased transcript levels of HSP 70a, Histone 1.9, and Copia.
Villarini et al. (2015)	Blood leukocytes from electric arc welders presumably exposed to 50-Hz EMF (mean 0.0078 mT; range: 0.00003-0.171 mT)	Decreased DNA strand beaks.
Wahab et al. (2007)	Human peripheral blood lymphocytes exposed to 50 Hz sinusoidal (continuous or pulsed) or square (continuous or pulsed) magnetic fields at 0.001 or 1 mT for 72 h.	A significant increase in the number of sister chromatid exchange/cell observed.
Xu C et al. (2012)	Arabidopsis (rockcrest) exposed to near-null magnetic field (hypomagnetic field)	Expressions of three cryptochrome-signaling-related genes affected (increased *PHYB*, and decreased *CO* and *FT* expression) (Affected reproductive growth and delayed flowering time under white light.)
Xu C et al. (2021)	Arabidopsis (rockcrest) exposed (from seed to 5 day old fruit).to near-null magnetic field (hypomagnetic field) (compared with plant grew in geomagnetic field)	Expressions of GA(gibberellin)20-oxidase (GA20ox) genes (GA20ox1 and GA20ox2) and GA3-oxidase (GA3ox) genes (GA3ox1 and GA3ox3) in fruits downregulated. Effects not found in cryptochrome double mutant, cry1/cry2 indicating that suppression of fruit growth by the near-null magnetic field is mediated by cryptochrome.

Zendehdel et al. (2019)	Peripheral blood cells of male power line workers in a power plant. The median value of the magnetic field at the working sites was 0.00085 mT.	Increased in DNA strand breaks.
Zhang Y et al. (2022)	Nilaparvata lugens (brown planthopper insect) exposed to hypomagnetic field (<0.005 mT) during developmental from juveniles to adults	Different reference gene expressions were observed at different stages of development.