



SECTION 5

Evidence for EMF Transcriptomics and Proteomics Research 2007-2012

2012 Supplement

Adamantia F. Fragopoulou, MSc, PhD
Department of Cell Biology and Biophysics
University of Athens,
Athens, Greece

Prof. Lukas H. Margaritis, PhD
Department of Cell Biology and Biophysics
University of Athens
Athens, Greece

Prepared for the BioInitiative Working Group
November 2012

I. INTRODUCTION

Daily exposure levels for non-ionizing electromagnetic radiation (NI-EMR) have significantly increased in the last few decades for human populations, and for wildlife, plants, and other living creatures on earth. NI-EMR includes a wide range of frequencies, as low as extremely low frequencies (ELF) magnetic fields deriving from the power lines up to microwave radiofrequencies (MW-RF). Within this range are FM and TV broadcast stations, wireless technology devices (mobile phones and masts, cordless phones, Wi-Fi routers and units).

The exposure to any of these frequencies individually, or in combination, raises concern about potentially harmful effects and is the subject of intensive scientific studies around the world. Such studies include epidemiological, clinical, *in vivo* and *in vitro* studies. The pace of scientific study accelerated after 2010, when the World Health Organization following the ELF agenda of 2007 (WHO, 2007), announced the implementation of the International EMF Project's RF Research Agenda as a "*research topic for measurement surveys to characterize population exposures from all radio frequency (RF) sources with a particular emphasis on new wireless technologies*" (WHO, 2010). The IARC (International Agency for Research on Cancer) under the auspices of the WHO classified RFR as a Possible Human Carcinogen (Group 2B) on 2011 (Baan et al., 2011).

The studies published so far have utilized various model systems and approaches but not in a coordinated manner, although there have been international efforts (i.e., INTERPHONE Final Study; Cardis et al., 2011).

As reviewed by Vlaanderen et al. (2009), OMICS technologies are relatively new biomarker discovery tools that can be applied to study large sets of biological molecules. (The English-language neologism omics informally refers to a field of study in biology ending in *-omics*, such as genomics, proteomics or metabolomics). Their applications in EMF and RFR research have become feasible in recent years due to a spectacular increase in the sensitivity, resolution and throughput of OMICS-based assays (Vlaanderen et al., 2009).

.Although, the number of OMIC techniques is ever expanding, the five most developed OMICS technologies are genotyping, transcriptomics, epigenomics, proteomics and metabolomics.

A number of reports have dealt with possible changes on gene/protein expression, either at an individual gene/protein level or using the high throughput “omics” approaches (T & P -transcriptomics and proteomics respectively) (for reviews see Xu & Chen, 2007; Blankenburg et al., 2009; McNamee & Chauhan, 2009; Mevissen M., 2011; Leszczynski et al., 2012). These T & P approaches have gained ground in the investigation of the possible EMF effects the last decade (Blankenburg et al., 2009), since they can screen the whole genome or proteome and may contribute on the elucidation of EMF mechanisms of action.

Following the work of Xu and Chen who gathered all studies on EMF research using T & P high throughput approaches up to 2006 in the BioInitiative Report (Xu & Chen, 2007), this supplemental chapter on Transcriptomics and Proteomics updates newly published work since that initial review in 2007.

II. EXTREMELY LOW FREQUENCY ELECTROMAGNETIC FIELDS (ELF-EMFS)

A. Transcriptomics

As explicitly described by M. Mevissen (2011), gene expression profiling is the identification and characterization of the mixture of mRNA that is present in a specific sample. Both the presence of specific forms of mRNA and the levels in which these forms occur are parameters that provide information on gene expression. A gene expression profile provides a quantitative overview of the mRNA transcripts that were present in a sample at the time of collection. Therefore, gene expression profiling can be used to determine which genes are differently expressed as a result of changes in environmental conditions. DNA Microarrays represent an innovative and comprehensive technology that allows researchers to assess the expression level of thousands of genes in a high-throughput fashion and has been exploited in EMF research studies.

Schwenzer et al. (2007) reported effects of static magnetic field on genome expression. Specifically, the researchers evaluated the influence of magnetic resonance imaging (MRI) on gene expression in embryonic human lung fibroblasts (Hel 299). The cells were exposed to the static magnetic field and to a turbo spin-echo sequence of an MR scanner at 3.0 Tesla. An MR group (exposed) and a control group

(sham-exposed) were set up using a special MR-compatible incubation system. The exposure time was two hours. Gene expression profiles were studied using a complementary deoxyribonucleic acid (cDNA) microarray containing 498 known genes involved in transcription, intracellular transport, structure/junction/adhesion or extracellular matrix, signalling, host defence, energetics, metabolism, cell shape, and death. No changes in gene expression were found in either group (exposed or sham-exposed cells) at the end of a two-hour exposure for any of the 498 tested protein genes. The results showed that MRI had no influence on protein–gene expression in eugenic human lung cells in this study.

The same year, Walther et al. (2007) analyzed the effects of BEMER type (combination of electromagnetic field and light therapy) electromagnetic field (BTEMF) on gene expression in human mesenchymal stem cells and chondrocytes. Primary mesenchymal stem cells from bone marrow and the chondrocyte cell line C28I2 were stimulated 5 times at 12-h intervals for 8 min each with BTEMF. RNA from treated and control cells was analyzed for gene expression using the affymetrix chip HG-U133A. A limited number of regulated gene products from both cell types, which control cell metabolism and cell matrix structure, was mainly affected. There was no increased expression though of cancer-related genes. RT-PCR analysis of selected transcripts partly confirmed array data. Results indicate that BTEMF in human mesenchymal stem cells and chondrocytes provide the first indications. A limitation of this study is the single array analysis which was performed. Therefore, as stated by the authors, the results should be regarded as a first hint on BTEMF effects on these cellular systems. Nevertheless, their findings indicate that matrix dynamics and cell metabolism/energy balance are processes that are affected by the electromagnetic field application.

In a follow-up study, using fibroblasts as in the study by Schwenzer et al. (2007), but exposing them to electric fields (EFs), Jennings et al. (2008) tried to elucidate the role of EFs during the course of normal wound healing. Fibroblasts at the wound edge are exposed to electric fields (EFs) ranging from 40 to 200 mV/mm and so various forms of EFs can influence fibroblast migration, proliferation, and protein synthesis and may contribute to fibroblast activation during wound repair. These authors compared gene expression in normal adult dermal fibroblasts exposed to a 100 mV/mm EF for 1 h to non-stimulated controls. Significantly increased expression of 162 transcripts and decreased expression of 302 transcripts was detected using

microarrays, with 126 transcripts above the level of 1.4-fold increase or decrease compared to the controls. Only 11 genes were significantly increased or decreased above the level of 2-fold, compared to controls. Many of these significantly regulated genes were associated with wound repair through the processes of matrix production, cellular signalling, and growth. Activity within specific cellular signalling pathways was noted, including TGF- β , G-proteins, and inhibition of apoptosis. In addition, RT-PCR analysis of the expression of KLF6, FN1, RGS2, and JMJD1C over continued stimulation and at different field strengths suggests that there are specific windows of field characteristics for maximum induction in the expression of these genes. EFs thus appeared to have an important role in controlling fibroblast activity in the process of wound healing. The authors highlight that 2-fold changes have traditionally and somewhat arbitrarily been designated as meaningful changes in gene expression, although there is little quantitative information connecting these values to changes in biological function. Therefore, multiple microarray experiments at different time points and field conditions may have revealed induction of different sets of genes under different experimental conditions. Follow-up studies should include proteomic analysis of altered protein production resulting from altered gene expression, alternative splicing in protein translation, and gene silencing studies to further delineate the mechanisms and locations of interaction between EFs and transcriptional regulators.

Kimura et al. (2008) using magnetic resonance imaging with high intensity static magnetic fields (SMFs) demonstrated in the nematode *Caenorhabditis elegans* that genes involved in motor activity, actin binding, cell adhesion, and cuticles were transiently and specifically induced following exposure to 3 or 5 T SMF in this metazoan experimental model. 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.

