SECTION 5

EVIDENCE FOR EFFECTS ON GENE AND PROTEIN EXPRESSION

(Transcriptomic and Proteomic Research)

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I. INTRODUCTION

Daily exposure to electromagnetic fields (EMF), including extremely low frequency magnetic fields (ELF MF) and radiofrequency (RF) EMF, in the environment has raised public concerns about whether they have harmful consequences on human health. Several epidemiological studies suggest that exposure to EMF might associate with an elevated risk of cancer and other diseases in humans (reviewed in [Feychting et al., 2005]). To explain and/or support epidemiological observations, many laboratory studies have been conducted, but the results were controversial and no clear conclusion could be drawn to assess EMF health risk.

It is reasoned that one of the priorities in EMF research is to elucidate the biological effects of EMF exposure and the underlining mechanisms of action. Gene and protein are key players in organisms, and it has been assumed that any biological impact of EMF must be mediated by alterations in gene and protein expression [Phillips et al., 1992; Wei et al., 1990]. For example, heat shock protein, c-myc, and c-jun have been identified as EMF responsive genes and/or proteins in certain biological systems. In order to reveal the global effects of EMF on gene and protein expression, transcriptomics and proteomics, as high-throughput screening techniques (HTSTs), were eventually employed in EMF research with an intention to screen potential EMF-responsive genes and/or proteins without any bias. In 2005, WHO organized a Workshop on Application of Proteomics and Transcriptomics in EMF Research in Helsinki, Finland to discuss the related problems and solutions in this field [Leszczynski 2006; Leszczynski and Meltz 2006]. Later the journal Proteomics published a special issue devoted to the application of proteomics and transcriptomics to EMF research. This review aims to summarize the current research progress and discuss the applicability of HTSTs in the field.

II. ELF MF

II A. TRANSCRIPTOMICS

Binninger and Ungvichian firstly measured purified mRNA levels of total RNA from MF- and sham-exposed yeast cells and reported that the levels of a significant proportion of mRNAs were altered in response to continuous exposure to 20 T 60 Hz MF over a period of approximately 15 cell generations (24 h) [Binninger and Ungvichian 1997]. Unfortunately, no reproducible genes (polypetides) were identified in this study although the authors consistently found different proportions of transcripts whose abundances were altered in all four replication experiments.

Wu *et al*. have applied differential display reverse transcriptase–polymerase chain reaction (DD-RT-PCR) and Northern blotting to screen MF-responsive gene in Daudi cells. The cells were exposed to 0.8 mT of 50 Hz MF for 24 h. The authors screened out two candidate genes in Daudi cells and one was identified as a MF-responsive gene *ceramide glucosyltransferase*. They further found time-dependent changes in the transcription of *ceramide glucosyltransferase* induced by 0.8 mT MF [Wu et al., 2000]. With the help of DD-RT-PCR, Olivares-Banuelos *et al* reported that exposure to 0.7 mT 60 Hz MF for 7 days，4 h a day (2 h in the morning and 2 h in the afternoon), changed the global transcription profile of chromaffin cells. Eight RT-PCR products which correspond to six genes were identified, including *phosphoglucomutase*-1, *neurofibromatosis-2 interacting protein*, *microtubule associated protein*-2, *thiamine pyrophosphokinase*, and two hypothetical proteins (RNOR02022103 and ROR01044577). In addition, the authors found that presumed regulatory regions of these genes contained CTCT-clusters [Olivares-Banuelos et al., 2004], which has been identified as an electromagnetic field-responsive DNA element regulating gene expression [Goodman and Blank 2002].

