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Pulsed Electromagnetic Field with Temozolomide Can Elicit an Epigenetic Pro-apoptotic Effect on Glioblastoma T98G Cells

FRANCESCA PASI[1,2*], LORENZO FASSINA[3,4*], MARIA EVELINA MOGNASCHI[3], GIUSEPPE LUPO[4,5], FRANCO CORBELLA[1], ROSANNA NANO[2] and ENRICA CAPELLI[4,5]

[1]Radiation Oncology Unit, Hospital San Matteo Foundation, Pavia, Italy;
[2]Laboratory of Neuro-Radio-Experimental Biology, Department of Biology and Biotechnology,
[3]Department of Electrical, Computer and Biomedical Engineering,
[4]Centre for Health Technologies and [5]Laboratory of Immunology and Genetic Analysis,
Department of Earth and Environmental Sciences, University of Pavia, Pavia, Italy

Abstract. *Treatment with pulsed electromagnetic fields (PEMFs) is emerging as an interesting therapeutic option for patients with cancer. The literature has demonstrated that low-frequency/low-energy electromagnetic fields do not cause predictable effects on DNA; however, they can epigenetically act on gene expression. The aim of the present work was to study a possible epigenetic effect of a PEMF, mediated by miRNAs, on a human glioblastoma cell line (T98G). We tested a PEMF (maximum magnetic induction, 2 mT; frequency, 75 Hz) that has been demonstrated to induce autophagy in glioblastoma cells. In particular, we studied the effect of PEMF on the expression of genes involved in cancer progression and a promising synergistic effect with temozolomide, a frequently used drug to treat glioblastoma multiforme. We found that electromagnetic stimulation in combination with temozolomide can elicit an epigenetic pro-apoptotic effect in the chemo- and radioresistant T98G glioblastoma cell line.*

Glioblastoma multiforme (GBM) is the most common and most aggressive primary brain tumor, with approximately 10,000 new diagnoses per year in the USA. At present, all therapies remain unsuitable and the median survival is 14 months (1), even with combined therapy of radiation treatment and temozolomide chemotherapy following surgery (2). As a consequence, there is a clear need for new physical, biological, and molecular approaches to treat this disease.

It has been demonstrated in *in vitro* studies that some electromagnetic fields (EMFs) are able to control the growth of various cancer cells (3) and some electric fields with 'tumor-specific modulation frequencies' can regulate cell migration and disrupt the mitotic spindle (4, 5).

Several studies showed a wide range of biological effects following exposure to extremely-low-frequency EMFs; these involve: cell functions and metabolism (6); dysregulation and risk for malignancy (7); intercellular and systemic effects (8); cell morphology, proliferation and differentiation (9); enzymatic (10) and pharmacological (11) effects.

Some of these studies have focused on negative impacts of EMF exposure, ranging from DNA damage to a role as a cancer promoter, whereas minor emphasis has been placed on the promising positive effects of controlled electromagnetic exposure.

Furthermore, studies examining DNA damage following EMF exposure have been contradictory: while one study showed double-strand breaks, evidence of chromosomal damage, and micronuclei formation (12), others reported no evidence of chromosomal damage or genotoxicity (13).

It has also been found that low-energy EMFs can act epigenetically on gene expression (14) and that electric fields with 'tumor-specific modulation frequencies' are able to block the growth of cancer cells (5). For example, the US Food and Drug Administration (FDA) has approved Tumor Treating Fields (TTFields) therapy as a treatment for glioblastoma. TTFields create low-intensity alternating electric fields that exert physical forces on electrically charged cellular components, preventing normal mitosis and causing cancer cell death (15, 16). In 2014, the FDA approved trials for TTFields in combination with temozolomide and, recently, with

*These Authors contributed equally to this study.

Correspondence to: Professor Enrica Capelli, Lab. of Immunology and Genetic Analysis, Dept. of Earth and Environmental Sciences, University of Pavia, viale Taramelli 24, 27100 Pavia, Italy. Tel: +39 0382987971, e-mail: enrica.capelli@unipv.it and Professor Rosanna Nano, Lab. of Neuro-Radio-Experimental Biology, Dept. of Biology and Biotechnology, University of Pavia, via Ferrata 9, 27100 Pavia, Italy. Tel: +39 0382986405, e-mail: rosanna.nano@unipv.it

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bevacizumab (Novocure with Radiation Therapy Oncology Group Foundation, 2016) (1, 17).