On 2010, Chung et al. conducted a study to investigate the possible effect of 60 Hz circularly polarized magnetic fields (MFs) as promoters of genetically initiated lymphoma in AKR mice. One hundred sixty female animals were divided into four different groups. They were exposed to four different intensities of circularly polarized MFs. Animals received exposure to 60 Hz circularly polarized MF at field strengths (rms-value) of 0 microT (sham control, T1, Group I), 5 microT (T2, Group II), 83.3 microT (T3, Group III), or 500 microT (T4, Group IV), for 21 h/day from the age of 4-6 weeks to the age of 44-46 weeks. There were no exposure-related changes in mean survival time, clinical signs, body weights, hematological values, micronucleus assay, gene expression arrays, analysis of apoptosis, and necropsy findings. Examination at the histopathological level, showed lymphoma in all the groups. The tumor incidence was 31/40(78%), 30/40(75%), 32/40(80%), and 31/40(78%) in sham control, 5, 83.3, and 500 microT groups, respectively. However, there were no differences in the tumor incidence between the sham control (T1) and circularly polarized MF exposure groups (T2-T4). In conclusion, there was no evidence that exposure to 60 Hz circularly polarized MF strengths up to 500 microT promoted lymphoma in AKR mice.

In a very recent attempt 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, Kirschenlohr et al. (2012) tried to determine if gene expression changes occur in white blood cells of volunteers exposed to an ELF-EMF. Each of 17 pairs of male volunteers age 20-30 was subjected either to a 50 Hz EMF exposure of $62.0 \pm 7.1 \mu\text{T}$ (approximately 600 mG) for 2 h or to a sham exposure ($0.21 \pm 0.05 \mu\text{T}$) at the same time (11:00 a.m. to 13:00 p.m.). The alternative regime for each volunteer was repeated on the following day and the two-day sequence was repeated 6 days later, with the exception that a null exposure ($0.085 \pm 0.01 \mu\text{T}$) replaced the sham exposure. Five blood samples (10 ml) were collected at 2 h intervals from 9:00 to 17:00 with five additional samples during the exposure and sham or null exposure periods on each study day. RNA samples were pooled for the same time on each study day for the group of 17 volunteers that were subjected to the ELF-EMF exposure/sham or null exposure sequence and were analyzed on Illumina microarrays. Time courses for 16 mammalian genes previously

reported to be responsive to ELF-EMF exposure, including immediate early genes, stress response, cell proliferation and apoptotic genes were examined in detail. No genes or gene sets showed consistent response profiles to repeated ELF-EMF exposures. A stress response was detected as a transient increase in plasma cortisol at the onset of either exposure or sham exposure on the first study day. The cortisol response diminished progressively on subsequent exposures or sham exposures, and was attributable to mild stress associated with the experimental protocol.

Commenting the above data, we note that the overall experimental design seems to lack real life conditions since a) the suspicion refers to childhood leukaemia and not to adults, b) exposure is not supposed to be just 2 hours a day but day long for children living in the vicinity of power lines, c) continuous daily exposure for years is the rationale behind the possibility of ELF's causing or increasing leukaemia.

B. Proteomics

Proteins are the key molecules that participate and regulate nearly all cellular functions. The number of each protein species in a given cell changes over time according to the metabolic and signalling demand and is subject to differential gene expression. Proteomics, is the science that explores by high throughput techniques the so called “protein expression profile” of proteins.

The reports on ELF and proteomics are practically absent in the last 5 years leaving only the old study by Seyyedi et al. (2007) in human fibroblast (using 3 Hz, sinusoidal continuous ELF electromagnetic fields, 3 h duration and 4 mT magnetic field intensity) and one more in 2011 by Sulpizio et al. The first study showed that some protein expressions were affected by radiation after comparing the 2-DE separated proteins from the exposed and sham (control) cells. The two proteins that their expression was reduced about 50% were determined as alpha 1 antitrypsin (A1AT) and Transthyretin (TTR) and has been concluded that application of ELF-EMF in therapeutic aspects may be accompanied by their side effects.

Along the “leukaemia ELF rationale” and in addition a possible ELF link with cancer, cardiovascular, and neurological disorders, Sulpizio et al. (2011) exposed human SH-SY5Y neuroblastoma cells to a 50 Hz, 1 mT (10 Gauss) sinusoidal ELF-MF at three duration schemes, 5 days (T5), 10 days (T10), and 15 days (T15). The effects of ELF-MF on proteome expression and biological behavior were investigated. Through comparative analysis between treated and control samples they identified

nine new proteins after a 15-day treatment. They suggested that the proteins were involved in a cellular defence mechanism and/or in cellular organization and proliferation such as peroxiredoxin isoenzymes (2, 3, and 6), 3-mercaptopyruvate sulfurtransferase, actin cytoplasmic 2, t-complex protein subunit beta, ropporin-1A, and profilin-2 and spindlin-1. These authors concluded that ELF-MFs exposure altered the proliferative status and other important cell biology-related parameters, such as cell growth pattern, and cytoskeletal organization and that ELF radiation could trigger a shift toward a more invasive phenotype.

III. RADIOFREQUENCY ELECTROMAGNETIC FIELDS (RF-EMFS)

A relatively small number of publications have dealt after 2007 with the effects of RF-EMF on the proteome and transcriptome of cells and even less number with the effects on animals.

A. Transcriptomics

Chauhan et al. (2007a) assessed non-thermal RF-field exposure effects on a variety of biological processes (including apoptosis, cell cycle progression, viability and cytokine production) in a series of human-derived cell lines (TK6, HL60 and Mono-Mac-6). Exponentially growing cells were exposed to intermittent (5 min on, 10 min off) 1.9 GHz pulse-modulated RF fields for 6 h at mean specific absorption rates (SARs) of 0, 1 and 10 W/kg. Concurrent negative (incubator) and positive (heat shock for 1 h at 43 degrees C) controls were included in each experiment. Immediately after the 6-h exposure period and 18 h after exposure, cell pellets were collected and analyzed for cell viability, the incidence of apoptosis, and alterations in cell cycle kinetics. The cell culture supernatants were assessed for the presence of a series of human inflammatory cytokines (TNFA, IL1B, IL6, IL8, IL10, IL12) using a cytometric bead array assay. No detectable changes in cell viability, cell cycle kinetics, incidence of apoptosis, or cytokine expression were observed in any of RF-field-exposed groups in any of the cell lines tested, relative to the sham controls. However, the positive (heat-shock) control samples displayed a significant decrease in cell viability, increase in apoptosis, and alteration in cell cycle kinetics (G(2)/M block). Overall, the researchers found no evidence that non-thermal RF-field exposure could elicit any detectable biological effect in three human-derived cell lines.

Chauhan et al. (2007b) have examined the effect of RF field exposure on the possible expression of late onset genes in U87MG cells after a 24 h RF exposure period. In addition, a human monocyte-derived cell-line (Mono-Mac-6, MM6) was exposed to intermittent (5 min ON, 10 min OFF) RF fields for 6 h and then gene expression was assessed immediately after exposure and at 18 h post exposure. Both cell lines were exposed to 1.9 GHz pulse-modulated RF fields for 6 or 24 h at specific absorption rates (SARs) of 0.1-10.0 W/kg (very high SAR value). In support of their previous results, they found no evidence that nonthermal RF field exposure could alter gene expression in either cultured U87MG or MM6 cells, relative to non irradiated control groups. However, exposure of both cell-lines to heat-shock conditions (43 degrees C for 1 h) caused an alteration in the expression of a number of well-characterized heat-shock proteins.

The same year, Zhao et al. (2007) investigated whether expression of genes related to cell death pathways are dysregulated in primary cultured neurons and astrocytes by exposure to a working GSM cell phone rated at a frequency of 1900 MHz. Primary cultures were exposed for 2h. Microarray analysis and real-time RT-PCR were applied and showed up-regulation of caspase-2, caspase-6 and Asc gene expression in neurons and astrocytes. Up-regulation occurred in both "on" and "stand-by" modes in neurons, but only in "on" mode in astrocytes. Additionally, astrocytes showed up-regulation of the Bax gene. The effects were specific since up-regulation was not seen for other genes associated with apoptosis, such as caspase-9 in either neurons or astrocytes, or Bax in neurons. The results showed that even relatively short-term exposure to cell phone radiofrequency emissions can up-regulate elements of apoptotic pathways in cells derived from the brain, and that neurons appear to be more sensitive to this effect than astrocytes.