Balcer-Kubiczek *et al*. have applied the two-gel cDNA library screening method (BIGEL) to screen MF-responsive genes, in which the gel arrays contained a total of

960 cDNAs selected at random from the cDNA library. The HL 60 cells were exposed to 2 mT of 60 Hz square wave MF for 24 h. Four candidate genes were shown responsive to the MF exposure, but could not be confirmed by following Northern analysis. Furthermore, the authors found that these four candidates and another four selected genes (*MYC*, *HSP70*, *RAN* and *SOD1*) did not react to either square wave or sine wave 60 Hz MF at 2 mT for 24 h [Balcer-Kubiczek et al., 2000]. However, the cellular responses to square wave and sine wave 60 Hz MF might be different. In order to systematically evaluate the effect of 60 Hz MF on gene expression in HL 60 cells, it is necessary for the authors to screen 60 Hz sine wave MF responsive candidate genes in HL 60 cells with BIGEL method as well, and then, perform validation with Northern blotting for these candidates.

Using cDNA arrays containing 588 cancer-related genes, Loberg *et al*. analyzed gene expression in normal (HME) and transformed (HBL-100) human mammary epithelial cells and human promyelocytic leukemia (HL60) cells after exposure to 60 Hz MF at intensity of 0.01 or 1.0 mT for 24 h. The authors reported that several genes were identified in MF-exposed cells whose expressions were increased by at least two folds or decreased by 50% or more, but no gene was found to be differentially expressed in each of three independent exposures for any cell type, and no relationship between exposure intensity and differential gene expression was found [Loberg et al., 2000].

In order to obtain a more global evaluation, genome-wide microarray screening methods were applied to identify genes responding to ELF MF in certain types of cells. By application of cDNA microarray, Nakasono *et al*. have investigated the effect of 50 Hz MF below 300 mT on gene expression in yeast. The authors reported that several genes were found differentially expressed in yeast cells with medium to low confidence level (CL) after exposure to 10, 150 and 300 mT for 24 h. Among these genes, seven showed a dose-response relationship in the normalized ratio data and three genes showed a reproducible change for all three intensities. They also proposed that these genes should be re-examined by methods with greater sensitivity or by quantitative methods, such as real-time PCR. On the other hand, no high-confidence expression

changes were observed for genes that are involved in heat-shock response, DNA repair, respiration, protein synthesis, or cell cycle. Thus, they concluded that 50 Hz MF up to 300 mT did not appear to affect gene expression linked to either defined cell processes stated above or unknown cell responses in investigated model eukaryotic cells [Nakasono et al., 2003]. Unfortunately, only single experiment for array analysis was performed in this study.

Recently, a similar study was conducted by Luceri *et al*. to investigate the global gene response to 50 Hz MF in human lymphocytes and yeast cells. These two types of cells were exposed to MF at intensity of 100 T, 10 T and 1 T for 18 h. As a result, in lymphocytes, one gene was found down-regulated at 100 T, one down-regulated gene and two up-regulated genes were screened out at 10 T, and no gene was detected changed at 1 T. As to the yeast cells, the results showed 2, 15 and 2 genes as differentially expressed (mainly down-regulated) after exposure to 100, 10 and 1 T, respectively, in which SPS100 gene was consistently up-regulated after exposure to 50 Hz MF at all three intensities. But no genes were found differentially expressed when the authors analyzed the data by other statistical methods. Thus, the authors concluded that 50 Hz MF did not affect gene expression in these two types of cells and the variations of a few genes mentioned above could be due to experimental noise [Luceri et al., 2005]. However, it is necessary to examine the candidates, especially the SPS100 gene, to validate whether they were real “un-responsive” genes.

In Henderson’s report, human umbilical vein endothelial cells (HUVEC) were exposed to various patterns and intensities of 50 Hz MF, including continuous exposure at a two intensities (10 and 700 T), intermittent exposure (60 min on/ 30 min off) at a single intensity (700 T), and continuous exposure to a variable-intensity fields (10-30 T). The transcriptional response of the cells was investigated using oligonucleotide microarrays containing up to 30, 000 unique features. Although different genes were identified where their expressions appeared to be affected by exposure to MF in individual experiments, none of these genes were regulated in the same manner in

subsequent repetition experiments [Henderson et al., 2006].