Other physical stimuli such as low-frequency/low-energy pulsed electromagnetic fields (PEMFs) (18) have been studied as promising therapeutic agents. PEMFs can, for example, improve the construction of calcified bone matrix (19), diminish the inflammation of joint cartilage (20), moderate brain inflammation *via* better use of endogenous adenosine (21), elicit a cytoprotective response in human neurons in terms of promotion of the non-amyloidogenic pathways (22), protect cells from oxidative stress (23), and induce useful autophagy in neurons (24).

Consequently, the core idea of the present work was to study the ability of a PEMF to modulate the expression of genes involved in tumor progression in a human cell model of chemo- and radioresistant glioblastoma (T98G cell line) and to investigate a potential synergy of PEMF with temozolomide, one of the drugs most frequently used to treat GBM.

Materials and Methods

Electromagnetic bioreactor. The electromagnetic bioreactor used here has been previously investigated in terms of biological effects (19, 22, 24-28) and in terms of numerical dosimetry and physical parameters (induced electric field, induced electric current, induced forces) (18). The setup was based on two air-cored solenoids [see Figure 1 in (18)] connected in series, placed inside a cell incubator and powered by a pulse generator (Biostim SPT; Igea, Carpi, Italy). The solenoids had a quasi-rectangular shape (length=17 cm; width=11.5 cm) and their planes were parallel with a distance of 10 cm, so that the cell cultures were placed 5 cm away from each solenoid. In this configuration, the magnetic induction field (module=2.0±0.2 mT; frequency=75±2 Hz) was perpendicular to the seeded cells. In particular, in our experimental setup: i) the electric current in the solenoid wire ranged from 0 to 319 mA in 1.36 ms; ii) in order to optimize the spatial homogeneity of the magnetic induction field, especially in the central region where the cells were stimulated, the two solenoids were supplied by the same electric current and their dimensions and distance were comparable; spatial homogeneity was calculated *in silico* (18) and verified inside the cell incubator by means of Hall-effect gaussmeter; iii) the maximum electromagnetic energy density applied to the cells was about 3.18 J/m^3 and, using a thermocouple, we observed no PEMF-induced heating; iv) during electromagnetic stimulation, control cells were placed into another identical incubator with no PEMF.

Drug preparation. Temozolomide (Temodal®) was purchased from Sigma-Aldrich (Milan, Italy) and 100 mg were dissolved in 1 ml of dimethyl sulfoxide (DMSO). The solution was diluted (1:10) with Eagle's minimum essential medium (EMEM), subdivided into stock aliquots stored at −20˚C. The solution was further diluted to appropriate concentration using EMEM immediately before use.

Cell culture. Human glioblastoma T98G cells were obtained from the European Collection of Authenticated Cell Cultures (Public Health England, Porton Down, Salisbury, UK). These cells were maintained in EMEM containing 10% calf serum, 100 units/ml penicillin-streptomycin, 1% sodium pyruvate and 2 mM L-glutamine (Sigma-Aldrich). The cells were maintained in exponential growth as monolayers in T75 plastic tissue-culture flasks (Corning, Oneonta, NY, USA) and kept in a humidified atmosphere with 5% CO_2 at 37˚C.

Treatments. Cells were exposed for 1 h to the PEMF described above. They were seeded 20-22 h before PEMF exposure in order to use the cells in the exponential phase of growth. The number of cells seeded in T75 flasks was 5×10^6. The control cultures were maintained and processed under the same conditions except for exposure to PEMF. 24 h after the end of PEMF treatment, 1×10^6 cells were seeded in 60 mm Petri dishes. Temozolomide was added to the cells for 24 h at a concentration of 10 μM. Control cells were treated under the same conditions except for drug administration. Proliferation and miRNA profiles were assessed at different times after temozolomide treatment.

Mitotic and apoptotic indices. To evaluate the mitotic index and the apoptotic index, cells grown on glass coverslips, with and without treatments, were stained with May-Grünwald-Giemsa method. The mitotic and apoptotic indices were assessed by examining ~10 consecutive high-power fields with an Olympus BX-41 microscope in a blind manner.