In an *in vitro* study focusing on the effects of low-level radiofrequency (RF) fields from mobile radio base stations employing the International Mobile Telecommunication 2000 (IMT-2000) cellular system, Hirose et al. (2007) tested the hypothesis that modulated RF fields act to induce phosphorylation and overexpression of heat shock protein hsp27. The study evaluated the responses of human cells to microwave exposure at a specific absorption rate (SAR) of 80 mW/kg, which corresponds to the limit of the average whole-body SAR for general public exposure defined as a basic restriction in the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines. Secondly, the study investigated whether

continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) modulated signal RF fields at 2.1425 GHz can induce activation or gene expression of hsp27 and other heat shock proteins (hsps). Human glioblastoma A172 cells were exposed to W-CDMA radiation at SARs of 80 and 800 mW/kg for 2-48 h, and CW radiation at 80 mW/kg for 24 h. Human IMR-90 fibroblasts from fetal lungs were exposed to W-CDMA at 80 and 800 mW/kg for 2 or 28 h, and CW at 80 mW/kg for 28 h. Under the RF field exposure conditions described above, no significant differences in the expression levels of phosphorylated hsp27 at serine 82 (hsp27[pS82]) were observed between the test groups exposed to W-CDMA or CW signal and the sham-exposed negative controls, as evaluated immediately after the exposure periods by bead-based multiplex assays. Moreover, no noticeable differences in the gene expression of hsps were observed between the test groups and the negative controls by DNA Chip analysis.

Paparini et al. (2008) found no evidence of major transcriptional changes in the brain of mice exposed to 1800 MHz GSM signal for 1 h at a whole body SAR of 1.1 W/kg. Gene expression was studied in the whole brain, where the average SAR was 0.2 W/kg, by expression microarrays containing over 22,600 probe sets. Comparison of data from sham and exposed animals showed no significant difference in gene expression modulation. However, when less stringent constraints were adopted to analyze microarray results, 75 genes were found to be modulated following exposure. Forty-two probes showed fold changes ranging from 1.5 to 2.8, whereas 33 were down-regulated from 0.67- to 0.29-fold changes, but these differences in gene expression were not confirmed by real-time PCR. Under these specific limited conditions, no consistent indication of gene expression changes in whole mouse brain was found associated to GSM 1800 MHz exposure. *We could possibly explain the lack of gene expression changes in this, as well in other studies, by the very short exposure duration used of 1 h.*

Nittby et al. (2008) applied Microarray hybridizations on Affymetrix rat2302 chips of RNA extracts from cortex and hippocampus of GSM 1800 exposed rats for just 6 h within TEM cells. Using four exposed and four control animals they found that a large number of genes were altered at hippocampus and cortex. The vast majority were downregulated. Since the genes that were differentially expressed between the two groups were responsible to membrane integral and signal transduction, the authors concluded that the change of their expression might be the cause of their

previous observations of blood-brain-barrier leakage and albumin transport through brain capillaries.

Huang et al. (2008a) monitored cellular and molecular changes in Jurkat human T lymphoma cells after irradiating with 1763 MHz RF radiation in order to test the effect on RF radiation in immune cells. Jurkat T-cells were exposed to RF radiation to assess the effects on cell proliferation, cell cycle progression, DNA damage and gene expression. Cells were exposed to 1763 MHz RF radiation at 10 W/kg specific absorption rate (SAR) and compared to sham exposed cells. RF exposure did not produce significant changes in cell numbers, cell cycle distributions, or levels of DNA damage. In genome-wide analysis of gene expressions, there were no genes changed more than 2-fold upon RF-radiation while ten genes changed from 1.3 to approximately 1.8-fold. Among these ten genes, two cytokine receptor genes such as chemokine (C-X-C motif) receptor 3 (CXCR3) and interleukin 1 receptor, type II (IL1R2) were down-regulated upon RF radiation. These results indicate that the alterations in cell proliferation, cell cycle progression, DNA integrity or global gene expression were not detected upon 1763 MHz RF radiation under 10 W/kg SAR for 24 h to Jurkat T cells.

In a follow-up study Huang et al. (2008b) chose HEI-OC1 immortalized mouse auditory hair cells to characterize the cellular response to 1763 MHz RF exposure, because auditory cells can be exposed to mobile phone frequencies. Cells were exposed to 1763 MHz RF at a 20 W/kg specific absorption rate (SAR) in a code division multiple access (CDMA) exposure chamber for 24 and 48 h to check for changes in cell cycle, DNA damage, stress response, and gene expression. Neither cell cycle changes nor DNA damage were detected in RF-exposed cells. The expression of heat shock proteins (HSP) and the phosphorylation of mitogen-activated protein kinases (MAPK) did not change, either. The researchers tried to identify any alteration in gene expression using microarrays. Using the Applied Biosystems 1700 full genome expression mouse microarray, they found that 29 genes (0.09% of total genes examined) were changed by more than 1.5-fold on RF exposure. From these results, they could not find any evidence of the induction of cellular responses, including cell cycle distribution, DNA damage, stress response and gene expression, after 1763 MHz RF exposure at an SAR of 20 W/kg (very high value) in HEI-OC1 auditory hair cells.

Concerning plant cell experiments Engelmann et al. (2008) searched for physiological processes of plant cells sensitive to RF fields. They reported significant changes (but not more than 2.5-fold) in transcription of 10 genes in cell suspension cultures of *Arabidopsis thaliana*, which were exposed for 24 h to an RF field protocol representing typical microwave exposition in an urban environment. The changes in transcription of these genes were compared with published microarray datasets and revealed a weak similarity of the microwave to light treatment experiments. Considering the large changes described in published experiments, it is questionable if the small alterations caused by a 24 h continuous microwave exposure would have any impact on the growth and reproduction of whole plants.

Using very low SAR values (0.9–3 mW/kg) Dawe et al. (2009) applied microarray technology in the nematode *C. elegans*. They compared five Affymetrix gene arrays of pooled triplicate RNA populations from sham-exposed L4/adult worms against five gene arrays of pooled RNA from microwave-exposed worms (taken from the same source population in each run). No genes showed consistent expression changes across all five comparisons, and all expression changes appeared modest after normalisation (< or =40% up- or down-regulated). The number of statistically significant differences in gene expression (846) was less than the false-positive rate expected by chance (1131). The authors concluded that the pattern of gene expression in L4/adult *C. elegans* is substantially unaffected by low-intensity microwave radiation and that the minor changes observed in this study could well be false positives. As a positive control, they compared RNA samples from N2 worms subjected to a mild heat-shock treatment (30 °C) against controls at 26 °C (two gene arrays per condition). As expected, heat-shock genes were strongly up-regulated at 30 °C, particularly an hsp-70 family member (C12C8.1) and hsp-16.2. Under these heat-shock conditions, they confirmed that an hsp-16.2::GFP transgene was strongly up-regulated, whereas two non-heat-inducible transgenes (daf-16::GFP; cyp-34A9::GFP) showed little change in expression. Preliminary work in our lab has indicated that this model organism is highly resistant to EMF sources including mobile phone, DECT and Wi-Fi radiation exposures, for reasons that are under investigation (Margaritis et al., unpublished).

RF exposure up to the limit of whole-body average SAR levels as specified in the ICNIRP guidelines is unlikely to elicit a general stress response in the tested cell lines

under these conditions as reported by Sekijima et al. (2010). These authors investigated the mechanisms by which radiofrequency (RF) fields exert their activity, and the changes in both cell proliferation and the gene expression profile in the human cell lines, A172 (glioblastoma), H4 (neuroglioma), and IMR-90 (fibroblasts from normal fetal lung) following exposure to 2.1425 GHz continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) RF fields at three field levels. During the incubation phase, cells were exposed at specific absorption rates (SARs) of 80, 250, or 800 mW/kg with both CW and W-CDMA RF fields for up to 96 h. Heat shock treatment was used as the positive control. No significant differences in cell growth or viability were observed between any test group exposed to W-CDMA or CW radiation and the sham-exposed negative controls. Using the Affymetrix Human Genome Array, only a very small (< 1%) number of available genes (ca. 16,000 to 19,000) exhibited altered expression in each experiment. According to the authors the results confirm that low-level exposure to 2.1425 GHz CW and W-CDMA RF fields for up to 96 h did not act as an acute cytotoxicant in either cell proliferation or the gene expression profile. These results suggest that RF exposure up to the limit of whole-body average SAR levels as specified in the ICNIRP guidelines is unlikely to elicit a general stress response in the tested cell lines under these conditions.

In order to investigate whether exposure to high-frequency electromagnetic fields (EMF) could induce adverse health effects, Trivino et al. (2012) cultured acute T-lymphoblastoid leukemia cells (CCRF-CEM) in the presence of 900 MHz MW-EMF generated by a transverse electromagnetic (TEM) cell at short and long exposure times and the effect of high-frequency EMF on gene expression has been evaluated. Significant changes in gene expression levels of genes involved in DNA repair, cell cycle arrest, apoptosis, chromosomal organization, and angiogenesis were observed. The authors have identified functional pathways influenced by 900 MHz MW-EMF exposure.