Antonini *et al* reported that intermittent exposure (5 min on/5 min off) to 50 Hz MF at flux densities of 2 mT for 16 h could change gene expression in human neuroblastoma cell line SH-SY5Y by application of whole-genome Human Unigene RZPD-2 cDNA array which contains about 75, 000 cDNA clones. Several genes were found down- or up-regulated at least five-fold after ELF MF exposure and the authors concluded that SH-SY5Y cells were sensitive to ELF MF [Antonini et al., 2006]. However, no reports indicated that these differentially expressed genes were confirmed by other methods.

Lupke *et al* investigated the effect of ELF MF on gene expression profiling in human umbilical cord blood-derived monocytes using the same Unigene RZPD-2. The results indicated that 0.1 mT 50 Hz MF exposure for 45 minutes altered the expressions of 986 genes involved in metabolism, cellular physiological processes, signal transduction, and immune response, among them, five genes were significantly regulated. Furthermore, the authors analyzed several genes by real-time RT-PCR and one ELF MF candidate responsive gene IL15RA was confirmed. However, this study only did single array analysis for pooling sample from 78 donors and two independent real-time RT-PCR analyses for samples from 5 and 6 different donors. The authors did not report the examinations of other candidates with real-time RT-PCR analysis [Lupke et al., 2006].

II B. PROTEOMICS

Nakasono *et al*. has investigated the effects of protein expression in model system such as *Escherichia coli* and *Saccharomyces cerevisiae* using two dimensional gels electrophoresis (2-DE) method. When the bacterial cells were exposed to each MF at 5-100 Hz under aerobic conditions (6.5 h) or at 50 Hz under anaerobic conditions (16 h) at the maximum intensity (7.8 to 14 mT), no reproducible changes were observed in the 2D gels. However, the stress-sensitive proteins did respond to most stress factors, including temperature change, chemical compounds, heavy metals, and nutrients. The authors concluded that the high-intensity ELF MF (14 mT at power frequency) did not act as a general stress factor [Nakasono and Saiki 2000]. When using *Saccharomyces cerevisiae* as a model system, Nakasono *et al*. reported that no reproducible changes in the 2D gels were observed in yeast cells after exposure to 50 Hz MF at the intensity up to 300 mT for 24 h [Nakasono et al., 2003]. In this study, only three sets of gels from three independent experiments were analyzed.

Li *et al*. have performed a proteomics approach to investigate the changes of protein expression profile induced by ELF MF in human breast cancer cell line MCF-7. With help of 2-DE and data analysis on nine gels for each group, 44 differentially expressed protein spots were screened in MCF-7 cells after exposure to 0.4 mT 50 Hz MF for 24 h. Three proteins were identified by LC-IT Tandem MS as RNA binding protein regulatory subunit, proteasome subunit beta type 7 precursor, and translationally controlled tumor protein, respectively [Li et al 2005]. Further investigations, such as Western blotting, are required to confirm these ELF responsive candidate proteins.

Using 2-D Fluorescence Difference Gel Electrophoresis (2-D DIGE) technology and MS in a blind study, Sinclair *et al* have investigated the effects of ELF MF on the proteomes of wild type *Schizosaccharomyces pombe* and a Sty1p deletion mutant which displays increased sensitivity to a variety of cellular stresses. The yeast cells were exposed to 50 Hz EMF at field strength of 1 mT for 60 min. While this study

identified a number of protein isoforms that displayed significant differential expressions across experimental conditions, there was no correlation between their patterns of expression and the ELF MF exposure regimen. The authors concluded that there were no significant effects of ELF MF on the yeast proteome at the sensitivity afforded by 2D-DIGE. They hypothesized that the proteins identified in the experiments must be sensitive to subtle changes in culture and/or handling conditions. Based on their experience, they suggested to the community that the interpretation of proteomic data in a biological context should be treated with caution [Sinclair et al., 2006].