RNA extraction. Total RNA was extracted from cell monolayers using the RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The quality of RNA was evaluated by determining the RIN (TapeStation; Agilent Technologies, Santa Clara, CA, USA). A quantitative RNA analysis was performed using fluorometric methods by means of the Qubit™ platform (Invitrogen, Carlsbad, CA, USA) using the Quant-iT RNA assay (declared assay range between 5-100 ng; sample starting concentration between 250 pg/μl and 100 ng/μl): 2 μl of RNA were added to 198 μl of working solution obtained by mixing 1 μl of Qubit™ RNA reagent with 199 μl of Qubit™ RNA buffer. The quantification was performed following the calibration of the instrument with the Quant-iT RNA standards.

Quantitative real-time reverse transcription polymerase chain reaction. Quantitative real-time reverse transcription PCR (qRT-PCR) was performed using the cDNA obtained *via* the reverse transcription reaction with miRCURY LNA™ Universal RT miRNA PCR kit: 4 μl of total RNA (5 ng/μl) were added to 4 μl of 5× reaction buffer, 2 μl of enzyme mix, 1 μl of synthetic spike-in and 9 μl of nuclease-free water. The reaction was performed using a MJ Mini thermal cycler (Bio-Rad Laboratories, Segrate, Italy) for one reaction cycle at 42˚C for 60 min, at 95˚C for 5 min and the reaction products were immediately cooled at 4˚C. To evaluate the miRNA expression, qRT-PCR reactions were performed using the Universal cDNA Synthesis and SYBR® Green Master Mix kits. Amplification was carried out in a 10 μl reaction mixture containing 4 μl of 1:80 diluted cDNA, 5 μl of SYBR Green Master Mix and 1 μl of specific LNA probe (*miR-17-3p, miR-21-5p* and *miR-421-5p*) provided by Exiqon (Vedbaek, Denmark), using the following reaction conditions: a first step at 95˚C for 10 min, 45 amplification cycles at 95˚C for 10 s followed by a step at 60˚C for 1 min. U6 small nuclear RNA (*snU6*) was used to normalize miRNA expression and each assay was completed in triplicate using an Eco Real-Time PCR instrument (Illumina, San Diego, CA, USA). The results were

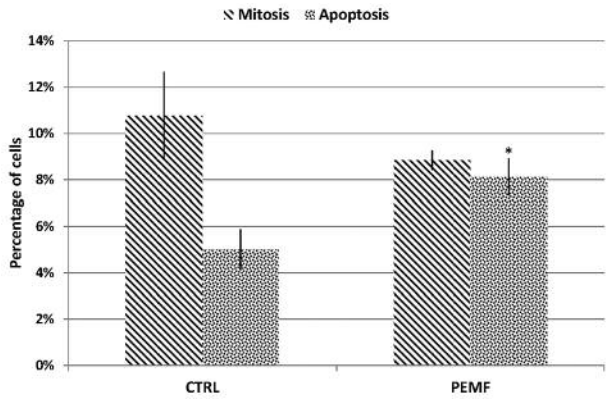

Figure 1. *Mitotic and apoptotic indices (%) in control (CTRL) cells and in pulsed electromagnetic field (PEMF)-exposed cells (observed 48 h after the end of PEMF treatment). Results are shown as the mean±SD, *p<0.05 Compared to the control, n=3.*

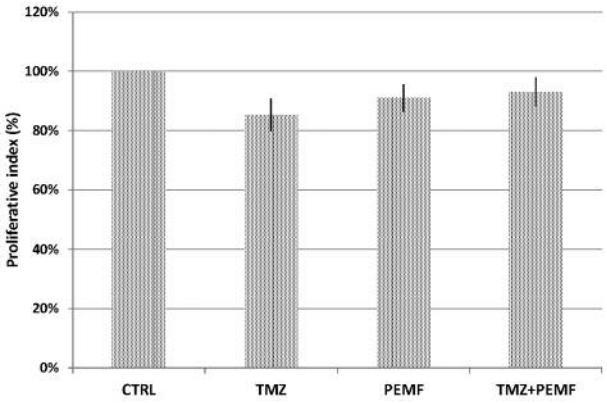

Figure 2. *Proliferative index (%) given as the number of cells relative to that of the control (CTRL) measured 48 h after the end of single and combined temozolomide (TMZ)/pulsed electromagnetic field (PEMF) treatment. Results are shown as the mean±SD, n=3.*

analyzed by the comparative C_T method ($\Delta\Delta C_T$) by the software package of the Eco Real-Time PCR system for the calculation of the $2^{-\Delta\Delta CT}$ value (29).