It is worth mentioning, although beyond the frequencies used in cellular communication, that changes were detected using millimeter-waves in 56 genes at 6 h exposure and 58 genes at 24 h exposure in rats as shown by Millenbaugh et al. (2008). The animals were subjected to 35 GHz millimeter waves at a power density of 75 mW/cm², to sham exposure and to 42 degrees Centigrade environmental heat. Skin

samples were collected at 6 and 24 h after exposure for Affymetrix Gene Chip analysis. The skin was harvested from a separate group of rats at 3-6 h or 24-48 h after exposure for histopathology analysis. Microscopic findings observed in the dermis of rats exposed to 35 GHz millimeter waves included aggregation of neutrophils in vessels, degeneration of stromal cells, and breakdown of collagen. Changes were detected in 56 genes at 6 h and 58 genes at 24 h in the millimeter-wave-exposed rats. Genes associated with regulation of transcription, protein folding, oxidative stress, immune response, and tissue matrix turnover were affected at both times. At 24 h, more genes related to extracellular matrix structure and chemokine activity were altered. Up-regulation of Hspa1a, Timp1, S100a9, Ccl2 and Angptl4 at 24 h by 35 GHz millimeter-wave exposure was confirmed by real-time RT-PCR. These results obtained from histopathology, microarrays and RT-PCR indicated that prolonged exposure to 35 GHz millimeter waves causes thermally related stress and injury in skin while triggering repair processes involving inflammation and tissue matrix recovery.

B. Proteomics

In a series of publications by Leszczynski's research group, consistently using human endothelial cell lines EA.hy926 and EA.hy926v1, protein expression changes occurred after exposure to 900 MHz.

The potential proteome expression changes by RF on the same cell line EA.hy926 have been further investigated by the same group in a follow-up study (Nylund et al., 2009), where they reported that 1h exposure to GSM 1800 MHz mobile phone radiation (SAR 2.0 W/kg) can also alter this cell line's proteome expression. Sham samples were produced simultaneously in the same conditions but without the radiation exposure. Cells were harvested immediately after 1-hour exposure to the radiation, and proteins were extracted and separated using 2-dimensional electrophoresis (2DE). In total, 10 experimental replicates were generated from both exposed and sham samples. About 900 protein spots were detected in the 2DE-gels using PDQuest software and eight of them were found to be differentially expressed in exposed cells ($p < 0.05$, t-test). Three out of these eight proteins were identified using Maldi-ToF mass spectrometry (MS). These proteins were: spermidine synthase (SRM), 78 kDa glucose-regulated protein (55 kDa fragment) (GRP78) and proteasome subunit alpha type 1 (PSA1). Due to the lack of the availability of

commercial antibodies the researchers were able to further examine expression of only GRP78. Using SDS-PAGE and western blot method they were not able to confirm the result obtained for GRP78 using 2DE. Additionally, no effects were reported this time for 1800GSM exposure on the expression of vimentin and Hsp27 - proteins that were affected by the 900 MHz GSM exposure in their earlier studies. The authors highlight that the observed discrepancy between the expression changes of GRP78 detected with 1DE and 2DE confirms the importance of validation of the results obtained with 2DE using other methods, e.g. western blot.

Using a higher definition technique, the 2D-DIGE, Leszczynski's group investigated whether GSM1800 radiation can alter the proteome of primary human umbilical vein endothelial cells and primary human brain microvascular endothelial cells (Nylund et al., 2010). The cells were exposed for 1 hour to 1800 MHz GSM mobile phone radiation at an average specific absorption rate of 2.0 W/kg. Following that, cells were harvested immediately and the protein expression patterns of the sham-exposed and radiation-exposed cells were examined using two dimensional difference gel electrophoresis based proteomics (2DE-DIGE). Numerous differences were observed between the proteomes of human umbilical vein endothelial cells and human brain microvascular endothelial cells (both sham-exposed). These differences are most likely representing physiological differences between endothelia in different vascular beds. However, the exposure of both types of primary endothelial cells to mobile phone radiation did not cause any statistically significant changes in protein expression. So, radiation did not provoke any proteome expression changes to these kinds of cells immediately at the end of the exposure and when the false discovery rate correction was applied to analysis. This observation agrees with earlier the earlier study of this group showing that the 1800 MHz GSM radiation exposure had only very limited effect on the proteome of human endothelial cell line EA.hy926, as compared with the effect of 900 MHz GSM radiation.

Another "omics" group exposing human lens epithelial cells detected heat-shock protein (HSP) 70 and heterogeneous nuclear ribonucleoprotein K (hnRNP K) to be upregulated following exposure to GSM 1800 MHz for 2 h (Li et al., 2007). In three separate experiments, HLECs were exposed and sham-exposed (six dishes each) to 1800-MHz GSM-like radiation for 2 h. The specific absorption rates were 1.0, 2.0, or 3.5 W/kg. Immediately after radiation, the proteome was extracted from the HLECs. Immobilized pH gradient two-dimensional polyacrylamide gel electrophoresis (2-DE;

silver staining) and PDQuest 2-DE analysis software were used to separate and analyze the proteome of exposed and sham-exposed HLECs. Four differentially expressed protein spots were selected and identified by using electrospray ionization tandem mass spectrometry (ESI-MS-MS). When the protein profiles of exposed cells were compared with those of sham-exposed cells, four proteins were detected as upregulated. After analysis by ESI-MS-MS and through a database search, heat-shock protein (HSP) 70 and heterogeneous nuclear ribonucleoprotein K (hnRNP K) were determined to be upregulated in the exposed cells.

Since the above *in vitro* effects cannot be easily translated into humans, in 2008, Leszczynski's group performed a pilot study on volunteers (Karinen et al., 2008) and showed that mobile phone radiation might alter protein expression in human skin cells. Small area of forearm's skin in 10 female volunteers was exposed to RF-EMF (specific absorption rate SAR = 1.3 W/kg) and punch biopsies were collected from exposed and non-exposed areas of skin. Proteins extracted from biopsies were separated using 2-DE and protein expression changes were analyzed using PDQuest software. Analysis has identified 8 proteins that were statistically significantly affected (Anova and Wilcoxon tests). Two of the proteins were present in all 10 volunteers. This suggests that protein expression in human skin might be affected by the exposure to RF-EMF. The number of affected proteins was similar to the number of affected proteins observed in this group's earlier *in vitro* studies. This is the first study showing that molecular level changes might take place in human volunteers in response to exposure to RF-EMF, although the overall conclusions were criticized by Leszczynski et al. (2012).

However, such a limited and non systematic number of publications using “omics” approaches does not allow for any conclusions to be drawn concerning the impact of mobile phone emitted radiation upon cell proteome, physiology and function (Nylund et al., 2009), as also pointed out by Vanderstraeten & Verschaeve (2008).

Kim et al. (2010) have monitored changes in protein expression profiles in RF-exposed MCF7 human breast cancer cells using two-dimensional gel electrophoresis. MCF7 cells were exposed to 849 MHz RF radiation for 1 h per day for three consecutive days at specific absorption rates (SARs) of either 2 W/Kg or 10 W/kg. During exposure, the temperature in the exposure chamber was kept in an isothermal condition. Twenty-four hours after the final RF exposure, the protein lysates from MCF cells were prepared and two-dimensional electrophoretic analyses were

conducted. The protein expression profiles of the MCF cells were not significantly altered as the result of RF exposure. None of the protein spots on the two-dimensional electrophoretic gels showed reproducible changes in three independent experiments. To determine effect of RF radiation on protein expression profiles more clearly, three spots showing altered expression without reproducibility were identified using electrospray ionization tandem mass spectrometry analysis and their expressions were examined with RT-PCR and Western blot assays. There was no alteration in their mRNA and protein levels. The authors concluded that it seems unlikely that RF exposure modulates the protein expression profile.

Since oxidative stress is gaining more and more ground as being the initial mechanism of action of EMFs, the review by Gaestel M. (2010) describes the (up to 2010) developments in analysing the influence of RF-EMFs on biological systems by monitoring the cellular stress response as well as overall gene expression. Recent data on the initiation and modulation of the classical cellular stress response by RF-EMFs, comprising expression of heat shock proteins and stimulation of stress-activated protein kinases, are summarised and evaluated. Since isothermic RF-EMF exposure is assumed rather than proven there are clear limitations in using the stress response to describe non-thermal effects of RF-EMFs. In particular, according to the authors further experiments are needed to characterise better the threshold of the thermal heat shock response and the homogeneity of the cellular response in the whole sample for each biological system used. Before then, it is proposed that the absence of the classical stress response can define isothermal experimental conditions and qualifies other biological effects of RF-EMFs detected under these conditions to be of non-thermal origin. To minimise the probability that by making this assumption valuable insights into the nature of biological effects of RF-EMFs could be lost, proteotoxic non-thermal RF-EMF effects should also be monitored by measuring activities of labile intracellular enzymes and/or levels of their metabolites before the threshold for the heat shock response is reached. In addition, non-thermal induction of the stress response via promoter elements distinct from the heat shock element (HSE) should be analysed using HSE-mutated heat shock promoter reporter constructs. Screening for non-thermal RF-EMF effects in the absence of a classical stress response should be performed by transcriptomics and proteomics. It is postulated that due to their high-throughput characteristics, these methods inherently generate false positive results and

require statistical evaluation based on quantitative expression analysis from a sufficient number of independent experiments with identical parameters. In future approaches, positive results must be confirmed by independent quantitative methods and should also be evaluated *in vivo* to prove possible non-thermal effects of RF-EMFs on living beings. If successful, this strategy should contribute to identification of new underlying molecular mechanisms of interaction between RF-EMFs and living beings distinct from absorption of thermal energy.