II C. SUMMARY

Generally, recent studies on global gene and protein expression responding to ELF MF have been conducted in different biological systems by applications of HTSTs. Only a few studies reported to identify ELF MF responsive genes successfully. For example Wu *et al*. identified *ceramide glucosyltransferase* as a MF-responsive gene in Daudi cells [Wu et al., 2000] and Olivares-Banuelos *et al.* identified six ELF MF genes in chromaffin cells [Olivares-Banuelos et al., 2004] with the help of DD-RT-PCR and Northern blotting analysis; by combining cDNA array analysis with real-time RT-PCR confimation, Lupke *et al*. identified IL15RA as ELF MF responsive genes in human monocytes [Lupke et al., 2006]. Although many transcriptome and proteome analysis showed that ELF MF exposure could change gene and/or protein expression in certain cell types [Antonini et al., 2006; Binninger and Ungvichian 1997; Li et al., 2005], there are lack of confirmation to determine if they are real ELF MF responsive genes or proteins. Therefore, it is a priority to conduct confirmation experiments to demonstrate the author’s findings.

As to those negative reports, few or no genes and proteins were found significantly changed according to their statistical analysis and screening standards. But these few genes and proteins were neither reproducible [Henderson et al., 2006; Nakasono et al.,

2003; Sinclair et al., 2006]nor confirmed by other methods [Balcer-Kubiczek et al., 2000], and the changes were not related to ELF MF exposure [Loberg et al., 2000; Luceri et al., 2005; Nakasono et al., 2003]. Therefore, these studies are also needed to be replicated or verified.

III. RF EMF

III A . TRANSCRIPTOMICS

In an initial study utilizing membrane-based cDNA microarray, Harvey and French studied the effects of 864.3 MHz (CW) on HMC-1 human monocytes. The exposure was carefully controlled and averaged at an SAR of 7 W/kg, almost double the exposure level of established adverse effects. Three 20 min exposures were performed at 4-h intervals daily for 7 days. cDNA microarray analyses revealed consistent alterations in steady-state mRNA levels of 3 of the 558 genes represented on the membranes including one proto-oncogene *c-kit* (increased), one apoptosis-associated gene *DAD-1* (decreased) and one potential tumor suppressor gene *NDPK* (decreased) [Harvey and French 1999]. However, there were considerable variabilities between the two experiments reported and the fold change of each differentially expressed gene was small (< 1.5 folds). Meanwhile, the authors did not use other methods to confirm the results.

Pacini *et al*. investigated the effect of gene expression in human skin fibroblasts by using cDNA arrays including 82 genes, and reported that exposure to GSM 902.4 MHz RF EMF at an average SAR of 0.6 W/kg for 1 h increased the expression of 14 genes which function in mitogenic signal transduction, cell growth and apoptosis controlling. The authors further demonstrated a significant increase in DNA synthesis and intracellular mitogenic second messenger formation which were matched the high expression of MAP kinase family genes [Pacini et al., 2002]. The authors suggested that the RF EMF exposure has significant biological effects on human skin fibroblasts.

However, only one experiment was performed in array analysis and no more experiment was made by the authors to confirm the array analysis result.

With help of cDNA microarray, Leszczynski *et al*. reported that exposure to GSM 900 MHz RF EMF at an average SAR of 2.4 W/kg for 1 h changed expression of 3600 genes, including down-regulated genes involved in forming the Fas/TNFa apoptotic pathway in human endothelial cell line EA.hy926 [Leszczynski et al., 2004]. The authors performed three separate experiments in array analysis, but no confirmation experiments were conducted to validate the array analysis result. Recently, Leszczynski group compared the global gene response of two human endothelial cells, EA.hy926 and its variant EA.hy926v1 to RF EMF and reported that the same genes were differently affected by the exposure to GSM 900 MHz RF EMF at an average SAR of 2.8 W/kg for 1 h in each of the cell lines [Nylund and Leszczynski 2006]. Similarly, no reports indicated that the differentially expressed genes in this study were confirmed by other methods.

Lee *et al*. used the serial analysis of gene expression (SAGE) method to measure the RF EMF effect on genome scale gene expression in HL 60 cells. The cells were exposed to 2.45 GHz RF EMF at an average SAR of 10 W/kg for 2 h and 6 h. The authors observed that 221 genes and 759 genes altered their expression after 2 h exposure and 6 h exposure respectively. Functional classification of the affected genes revealed that apoptosis-related genes were among the up-regulated ones and the cell cycle genes among the down-regulated ones, but no significant increase in the expression of heat shock genes were found [Lee et al., 2005]. However, the SAGE experiment was repeated only once and only one control with 2 h sham exposure was used. No confirmation experiment was reported to validate these differentially expressed genes.