Statistics. Results are presented as the mean±SD. Statistical significance was analyzed by one-way ANOVA using GraphPad QuickCalcs (GraphPad Software, La Jolla, CA, USA), electing a significance level of 0.05.

Results

In the present work, the ability of a PEMF to counteract tumor growth was tested in an *in vitro* model of glioblastoma, the T98G cell line. This line is resistant to chemo- and radiotherapy and represents a useful model for evaluating the effect of EMFs on tumor growth. The PEMF was the same which was found to induce autophagy in glioblastoma cells (24). According to this study, the cells were exposed to PEMF for 1 h at 37˚C and, every 2 days, a sample of PEMF-exposed culture and a sample of control, unexposed culture were sacrificed for comparison.

In order to assess whether PEMF inhibits tumor growth, the cell recovery was evaluated during the 8 days after the end of treatment by measuring the cell number in PEMF-treated cultures compared to the unexposed ones: a slight decrease in cell growth (~20%) was observed 2 days after the end of PEMF stimulation, followed by cell recovery and a slight increase in cell growth after 8 days. On the other hand, temozolomide was always found to reduce the cell number with respect to the control.

In comparison with the control, the mitotic index slightly, non-significantly decreased 48 h after the end of PEMF exposure, whereas the apoptotic index significantly increased ($p<0.05$) at the same time (Figure 1).

We then evaluated a possible synergistic effect of PEMF and temozolomide. Cells were treated with PEMF with/without temozolomide according to two different protocols: i) PEMF conditioning (1 h), 24 h without PEMF, temozolomide treatment (10 μM for 24 h); or ii) temozolomide treatment (10 μM for 24 h) immediately after PEMF exposure (1 h).

The proliferative index (number of cells relative to the control) was evaluated 48 h after the end of temozolomide/PEMF treatment. The results obtained are reported in Figure 2. Temozolomide alone, PEMF alone, and the combined treatment induced only a slight reduction of cell recovery, indicating a weak ability of all treatments to counteract cell proliferation. The greatest effect was observed when the cells were treated with temozolomide alone.

We then studied some aspects of the epigenetic regulation of cell growth/survival and of oncogenesis in terms of the expression of miRNAs. Such miRNAs are related to mitochondrial antioxidant activities (*miR-17*), cell-cycle progression (*miR-21*) and to repair of DNA damaged by chemical mutagens (*miR-421*). *miR-17* down-regulates the expression of three critical primary mitochondrial antioxidant enzymes (manganese superoxide dismutase, glutathione peroxidase 2 and thioredoxin reductase 2) and is reported to act as a tumor promoter *e.g.* it enhances prostate tumor growth by increasing cell proliferation, colony formation, cell survival and invasion (30). *miR-21* down-regulates the expression of the activator transcriptional factors E2F1 and E2F2 involved in cell cycle progression and, finally, the expression of ataxia telangiectasia mutated (*ATM*) gene, fundamental in DNA repair. *miR-421* targets the 3' untranslated region of ataxia telangiectasia mutated mRNA leading to greater radiosensitivity (31).

The expression of these miRNAs was studied 48 h after the end of temozolomide/PEMF treatment; the results are reported in Figure 3. *miR-17* expression showed a significant increase over the control ($p<0.05$) only for temozolomide treatment; *miR-21* synthesis was significantly ($p<0.05$) reduced only with the combined treatment (temozolomide administered immediately after PEMF); *miR-421* expression significantly increased over the control ($p<0.05$) after temozolomide alone and after PEMF alone. Expression of all studied miRNAs was drastically reduced ($p<0.05$) when the cells were treated with temozolomide immediately after PEMF exposure ($p<0.05$).