In the review by Leszczynski et al., (2012) the authors have analyzed all available data up through the end of 2010 and have raised a number of concerns regarding the handling of proteomics technology, such as the different proteome analysis methods used, the low number of replicates, the posttreatment sampling (one or very few time points), the large number of protein analyzed, the huge differences in the dynamic range of protein concentrations in cells or plasma, the variety of posttranslational modifications, the lack of validation of the results with a second method, as well as the various SAR/exposure conditions/duration/frequency dependencies in order to properly evaluate the EMF impact. The authors agree along with Gerner et al. (2010) that protein expression per se may be a reliable way to explain EMF effects. We might add that in terms of protein synthesis dynamics, the quantity of any protein species at a given time point (as detected by proteomics) should take into account the protein stability and turnover (as pointed out by Eden et al., 2011) as well as mRNA stability and maturation/translational-posttranslational control. In a hypothetical scenario that EMFs affect gene activation /deactivation (see Blank & Goodman, 2008), the end effect may not be seen by proteomics, since no net quantity change is taking place immediately but (possibly) a few hours following exposure and (also hypothetically) normal levels come back a few days or weeks later due homeostatic mechanisms.

Our own contribution to the field of RF-EMF induced protein expression changes was performed in mice exposed to mobile phone and wireless DECT base radiation under real-time exposure conditions and analyzing thereafter the proteome of three critical brain regions; hippocampus, cerebellum and frontal lobe (Fragopoulou et al. 2012). Three equally divided groups of Balb/c mice (6 animals/group) were used; the first group was exposed to a typical mobile phone, at a SAR level range of 0.17-0.37 W/kg for 3 h daily for 8 months, the second group was exposed to a wireless DECT

base (Digital Enhanced Cordless Telecommunications Telephone) at a SAR level range of 0.012-0.028 W/kg for 8 h/day for 8 months and the third group comprised the sham-exposed animals. Comparative proteomics analysis revealed that long-term irradiation from both EMF sources significantly altered (p< 0.05) the expression of 143 proteins in total (as low as 0.003 fold downregulation up to 114 fold overexpression). Several neural function related proteins (i.e., Glial Fibrillary Acidic Protein (GFAP), Alpha-synuclein, Glia Maturation Factor beta (GMF), and apolipoprotein E (apoE)), heat shock proteins, and cytoskeletal proteins (i.e., Neurofilaments and tropomodulin) are included in this list as well as proteins of the brain metabolism (i.e., Aspartate aminotransferase, Glutamate dehydrogenase) to nearly all brain regions studied. Western blot analysis on selected proteins confirmed the proteomics data. The observed protein expression changes may be related to brain plasticity alterations, indicative of oxidative stress in the nervous system or involved in apoptosis and might potentially explain human health hazards reported so far, such as headaches, sleep disturbance, fatigue, memory deficits, and long-term induction of brain tumors under similar exposure conditions.

As mentioned earlier, beyond the mobile phone frequencies, 35 GHz radiation had effects on gene expression. Similarly, Sypniewska et al. (2010) using proteomics reported that this frequency can also alter the proteome of NR8383 rat macrophages. Two-dimensional polyacrylamide gel electrophoresis, image analysis, and Western blotting were used to analyze approximately 600 protein spots in the cell lysates for changes in protein abundance and levels of 3-nitrotyrosine, a marker of macrophage stimulation. Proteins of interest were identified using peptide mass fingerprinting. Compared to plasma from sham-exposed rats, plasma from environmental heat- or millimeter wave-exposed rats increased the expression of 11 proteins, and levels of 3-nitrotyrosine in seven proteins, in the NR8383 cells. These altered proteins are associated with inflammation, oxidative stress, and energy metabolism. Findings of this study indicate both environmental heat and 35 GHz millimeter wave exposure elicit the release of macrophage-activating mediators into the plasma of rats.

Interestingly, there is a wealth of information regarding proteome and/or transcriptomics studies following exposure to ionizing radiation. In the perspective of similar mechanisms of action between NIR and IR, it is worth mentioning just one study using very low dose ionizing radiation by Pluder et al., 2011. In this study low-

dose radiation induced rapid and time-dependent changes in the cytoplasmic proteome of the human endothelial cell line EA.hy926 (used by Dariusz Leszczynski and his group in their EMF studies). The proteomes were investigated at 4 and 24 h after irradiation at two different dose rates (Co-60 gamma ray total dose 200 mGy; 20 mGy/min and 190 mGy/min) using 2D-DIGE technology. The researchers identified 15 significantly differentially expressed proteins, of which 10 were upregulated and 5 down-regulated, with more than ± 1.5 -fold difference compared with unexposed cells. Pathways influenced by the low-dose exposures included the Ran and RhoA pathways, fatty acid metabolism and stress response which are reminiscent of EMF impact studies.

Concerning proteomics techniques, a recent review by Damm et al., (2012) re-evaluates the putative advantages of microwave-assisted tryptic digests compared to conventionally heated protocols performed at the same temperature. An initial investigation of enzyme stability in a temperature range of 37-80°C demonstrated that trypsin activity declines sharply at temperatures above 60°C, regardless if microwave dielectric heating or conventional heating is employed. Tryptic digests of three proteins of different size (bovine serum albumin, cytochrome c and β -casein) were thus performed at 37°C and 50°C using both microwave and conventional heating applying accurate internal fiber-optic probe reaction temperature measurements. The impact of the heating method on protein degradation and peptide fragment generation was analyzed by SDS-PAGE and MALDI-TOF-MS. Time-dependent tryptic digestion of the three proteins and subsequent analysis of the corresponding cleavage products by MALDI-TOF provided virtually identical results for both microwave and conventional heating. In addition, the impact of electromagnetic field strength on the tertiary structure of trypsin and BSA was evaluated by molecular mechanics calculations. These simulations revealed that the applied field in a typical laboratory microwave reactor is 3-4 orders of magnitude too low to induce conformational changes in proteins or enzymes.

IV. SUMMARY

The papers analyzed in this review have dealt with a very difficult research problem, which is EMF effects as measured by the highthroughput techniques of transcriptomics and proteomics. It is a very difficult task because the technical

complexity of the approaches is added to the enormous variations of the exposure details (duration, frequency, pulses, repetition, intensity, peak values, e.t.c). In total there were 29 original articles from 2007. Eight (8) of them were in the ELF frequencies, where the three of them indicate an effect in gene expression, the other three indicate no effect in gene expression and two studies show an effect in protein expression. Regarding radiofrequency studies (RF-EMF) a total of 21 papers were published in this area since 2007. Thirteen (13) dealt with transcriptomics [eight (8) effect- five (5) no effect] and eight (8) in proteomics [six (6) show effect and two (2) show no effect]. So, in total, 66% of the studies reveal an effect of EMF on transcriptome and proteome expression (Table 1).

Table 1
EMF Transcriptomics and Proteomics studies 2007-2012

(E=effect, NE= no effect)

The classification of the studies to the category “Effect – No effect” is based on the general conclusions of each article, although different conditions are used in exposure setup, biological system, duration, approaches. It is also considered as an effect even if a single gene or protein is affected by exposure to EMF.