Huang *et al*. investigated the effect of 1763 MHz RF EMF on gene expression in Jurkat cells by Applied Biosystems 1700 full genome expression microarray. The authors

found that 68 genes were differentially expressed in the cells after exposure to RF EMF at SAR of 10 W/kg for 1 h and harvested immediately or after 5 h [Huang et al., 2006]. The authors repeated sets of experiment five times to collect biological triplicates in every sample but the differentially expressed genes were not confirmed by other methods.

Whitehead *et al.* have performed *in vitro* experiments with C3H 10T(1/2) mouse cells to determine whether Frequency Division Multiple Access (FDMA) or Code Division Multiple Access (CDMA) modulated RF radiations can induce changes in gene expression using the Affymetrix U74Av2 GeneChip. The GenesChip data showed the number of probe sets with an expression change greater than 1.3-fold was less than or equal to the expected number of false positives in C3H 10T(1/2) mouse cells after 835.62 MHz FDMA or 847.74 MHz CDMA modulated RF EMF exposure at SAR of 5 W/kg for 24 h. The authors concluded that the 24 h exposures to FDMA or CDMA RF radiation at 5 W/kg had no statistically significant effect on gene expression [Whitehead et al., 2006a; Whitehead et al., 2006b]. However, the authors did not demonstrate that these differentially expressed genes were real “false positive” with other methods.

In Gurisik’s report, human neuroblastoma cells (SK-N-SH) were exposed to GSM 900 MHz RF signal at SAR of 0.2 W/kg for 2 h and recovered without field for 2 h post-exposure. Gene expression were examined by Affymetrix Human Focus Gene Arrays including 8400 genes and followed by real-time RT-PCR of the genes of interest. Only six genes were found to be slightly down-regulated in response to RF exposure comparing with mock-exposed cells. Furthermore, these genes can not be confirmed by real-time RT-PCR analysis. Thus, the authors concluded that the RF EMF exposure applied in this study could not change gene expression in SK-N-SH cells [Gurisik et al., 2006]. However, the array analysis experiment was repeated only once and only one array for exposure or sham exposure group.

Qutob *et al* have assessed the ability of exposure to a 1.9 GHz pulse-modulated RF field

to affect global gene expression in U87MG glioblastoma cells by application of Agilent Human 1A (v1) oligonucleotide 22K microarray slides. The U87MG cells were exposed to 1.9 GHz pulse-modulated (50 Hz, 1/3 duty cycle) RF field at an average SAR of 0.1, 1.0 and 10.0 W/kg for 4 hours, and incubated for an additional 6 hours. The authors found no evidence that exposure to RF fields under different exposure conditions can affect gene expression in cultured U87MG cells. In this paper, the authors performed five experiments, each containing a single replicate and some of genes were confirmed as real “un-effected genes” [Qutob et al., 2006].

Zeng *et al*. have investigated gene expression profile in MCF-7 after exposing to GSM 1800 MHz RF EMF using Affymetrix Genechip U133A. The result showed that no gene with 100% consistency change were found in MCF-7 cells after intermittent exposure (5 min on/ 10 min off) to RF EMF at an average SAR of 2.0 W/kg for 24 h while five genes with 100% consistency change were found in MCF-7 at same exposure conditions but at SAR of 3.5 W/kg. However, these five differentially transcribed genes could not be further confirmed by real-time RT-PCR assay. Thus, this study did not provide evidence that RF EMF exposure can produce distinct effects on gene expression in the MCF-7 cells [Zeng et al., 2006].