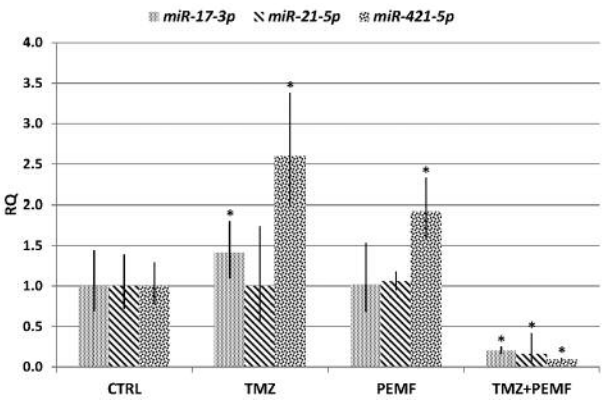

Figure 3. *Quantitative expression (RQ) with respect to that of the control (CTRL) of miR-17-3p, miR-21-5p and miR-421-5p measured 48 h after the end of single and combined temozolomide (TMZ)/pulsed electromagnetic field treatment. Results are shown as the mean±SD, *p<0.05 Compared with the control, n=3.*

Discussion

The results of our study demonstrate that PEMF exposure can affect gene expression, with an epigenetic action mediated by miRNAs, and that the combined treatment with temozolomide and PEMF (temozolomide administered immediately after PEMF) induces a modulation of expression of genes that regulate tumor progression. In particular, PEMF caused increased expression only of *miR-421*, whereas a similar increase was observed when cells were treated only with temozolomide, except for *miR-21*. The treatment with temozolomide applied 24 h after PEMF exposure did not have a clear effect (data not shown), but when glioblastoma cells were exposed to PEMF then soon after to temozolomide, expression of all studied miRNAs decreased.

A higher expression of *miR-17* is associated with tumor cells (32): miR-17 targets the tumor-suppressor gene phosphatase and tensin homolog (*PTEN*) and down-regulates antioxidant mitochondrial activities. Expression of *miR-21* has been found to be deregulated in almost all types of cancer and, therefore, it was classified as an oncomiR (33) (this miRNA was first noted as an apoptotic suppressor in various cell lines). In addition, *miR-421* has been reported to be up-regulated in several human cancer types (34), moreover, its overexpression induces apoptosis resistance (35).

Glioblastoma cells treated with temozolomide plus PEMF showed an evident decrease of these miRNAs, thus manifesting an epigenetic pro-apoptotic effect.

The use of electric and PEMFs for cancer therapy is based on the ability to block mitosis and to make tumor cells more sensitive to chemotherapy. TTField therapy is an FDA-approved treatment for patients with glioblastoma (36). On the other hand, the PEMF used here can act at the epigenetic level, that is, it can influence gene expression without changing the codifying sequence.

Only a few studies on epigenetic effects of PEMFs have been conducted: Liu *et al.* reported that PEMFs induce a variation in the methylation pattern of mouse spermatocyte-derived cells (37) and influence miRNA expression (14).

Our study showed that PEMF can epigenetically affect the regulation of oncogenes and tumor suppressors: the results indicate that PEMF coupled with chemotherapy (temozolomide plus PEMF) can trigger epigenetic mechanisms to slow down the neoplastic proliferation.

The T98G glioblastoma cell line is chemo- and radioresistant, making our findings more meaningful. The future challenge is to better understand the networks regulated by the miRNAs involved in tumor growth and influenced by PEMFs, also bearing in mind the duration and the cycles of electromagnetic treatment.

References

1 Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E and Mirimanoff RO: Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med *352*: 987-996, 2005.

2 Becker KP and Yu J: Status quo--standard-of-care medical and radiation therapy for glioblastoma. Cancer J 18: 12-19, 2012.

3 Huang L, Dong L, Chen Y, Qi H and Xiao D: Effects of sinusoidal magnetic field observed on cell proliferation, ion concentration, and osmolarity in two human cancer cell lines. Electromagn Biol Med *25*: 113-126, 2006.

4 Zimmerman JW, Pennison MJ, Brezovich I, Yi N, Yang CT, Ramaker R, Absher D, Myers RM, Kuster N, Costa FP, Barbault A and Pasche B: Cancer cell proliferation is inhibited by specific modulation frequencies. Br J Cancer *106*: 307-313, 2012.

5 Zimmerman JW, Jimenez H, Pennison MJ, Brezovich I, Morgan D, Mudry A, Costa FP, Barbault A and Pasche B: Targeted treatment of cancer with radiofrequency electromagnetic fields amplitude-modulated at tumor-specific frequencies. Chin J Cancer *32*: 573-581, 2013.

6 Manni V, Lisi A, Pozzi D, Rieti S, Serafino A, Giuliani L and Grimaldi S: Effects of extremely low frequency (50 Hz) magnetic field on morphological and biochemical properties of human keratinocytes. Bioelectromagnetics *23*: 298-305, 2002.