	Exposed biological model	Exposure set-up	SAR or/and power density or intensity of magnetic field	Duration of exposure / Time of sampling	Method of analysis	Category “Effect-No effect”	Comments	Reference/ Journal
ELF –EMF Transcriptomics	Primary human mesenchymal stem cells from the bone marrow and chondrocytes (cell line C28I2)	BTEMF (combination of electromagnetic field and light therapy) Coil system	35 μ T	Stimulated 5 times at 12-h intervals for 8 min each	Affymetrix GeneChip System, HG-U133A /RT-PCR partially confirmed the data	E	A limited number of regulated gene products from both cell types, which control cell metabolism and cell matrix structure,	Walther et al. (2007) <i>EBM</i>

							was mainly affected. There was no increased expression though of cancer-related genes	
	Adult human dermal fibroblasts <i>(scope: wound healing)</i>	Direct current field	100 mV/mm EF	1 h	Microarrays /RT-PCR validated 4 genes	E	Significantly increased expression of 162 transcripts and decreased expression of 302 transcripts was detected (126 transcripts above the level of 1.4-fold, 11 above the level of 2-	Jennings et al. (2008) <i>Bioelectromagnetics</i>

						fold)	
<i>Caenorhabditis elegans</i>	Static magnetic field (SMF) Magnetic resonance imaging	3 and 5 T	4 and 24 h	Affymetrix whole-genome array /qRT-PCR confirmed changes	E	Genes involved in motor activity, actin binding, cell adhesion, and cuticles, hsp12, hsp16 were transiently and specifically induced following exposure. Several genes encoding apoptotic cell-death activators and secreted surface proteins were	Kimura et al. (2008) <i>Bioelectromagnetics</i>

						upregulated after IR, but were not induced by SMFs.	
Embryonic human lung fibroblasts (Hel 299)	MR scanner	3.0 Tesla	2 h	cDNA microarray containing 498 known genes	NE		Schwenzer et al. (2007) <i>Journal of Magnetic Resonance imaging</i>
AKR mice	60 Hz Circularly polarized MFs	0 microT (sham control, T1, Group I), 5 microT (T2, Group II), 83.3 microT (T3, Group III), or 500 microT (T4, Group IV)	21 h/day from the age of 4-6 weeks to the age of 44-46 weeks	Affymetrix GeneChip Mouse Gene 1.0 ST assay	NE		Chung et al. (2010) <i>Bioelectromagnetics</i>
White blood cells of volunteers	50 Hz Sinusoidal ELF-MF	62.0 ± 7.1 µT	2 h, repeated on the followi	Illumina microarrays	NE		Kirschenlohr et al. (2012) <i>Radiat Res</i>

Proteomics				ng day and the two-day sequence was repeated 6 days later, 5 time points			
	Human fibroblasts	3 Hz continuous ELF, sinusoidal	4 mT	3 h	2-DE	E	Alpha 1 antitrypsin (A1AT) and Transthyretin (TTR) reduced their expression Seyyedi et al. (2007) <i>Pak J Biol Sci</i>
	Human SH-SY5Y neuroblastoma cells	50 Hz Sinusoidal ELF-MF	1 mT	5, 10, 15 days	2-DE /Western blot and immunohistochemical confirmation	E	Nine new proteins involved in cellular defence mechanism and/or in cellular organization Sulpizio et al. (2011) <i>J Cell Biochem</i>

							and proliferation	
RF-EMF Transcriptomics	Primary cultured neurons and astrocytes	GSM 1900 MHz Real-life exposure conditions	Not calculated	2 h	Microarray analysis /RT-PCR	E	Up-regulation of caspase-2, caspase-6 and Asc gene expression in neurons and astrocytes (and Bax upregulation in astrocytes)	Zhao et al. (2007) <i>Neurosci Lett</i>
	Rat cortex and hippocampus	GSM mobile test phone at 1800 MHz	Whole-body SAR- 13 mW/kg brain SAR- 30 mW/kg	6 h	Microarray hybridizations on Affymetrix rat2302 chips	E	Altered gene categories in both cortex and hippocampus : extracellular region, signal transducer activity, intrinsic to	Nittby et al. (2008) <i>Environmentalist</i>

						membrane, and integral to membrane	
Jurkat human T lymphoma cells	1763 MHz CDMA exposure chamber	10 W/kg	24 h	Applied Biosystems microarrays	E	Ten genes changed from 1.3 to approximately 1.8-fold	Huang et al. (2008a) <i>Int J Radiat Biol</i>
HEI-OC1 immortalized mouse auditory hair cells	1763 MHz CDMA exposure chamber	20 W/kg	24 h, 48 h	Applied Biosystems 1700 full genome expression mouse microarray	E	29 genes (0.09% of total genes examined) were changed by more than 1.5-fold on RF exposure	Huang et al. (2008b) <i>Int J Radiat Biol</i>
<i>Arabidopsis thaliana</i>	RF field protocol representing typical microwave exposition in an urban environment	2 and 0.75 W/kg	24 h	RNA-extraction, microarray hybridization, and quantitative RT-PCR	E	Significant changes (but not more than 2.5-fold) in transcription of 10 genes	Engelmann et al. (2008) <i>Computational Biology and Chemistry</i>

	nt						
Rats (skin)	35 GHz mm-waves	75 mW/cm ²	6 h, 24 h	Affymetrix Gene Chip analysis	E	Expression changes in 56 genes at 6 h exposure and 58 genes at 24 h exposure	Millenbaugh et al. (2008) <i>Radiat Res</i>
Cultured acute T- lymphoblast oid leukemia cells (CCRF- CEM)	900 MHz CW TEM cells	3.5 mW/Kg 3 V/m 1 mW in the cell culture dishes	2 h and 48 h	cDNA- microarray analysis /Western blot confirmatio n	E	DNA repair genes activated from 2 hrs, apoptotic genes overexpresse d, cell cycle arrest genes activated. Surprisingly effects with	Trivino et al. (2012) <i>EBM</i>

						very low dose	
Human cell lines, A172 (glioblastoma), H4 (neuroglioma), and IMR-90 (fibroblasts from normal fetal lung)	W-CDMA CW 2.1425 GHz	80, 250, or 800 mW/kg	For up to 96 h	Affymetrix Human Genome Array	E	A very small (< 1%) number of available genes (ca. 16,000 to 19,000) exhibited altered expression	Sekijima et al. (2010) <i>J. Radiat. Res</i>
Human-derived cell lines (TK6, HL60 and Mono-Mac-6)	1.9 GHz pulse-modulated RF fields	0, 1 and 10 W/kg	Intermittent (5 min ON, 10 min OFF) for 6 h	Cell cycle, apoptosis, viability, cytokines tested at 0 and 18h after exposure	NE		Chauhan et al. (2007a) <i>Rad. Research</i>

U87MG cells			24 h				
Mono-Mac-6, MM6	1.9 GHz pulse-modulated RF fields	0.1-10.0 W/kg	intermittent (5 min ON, 10 min OFF) for 6 h	Microarrays analysis 18 h after exposure	NE		Chauhan et al. (2007b) <i>Proteomics</i>
Human glioblastoma A172 cells	W-CDMA CW 2.1425 GHz	80 and 800 mW/kg 80 mW/kg	2-48 h 24 h	DNA Chip analysis	NE		Hirose et al. (2007) <i>Bioelectromagnetics</i>
Human IMR-90 fibroblasts		80 and 800 mW/kg 80 mW/kg	2h, 28h 28h				
Mouse brain	GSM 1800 MHz	Whole body SAR of 1.1 W/kg	1 h	Microarrays containing over	NE	75 genes were found to be modulated	Paparini et al. (2008) <i>Bioelectromagnetics</i>

			brain SAR 0.2 W/kg		22,600 probe sets RT-PCR		but since they were not confirmed ⇒ no effect	
Proteomics	<i>C. elegans</i>	1.0 GHz, 0.5W power input	0.9–3 mW/kg	1.5h 2.5h	Five Affymetrix gene arrays of pooled triplicate RNA populations from L4/adult worms from each group (sham and exposed)	NE	Minor changes in gene expression, probably false positives. Strange intensity window effect, no effect in high dose.	Dawe et al. (2009) <i>Bioelectrom agnetics</i>
	Human endothelial cell line EA.hy926	GSM 1800 MHz	2.0 W/kg	1h	2-DE /Western blot confirmed selected proteins	E		Nylund et al. (2009) <i>Journal of Proteomics and Bioinformati cs</i>

Human lens epithelial cells	GSM-like 1800-MHz	1.0, 2.0, or 3.5 W/kg.	2 h	2-DE	E	hnRNP K and HSP70 upregulated	Li et al. (2007) <i>Jpn. J. Ophthalmol</i>
Human skin cells.	Mobile phone GSM 900MHz	1.3 W/kg	1 h	2D in skin punch biopsies	E	8 proteins were affected	Karinen et al. (2008) <i>BMC Genomics</i>
Plasma from exposed rats causes changes in protein expression and levels of 3-NT in a rat alveolar macrophage cell line.NR8383 macrophages	Generator 35 GHz	Peak incident power density of 75 mW/cm ²	46 min	<i>in vitro</i> bioassay and proteomic screening	E	Increased the expression of 11 proteins, and levels of 3-nitrotyrosine in seven proteins, in the NR8383 cells. These altered proteins are associated with inflammation, oxidative stress, and energy metabolism	Sypniewska et al. (2010) <i>Bioelectromagnetics</i>
Human Jurkat T-	Modulated GSM 1800	2 W/kg	Intermittent	Autoradiography of 2-	E	Rate of protein	Gerner et al. (2010)