Remondini *et al*. have investigated the effect of RF EMF on gene expression profile in six different cell lines or primary cells, and found various types of cell reacted differently in RF EMF exposure). RF EMF exposure changed gene expression in 900 MHz-exposed EA.hy926 endothelial cells (22 up-regulations, ten down-regulations), 900 MHz-exposed U937 lymphoblastoma cells (32 up-regulations, two down-regulations), and 1800 MHz-exposed HL-60 leukemia cells (11 up-regulations, one down-regulation) while NB69 neuroblastoma cells, T-lymphocytes, and CHME5 microglial cells did not show significant changes in gene expression. The authors concluded that there were alterations in gene expression in some human cells types exposed to RF-EMF but these chenges depended on the type of cells and RF-EMF signal [Remondini et al., 2006]. However, these RF responsive candidate genes in

different types of cells were not confirmed yet.

Very recently, Zhao *et al*. have investigated the effects of RF EMF on gene expression of *in vitro* cultured rat neuron with Affymetrix Rat Neurobiology U34 array. Among 1200 candidate genes, 24 up-regulted genes and 10 down-regulated genes were identified after 24-h intermittent exposure (5 min on/ 10 min off) at an average SAR of 2.0 W/kg, which are associated with multiple cellular functions. The changes of most of genes were successfully validated by real-time RT-PCR, including genes involved in cytoskeleton, signal transduction pathway, metabolism [Zhao et al., 2007].

Belyaev et al. analyzed gene expression profile in RF exposed animals. Rats were exposed or sham exposed to GSM 915 MHz at whole body average SAR of 0.4 mW/g for 2 h and total RNA was extracted from cerebellum. Gene expression profiles were obtained by Affymetrix U34 GeneChips representing 8800 rat genes and analyzed with the Affymetrix Microarray Suite (MAS) 5.0 software. The results showed that 11 genes were up-regulated in a range of 1.34-2.74 folds and one gene was down-regulated 0.48-fold. The induced genes encode proteins with diverse functions including neurotransmitter regulation, blood-brain barrier (BBB), and melatonin production [Belyaev et al., 2006]. In this study, triplicate arrays were applied for three exposed samples or three sham exposed samples. But the differentially expressed genes were not confirmed by other methods.

III B . PROTEOMICS

Leszczynski *et al*. have provided perhaps some of the most relevant *in vitro* data by studying the effects of GSM 900 MHz RF EMF exposure [Leszczynski et al., 2002; Nylund and Leszczynski 2004; Nylund and Leszczynski 2006]. Firstly, the EA.hy926 cells were exposed to RF EMF at SAR of 2.0 W/kg over a one-hour period and the data indicated the RF exposure changed protein expression at a proteome scale, and up-regulated the level of HSP 27 protein and induced its hyper-phosphorylation. The

activation of p38 mitogen activated kinase (MAPK) was partially responsible for the phosphorylation of the HSP. They confirmed HSP27 protein expression, phosphorylation and cellular distribution by independent protein analytical techniques including western blotting and indirect immunofluorescence [Leszczynski et al., 2002]. Secondly, the group screened 38 proteins with statistically significantly altered expression in the same cell line after GSM 900 MHz exposure at SAR of 2.4 W/kg for 1 h. An isoform of vimentin was confirmed as a responsive protein by Western blotting and indirect immunofluorescence. The authors concluded that the cytoskeleton might be one of the mobile phone radiation-responding cytoplasmic structures [Nylund and Leszczynski 2004]. Furthermore, they compared *in vitro* response to GSM 900 MHz RF EMF in EA.hy926 with its variant EA.hy926v1 by examination of protein expression using 2-DE. The results showed protein expression profiles were altered in both examined cell lines after RF EMF exposure. However, the affected proteins were differently in each of the cell lines, 38 and 45 differentially expressed proteins were found in EA.hy926 and EA.hy926v1 respectively. Several differentially expressed proteins in EA.hy926 cells were confirmed by other methods, but no differentially expressed protein in EA.hy926v1 cells was confirmed. Base on the transcriptome and proteome analysis data, the authors concluded that the response might be genome- and proteome-dependent [Nylund and Leszczynski 2006]. One thing should be mentioned that all the 2-DE analyses in Leszczynski group reports were replicated ten times.