7 Zhao G, Lin X, Zhou M and Zhao J: Relationship between exposure to extremely low-frequency electromagnetic fields and breast cancer risk: a meta-analysis. Eur J Gynaecol Oncol *35*: 264-269, 2014.

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

9 Pasi F, Sanna S, Paolini A, Alquati M, Lascialfari A, Corti ME, Di Liberto R, Cialdai F, Monici M and Nano R: Effects of extremely low-frequency magnetotherapy on proliferation of human dermal fibroblasts. Electromagn Biol Med [In Press]: 1-10, 2016.

10 Zwirska-Korczala K, Jochem J, Adamczyk-Sowa M, Sowa P, Polaniak R, Birkner E, Latocha M, Pilc K and Suchanek R: Effect of extremely low frequency of electromagnetic fields on cell proliferation, antioxidative enzyme activities and lipid peroxidation in 3T3-L1 preadipocytes – an *in vitro* study. J Physiol Pharmacol *56(Suppl 6)*: 101-108, 2005.

11 Buldak RJ, Polaniak R, Buldak L, Zwirska-Korczala K, Skonieczna M, Monsiol A, Kukla M, Dulawa-Buldak A and 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*: 641-651, 2012.

12 Winker R, Ivancsits S, Pilger A, Adlkofer F and Rudiger HW: Chromosomal damage in human diploid fibroblasts by intermittent exposure to extremely low-frequency electromagnetic fields. Mutat Res *585*: 43-49, 2005.

13 Morandi MA, Pak CM, Caren RP and Caren LD: Lack of an EMF-induced genotoxic effect in the Ames assay. Life Sci *59*: 263-271, 1996.

14 Liu Y, Liu WB, Liu KJ, Ao L, Cao J, Zhong JL and Liu JY: Extremely low-frequency electromagnetic fields affect the miRNA-mediated regulation of signaling pathways in the GC-2 cell line. PLoS One *10*: e0139949, 2015.

15 Kirson ED, Gurvich Z, Schneiderman R, Dekel E, Itzhaki A, Wasserman Y, Schatzberger R and Palti Y: Disruption of cancer cell replication by alternating electric fields. Cancer Res *64*: 3288-3295, 2004.

16 Kirson ED, Dbaly V, Tovarys F, Vymazal J, Soustiel JF, Itzhaki A, Mordechovich D, Steinberg-Shapira S, Gurvich Z, Schneiderman R, Wasserman Y, Salzberg M, Ryffel B, Goldsher D, Dekel E and Palti Y: Alternating electric fields arrest cell proliferation in animal tumor models and human brain tumors. Proc Natl Acad Sci USA *104*: 10152-10157, 2007.

17 Ansstas G and Tran DD: Treatment with tumor-treating fields therapy and pulse dose bevacizumab in patients with bevacizumab-refractory recurrent glioblastoma: A case series. Case Rep Neurol *8*: 1-9, 2016.

18 Mognaschi ME, Di Barba P, Magenes G, Lenzi A, Naro F and Fassina L: Field models and numerical dosimetry inside an extremely-low-frequency electromagnetic bioreactor: the theoretical link between the electromagnetically induced mechanical forces and the biological mechanisms of the cell tensegrity. Springerplus *3*: 473, 2014.

19 Fassina L, Visai L, Benazzo F, Benedetti L, Calligaro A, Cusella De Angelis MG, Farina A, Maliardi V and Magenes G: Effects of electromagnetic stimulation on calcified matrix production by SAOS-2 cells over a polyurethane porous scaffold. Tissue Eng *12*: 1985-1999, 2006.

20 De Mattei M, Varani K, Masieri FF, Pellati A, Ongaro A, Fini M, Cadossi R, Vincenzi F, Borea PA and Caruso A: Adenosine analogs and electromagnetic fields inhibit prostaglandin E2 release in bovine synovial fibroblasts. Osteoarthritis Cartilage *17*: 252-262, 2009.

21 Varani K, Vincenzi F, Targa M, Corciulo C, Fini M, Setti S, Cadossi R and Borea PA: Effect of pulsed electromagnetic field exposure on adenosine receptors in rat brain. Bioelectromagnetics *33*: 279-287, 2012.

22 Osera C, Fassina L, Amadio M, Venturini L, Buoso E, Magenes G, Govoni S, Ricevuti G and Pascale A: Cytoprotective response induced by electromagnetic stimulation on SH-SY5Y human neuroblastoma cell line. Tissue Eng Part A *17*: 2573-2582, 2011.