<p>cells Primary human diploid fibroblasts Peripheral blood mononuclear cells</p>	<p>MHz</p>		<p>exposure 8h (5min ON 10 min OFF)</p>	<p>DE gel</p>		<p>synthesis in proliferating cells is increased by long-term (8 h) RF-EME, while no effect was detectable in quiescent white blood cells treated in the same manner.</p>	<p><i>Int. Arch. Occup. Environ. Health</i></p>
<p>Balb/c mice (hippocampus, frontal lobe, cerebellum)</p>	<p>GSM 900 MHz Mobile phone, 1880 MHz Wireless DECT base</p>	<p>0.17-0.37 W/kg 0.012-0.028 W/kg</p>	<p>3 h/day x 8 months 8 h/day x 8 months</p>	<p>2-De /Western blot confirmed selected proteins</p>	<p>E</p>	<p>Real-life exposure conditions</p>	<p>Fragopoulou et al. (2012) <i>EBM</i></p>
<p>Human primary umbilical vein endothelial cells and</p>	<p>1800 MHz GSM</p>	<p>2.0 W/kg</p>	<p>1 h</p>	<p>2-DE</p>	<p>NE</p>		<p>Nylund et al. (2010) <i>Proteome Sci</i></p>

primary human brain Microvascular endothelial cells							
Human breast cancer MCF-7 cells	849 MHz CDMA	2 and 10 W/kg	1 h/day x 3 days	2D, 24 h after exposure, Rt-PCR, Western blot	NE		Kim et al. (2010) <i>J Radiat Res</i>

V. CONCLUSIONS

It is clear that the effects of EMFs are very difficult to predict in the cells, and that SAR values do not provide any information about the molecular mechanisms likely to take place during exposure. Unlike drugs, EMFs are absorbed in a variety of different, diverse and non-linear ways depending on the “microenvironment” receiving the radiation, the orientation of the molecular targets and their shape, the metabolic state at the moment of exposure, the energy absorbance at the microscale of the cell and the modulation of the waves. On this basis, it is rather difficult to replicate experiments under different conditions and cell systems, which may explain the discrepancy of the results among research groups.

As far as changes in gene expression are concerned, they are observed within specific time duration with and without recovery time. As mentioned in some studies i.e., the same endothelial cell line responded to 1800 MHz intermittent exposure, but not to continuous exposure. Exposure time, exposure pattern and type of biological system (organism, tissue, cell) and experimental techniques may also play a key role in the end effect (Mevisen M., 2011).

In addition, we point out that all “averaging approaches” like proteomics and transcriptomics provide a mean value of changes in a specific protein/gene from all cell types of the tissue examined. The same is true for western blotting, RT-PCR and the entire battery of biochemical/molecular biological techniques. Of course, newly developed high sensitivity proteomics and transcriptomics might be able to analyse small quantities from individual cell types, since cell protein/gene expression changes would be the approach of choice in future experiments utilizing sophisticated state of the art microscopical techniques. Under these conditions, we will be able to understand why one cell type responds to EMF whereas another cell type is not responding, thus leading to a net “no effect” in case the second cell type is outnumbered.

Therefore the issue of examining by proteomics various time points during (or after) exposure is of utmost importance in order to unravel the mechanism(s) of EMF action. Approaches including 2D-autoradiography might be in addition very useful in this direction since the actual protein synthetic profile will be revealed (Gerner et al., 2010). As stated by these authors their findings of an association between metabolic activity and the observed cellular reaction to low intensity RF-EMF may reconcile conflicting results of previous studies. They further postulated that the observed

increased protein synthesis reflects an increased rate of protein turnover stemming from protein folding problems caused by the interference of radiofrequency electromagnetic fields with hydrogen bonds. These observations of course do not directly imply a health risk.

Needless to mention that a combination of all available high throughput techniques in the same system under identical exposure conditions will provide better data, especially if different laboratories replicate the results.

Taking into account that many studies using normal exposure conditions have revealed protein and gene expression changes, health hazards are possible.

It is clear that the existing guidelines are inadequate as pointed out by other studies as well (Fragopoulou et al., 2010). The transcriptomics and proteomics data reviewed here report that 66% of the papers published after 2007 show an effect. This is a clear indication of expression changes of proteins and genes at intensity levels commonly used by the wireless devices. Prudent avoidance of excessive usage of these devices is thus recommended.

Concerning the question of which model system is more suitable for such experiments in order to translate the effects into human EMF hazards, we might agree with Leszczynski's point that human volunteer skin is more suitable, but the major target of interest regarding EMF impacts is the brain which consists of an enormous complexity of nerve cell interactions far away from constituents of skin. Therefore, we argue that the system of choice for omics approaches should be rats or mice (preferably the second due to the possibility of handling transgenic material) as evolutionary very close to humans without neglecting the important work that has been (or will be) done using other biological systems, especially cell cultures.

VI. REFERENCES

- Baan R, Grosse Y, Lauby-Secretan B, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, et al. (2011) Carcinogenicity of radiofrequency electromagnetic fields. WHO International Agency for Research on Cancer Monograph Working Group. *Lancet Oncol.*, 12(7):624-626.
- Blank M, Goodman R, (2008) A mechanism for stimulation of biosynthesis by electromagnetic fields: charge transfer in DNA and base pair separation. *J Cell Physiol*, 214(1):20-26 [Review].
- Blankenburg M, Haberland L, Elvers HD, Tannert C, Jandrig B, (2009) High-Throughput Omics Technologies: Potential Tools for the Investigation of Influences of EMF on Biological Systems. *Curr Genomics*, 10:86-92.
- Cardis E, Richardson L, Deltour I, Armstrong B, Feychting M, Johansen C, et al. (2007) The INTERPHONE study: design, epidemiological methods, and description of the study population. *Eur J Epidemiol.*, 22(9):647-664.
- Chauhan V, Mariampillai A, Kutzner BC, Wilkins RC, Ferrarotto C, Bellier PV, et al. (2007a) Evaluating the biological effects of intermittent 1.9 GHz pulse-modulated radiofrequency fields in a series of human-derived cell lines. *Radiat Res.*, 167(1):87-93.
- Chauhan V, Qutob SS, Lui S, Mariampillai A, Bellier PV, Yauk CL, et al. (2007b) Analysis of gene expression in two human-derived cell lines exposed in vitro to a 1.9 GHz pulse-modulated radiofrequency field. *Proteomics*, 7(21):3896-3905.
- Chung MK, Yu WJ, Kim YB, Myung SH, (2010) Lack of a co-promotion effect of 60 Hz circularly polarized magnetic fields on spontaneous development of lymphoma in AKR mice. *Bioelectromagnetics*, 31(2):130-139.
- Damm M, Nussold C, Cantillo D, Rechberger GN, Gruber K, Sattler W, Kappe CO. (2012) Can electromagnetic fields influence the structure and enzymatic digest of proteins? A critical evaluation of microwave-assisted proteomics protocols. *J Proteomics*, 75(18):5533-5543.
- Dawe AS, Bodhicharla RK, Graham NS, May ST, Reader T, Loader B, et al., (2009) Low-intensity microwave irradiation does not substantially alter gene expression in late larval and adult *Caenorhabditis elegans*. *Bioelectromagnetics*, 30:602–612.
- Eden E, Geva-Zatorsky N, Issaeva I, Cohen A, Dekel E, Danon T, et al. (2011) Proteome half-life dynamics in living human cells. *Science*, 331(6018):764-768.
- Engelmann JC, Deeken R, Mueller T, Nitz G, Roelfsema MRG, Hedrich R, (2008) Is gene activity in plant cells affected by UMTS-irradiation? A whole genome approach. *Computational Biology and Chemistry: Advances and Applications*, 1:71-83.
- Fragopoulou A, Grigoriev Y, Johansson O, Margaritis LH, Morgan L, Richter E, Sage C, (2010) Scientific panel on electromagnetic field health risks – consensus points, recommendations and rationales. *Reviews on Environmental Health*, 25(4):307-317.
- Fragopoulou AF, Samara A, Antonelou MH, Xanthopoulou A, Papadopoulou A, Vougas K, et al. (2012) Brain proteome response following whole body exposure of mice to mobile phone or wireless DECT base radiation. *Electromagnetic Biology & Medicine*, 31(4):250-274.