Zeng *et al*. systematically explored the effects of 1800 MHz RF EMF on protein expression in MCF-7 cells by 2-DE, and revealed that a few but different proteins were differentially expressed under continuous or intermittent RF EMF exposure at SAR of 3.5 W/kg for 24 h or less, implying that the observed effects might have occurred by chance. By combination with the transcriptomics analysis data, this study did not provide convincing evidence that RF EMF exposure could produce distinct effects on gene and protein expression in the MCF-7 cells. The authors supposed that the MCF-7 cells may be less sensitive to RF EMF exposure [Zeng et al., 2006]. However, in this study, only triplicate gels were performed in each exposure condition experiment.

III C . SUMMARY

The effects of RF EMF on global gene and protein expression have been investigated in different biological systems, and most of studies were focused on the mobile phone utilization frequency (800-2000 MHz) at relative low exposure density (average SAR near 2.0 W/kg). Some studies reported negative results of RF EMF exposure on gene expression. For example, Whitehead *et al*. did not find differentially expressed genes in RF exposed C3H 10T(1/2) mouse cells [Whitehead et al., 2006a; Whitehead et al., 2006b]. Remondini *et al*. reported that NB69 cells, T lymphocytes, and CHME5 cells did not show significant changes in gene expression after RF EMF exposure [Remondini et al., 2006]. In Gurisik *et al.* [Gurisik et al., 2006]and Zeng *et al.* [Zeng et al., 2006]study, although they screened out several RF EMF-responsive candidate genes, they could not confirm these genes by real-time RT-PCR method.

Meanwhile, several groups claimed that RF EMF exposure can change gene and protein expression profile in certain types of cells and identified certain EMF responsive genes and proteins. Only one report found RF EMF exposure changed gene expression profile in neurons and most of changed genes were confirmed by real-time RT-PCR [Zhao et al 2007]. As to proteome analysis, only two groups have analyzed protein expression by proteomic approaches, including 2-DE and Mass Spectrum. Zeng *et al*. systematically explored the effects of 1800 MHz RF EMF on protein expression in MCF-7 cells by 2-DE, and revealed that a few but different proteins were differentially expressed under different exposure conditions, implying that the observed effects might have occurred by chance [Zeng et al., 2006]. However, in this study, only triplicate gels were performed in each exposure condition experiment. In contrast, Leszczynski group identified two RF EMF responsive proteins in EA.hy926 cells, i.e. HSP27 [Leszczynski et al., 2002] and vimentin [Leszczynski et al., 2004] with help of 2-DE and MS analysis. This group further confirmed the expression and cellular distribution of HSP27 and vimentin in RF exposed EA.hey926 cells by other methods including Western blotting and indirect immunofluorescence staining. Furthermore, they reported the changes of these RF EMF molecular targets had

down-stream impact on cell physiology [Leszczynski et al., 2002; Leszczynski et al., 2004].

Generally, it seems that the response of a cell to RF EMF exposure depends on exposure condition, cell type, and/or the cell's genome- and proteome [[Remondini et al., 2006; Nylund and Leszczynski 2006].

IV. Overall Conclusion

Based on current available literature, it is justified to conclude that EMF exposure can change gene and/or protein expression in certain types of cells, even at intensities lower than ICNIRP recommended values. However, the biological consequences of most of the changed genes/proteins are still unclear, and need to be further explored. Thus, it is not the time point yet to assess the health impact of EMF based on the gene and protein expression data. The IEEE and WHO data bases do not include the majority of ELF studies; they do include the majority of the RF studies.

Currently, controversial data exist in the literature. The EMF research community should pay equal attention to the negative reports as to the positive ones. Not only the positive findings need to be replicated, all the negative ones are also needed to be validated.

It is noteworthy that low intensity EMF is a weak physical stimulus for a cell or organism, and high throughput screening techniques (HTSTs) would sacrifice its sensitivity to ensure its high throughput. It has been recognized there is methodological defects while analyzing weak effect with HTSTs, such as reproducibility and variability. Thus, more experimental replications are needed to reduce the ratio of noise over signal. Meanwhile, confirmation study must be included to assure the validity of the data.

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