23 Osera C, Amadio M, Falone S, Fassina L, Magenes G, Amicarelli F, Ricevuti G, Govoni S and Pascale A: Pre-exposure of neuroblastoma cell line to pulsed electromagnetic field prevents H_2O_2-induced ROS production by increasing MnSOD activity. Bioelectromagnetics *36*: 219-232, 2015.

24 Marchesi N, Osera C, Fassina L, Amadio M, Angeletti F, Morini M, Magenes G, Venturini L, Biggiogera M, Ricevuti G, Govoni S, Caorsi S, Pascale A and Comincini S: Autophagy is modulated in human neuroblastoma cells through direct exposition to low frequency electromagnetic fields. J Cell Physiol *229*: 1776-1786, 2014.

25 Fassina L, Saino E, Visai L, Silvani G, Cusella De Angelis MG, Mazzini G, Benazzo F and Magenes G: Electromagnetic enhancement of a culture of human SAOS-2 osteoblasts seeded onto titanium fiber-mesh scaffolds. J Biomed Mater Res A *87*: 750-759, 2008.

26 Fassina L, Saino E, Sbarra MS, Visai L, Cusella De Angelis MG, Mazzini G, Benazzo F and Magenes G: Ultrasonic and electromagnetic enhancement of a culture of human SAOS-2 osteoblasts seeded onto a titanium plasma-spray surface. Tissue Eng Part C Methods *15*: 233-242, 2009.

27 Fassina L, Saino E, Sbarra MS, Visai L, Cusella De Angelis MG, Magenes G and Benazzo F: *In vitro* electromagnetically stimulated SAOS-2 osteoblasts inside porous hydroxyapatite. J Biomed Mater Res A *93*: 1272-1279, 2010.

28 Saino E, Fassina L, Van Vlierberghe S, Avanzini MA, Dubruel P, Magenes G, Visai L and Benazzo F: Effects of electromagnetic stimulation on osteogenic differentiation of human mesenchymal stromal cells seeded onto gelatin cryogel. Int J Immunopathol Pharmacol *24*: 1-6, 2011.

29 Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods *25*: 402-408, 2001.

30 Yang X, Du WW, Li H, Liu F, Khorshidi A, Rutnam ZJ and Yang BB: Both mature miR-17-5p and passenger strand miR-17-3p target TIMP3 and induce prostate tumor growth and invasion. Nucleic Acids Res *41*: 9688-9704, 2013.

31 Mansour WY, Bogdanova NV, Kasten-Pisula U, Rieckmann T, Kocher S, Borgmann K, Baumann M, Krause M, Petersen C, Hu H, Gatti RA, Dikomey E, Dork T and Dahm-Daphi J: Aberrant overexpression of miR-421 down-regulates ATM and leads to a pronounced DSB repair defect and clinical hypersensitivity in SKX squamous cell carcinoma. Radiother Oncol *106*: 147-154, 2013.

32 Shan SW, Fang L, Shatseva T, Rutnam ZJ, Yang X, Du W, Lu WY, Xuan JW, Deng Z and Yang BB: Mature miR-17-5p and passenger miR-17-3p induce hepatocellular carcinoma by targeting PTEN, GalNT7 and vimentin in different signal pathways. J Cell Sci *126*: 1517-1530, 2013.

33 Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR and Golub TR: MicroRNA expression profiles classify human cancers. Nature *435*: 834-838, 2005.

34 Jiang Z, Guo J, Xiao B, Miao Y, Huang R, Li D and Zhang Y: Increased expression of miR-421 in human gastric carcinoma and its clinical association. J Gastroenterol *45*: 17-23, 2010.

35 Chen L, Tang Y, Wang J, Yan Z and Xu R: miR-421 induces cell proliferation and apoptosis resistance in human nasopharyngeal carcinoma *via* down-regulation of *FOXO4*. Biochem Biophys Res Commun *435*: 745-750, 2013.

36 Gutin PH and Wong ET: Noninvasive application of alternating electric fields in glioblastoma: a fourth cancer treatment modality. American Society of Clinical Oncology educational book/ASCO American Society of Clinical Oncology Meeting: 126-131, 2012.

37 Liu Y, Liu WB, Liu KJ, Ao L, Zhong JL, Cao J and 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.

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