- Gaestel M, (2010) Biological monitoring of non-thermal effects of mobile phone radiation: recent approaches and challenges. *Biol Rev Camb Philos Soc.*, 85(3):489-500.
- Gerner C, Haudek V, Schnadl U, Bayer E, Gundacker N, Hutter HP, Mosgoeller W, (2010) Increased protein synthesis by cells exposed to a 1800 MHz radiofrequency mobile phone electromagnetic field detected by proteome profiling. *Int. Arch. Occup. Environ. Health*, 83:691–702.
- Hirose H, Sakuma N, Kaji N, Nakayama K, Inoue K, Sekijima M, et al. (2007) Mobile phone base station-emitted radiation does not induce phosphorylation of Hsp27. *Bioelectromagnetics*, 28(2):99-108.
- Huang TQ, Lee MS, Oh E, Zhang BT, Seo JS, Park WY, (2008a) Molecular responses of Jurkat T-cells to 1763 MHz radiofrequency radiation. *Int J Radiat Biol.*, 84(9):734-741.
- Huang TQ, Lee MS, Oh EH, Kalinec F, Zhang BT, Seo JS, Park W, (2008b) Characterization of biological effect of 1763 MHz radiofrequency exposure on auditory hair cells. *Int. J. Rad. Biol.*, 84:900–915.
- Jennings J, Chen D, Feldman D, (2008) Transcriptional Response of Dermal Fibroblasts in Direct Current Electric Fields. *Bioelectromagnetics*, 29:394-405.
- Karinen A, Heinavaara S, Nylund R, Leszczynski D, (2008) Mobile phone radiation might alter protein expression in human skin. *BMC Genomics*, 9:77–81.
- Kim KB, Byun HO, Han NK, Ko YG, Choi HD, Kim N, et al. (2010) Two-dimensional electrophoretic analysis of radio-frequency radiation-exposed MCF7 breast cancer cells. *J Radiat Res.*, 51(2):205-213.
- Kimura T, Takahashi K, Suzuki Y, Konishi Y, Ota Y, Mori C, et al. (2008) 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.
- Kirschenlohr H, Ellis P, Hesketh R, Metcalfe J, (2012) Gene expression profiles in white blood cells of volunteers exposed to a 50 Hz electromagnetic field. *Radiat Res.*, 178(3):138-149.
- Leszczynski D, de Pomerai D, Koczan D, Stoll D, Franke H, Albar JP, (2012) Five years later: the current status of the use of proteomics and transcriptomics in EMF research. *Proteomics*, 12(15-16):2493-2509.
- Li HW, Yao K, Jin HY, Sun LX, Lu DQ, Yu YB, (2007) Proteomic analysis of human lens epithelial cells exposed to microwaves. *Jpn. J. Ophthalmol.*, 51:412–416.
- McNamee JP, Chauhan V, (2009) Radiofrequency radiation and gene/protein expression: a review. *Radiat Res.*, 172(3):265-287.
- Mevissen M, (2011) Transcriptomics Approach in RF EMF Research, in *Cancer Risk Evaluation: Methods and Trends* (eds G. Obe, B. Jandrig, G. E. Marchant, H. Schütz and P. M. Wiedemann), Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany. doi: 10.1002/9783527634613.ch16.
- Millenbaugh NJ, Roth C, Sypniewska R, Chan V, Eggers JS, Kiel JL, et al., (2008) Gene expression changes in the skin of rats induced by prolonged 35 GHz millimeter-wave exposure. *Radiat Res.*, 169(3):288-300.

- Nittby H, Widegren B, Krogh M, Grafström G, Berlin H, Rehn G, et al. (2008) Exposure to radiation from global system for mobile communications at 1,800 MHz significantly changes gene expression in rat hippocampus and cortex, *Environmentalist*, 28:458-465.
- Nylund R, Tammio H, Kuster N, Leszczynski D, (2009) Proteomic analysis of the response of human endothelial cell line EA.hy926 to 1800 GSM mobile phone radiation. *J Proteom. Bioinform.*, 2:455–462.
- Nylund R, Kuster N, Leszczynski D, (2010) Analysis of proteome response to the mobile phone radiation in two types of human primary endothelial cells. *Proteome Sci.*, 8:52–58.
- Paparini A, Rossi P, Gianfranceschi G, Brugaletta V, Falsaperla R, De Luca P, Romano Spica V, (2008) No evidence of major transcriptional changes in the brain of mice exposed to 1800 MHz GSM signal. *Bioelectromagnetics*, 29:312–323.
- Pluder F, Barjaktarovic Z, Azimzadeh O, Mörtl S, Krämer A, Steininger S, et al. (2011). Low-dose irradiation causes rapid alterations to the proteome of the human endothelial cell line EA.hy926. *Radiat Environ Biophys.*, 50(1):155-166.
- Schwenzer NF, Bantleon R, Maurer B, Kehlbach R, Schraml C, Claussen CD, Rodegerdts E, (2007) Do Static or Time-Varying Magnetic Fields in Magnetic Resonance Imaging (3.0 T) Alter Protein–Gene Expression?—A Study on Human Embryonic Lung Fibroblasts. *Journal of Magnetic Resonance imaging*, 26:1210-1215.
- Sekijima M, Takeda H, Yasunaga K, Sakuma N, Hirose H, Nojima T, Miyakoshi J, (2010) 2-GHz Band CW and W-CDMA Modulated Radiofrequency Fields Have No Significant Effect on Cell Proliferation and Gene Expression Profile in Human Cells. *J. Radiat. Res.*, 51:277–284.
- Seyyedi SS, Dadras MS, Tavirani MR, Mozdarani H, Toossi P, Zali AR, (2007) Proteomic analysis in human fibroblasts by continuous exposure to extremely low-frequency electromagnetic fields. *Pak J Biol Sci.*, 10(22):4108-4112.
- Sulpizio M, Falone S, Amicarelli F, Marchisio M, Di Giuseppe F, Eleuterio E, et al. (2011) Molecular basis underlying the biological effects elicited by extremely low-frequency magnetic field (ELF-MF) on neuroblastoma cells. *J Cell Biochem*, 112(12):3797-3806.
- Sypniewska RK, Millenbaugh NJ, Kiel JL, Blystone RV, Ringham HN, Mason PA, Witzmann FA, (2010) Protein changes in macrophages induced by plasma from rats exposed to 35 GHz millimeter waves. *Bioelectromagnetics*, 31(8):656-663.
- Trivino Pardo JC, Grimaldi S, Taranta M, Naldi I, Cinti C, (2012) Microwave electromagnetic field regulates gene expression in T-lymphoblastoid leukemia CCRF-CEM cell line exposed to 900 MHz. *Electromagn Biol Med.*, 31(1):1-18.
- Walther M, Mayer F, Kafka W, Schütze N, (2007) Effects of Weak, Low-Frequency Pulsed Electromagnetic Fields (BEMER Type) on Gene Expression of Human Mesenchymal Stem Cells and Chondrocytes: An In Vitro Study. *Electromagnetic Biology and Medicine*, 26:179–190.
- World Health Organization. Extremely Low Frequency Electromagnetic fields (EMF) [online]. (2007). Available at http://www.who.int/peh-emf/research/elf_research_agenda_2007.pdf

- World Health Organization. Research Agenda for Radiofrequency Electromagnetic Fields (EMF) [online]. (2010). Available at http://whqlibdoc.who.int/publications/2010/9789241599948_eng.pdf
- Vlaanderen J, Moore LE, Smith MT, Lan Q, Zhang L, Skibola CF, et al. (2010) Application of OMICS technologies in occupational and environmental health research; current status and projections. *Occup Environ Med.*, 67(2):136-43.
- Vanderstraeten J, Verschaeve L, (2008) Gene and protein expression following exposure to radiofrequency fields from mobile phones. *Environ Health Perspect.*, 116(9):1131-1135. Review.
- Xu Z, Chen G. (2007) Evidence for effects on gene and protein expression. In *BioInitiative Report. A rationale for biologically-based public exposure standard for electromagnetic fields (ELF and RF)*. Carpenter DO, Sage C (eds). ISBN: 978-1-4276-3105-3, Vol. 1, Section 5, pp 91-104.
- Zhao TY, Zou SP, Knapp PE, (2007) Exposure to cell phone radiation up-regulates apoptosis genes in primary cultures of neurons and astrocytes. *Neurosci Lett.*, 412(1):34-38.