
Global Protein Expression in Response to Extremely Low Frequency Magnetic Fields

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Abstract

Daily exposure to extremely low frequency magnetic fields (ELF MF) in the environment has raised public concerns on human health. Epidemiological studies suggest that exposure to ELF MF might associate with an elevated risk of cancer and other diseases in humans. To explain and/or support epidemiological observations, many laboratory studies have been conducted to elucidate the biological effects of ELF MF exposure and the underlying mechanisms of action. In order to reveal the global effects of ELF MF on protein expression, the proteomics approaches has been employed in this research field. In 2005, WHO organized a Workshop on Application of Proteomics and Transcriptomics in electromagnetic fields (EMF) Research in Helsinki, Finland to discuss the related problems and solutions. Later the journal *Proteomics* published a special issue devoted to the application of proteomics to EMF research. This chapter aims to summarize the current research progress and discuss the applicability of proteomics approaches in studying on ELF MF induced biological effects and the underlying mechanisms.

Keywords

Proteome • Protein expression • Non-ionizing radiation • Electromagnetic fields • EMF • Extremely low frequency magnetic fields • ELF-MF • Two-dimensional gel electrophoresis • Mass spectrometry • Yeast

7.1 Introduction

Daily exposure to electromagnetic fields (EMF), including extremely low frequency magnetic fields (ELF MF) 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. [1]). 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.

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It is reasoned that one of the priorities in EMF research is to elucidate the biological effects of EMF exposure and the underlying mechanisms of action. Proteins are key players in organisms, and it has been assumed that any biological impact of EMF must be mediated by alterations in protein expression [2, 3]. For example, heat shock proteins have been identified as EMF responsive genes and/or proteins in certain biological systems [4]. In order to reveal the global effects of EMF on 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 [5, 6]. 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 proteomics approaches in the investigations on ELF MF induced biological effects and the underlying mechanisms.

7.2 Model Organism

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 (7.8–14 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 [7]. 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 [8]. In this study, only three sets of gels from three independent experiments were analyzed.

Using 2-D Fluorescence Difference Gel Electrophoresis (2-D DIGE) technology and mass spectrum (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 [9]. 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 [9].

7.3 Mammalian Cells

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 mass spectrum (MS) as RNA binding protein regulatory subunit, proteasome subunit beta type 7 precursor, and translationally controlled tumor protein, respectively [10]. Further investigations, such as Western blotting, are required to confirm these ELF responsive candidate proteins.

Kanitz et al. used proteomic methods to investigate the biochemical effects induced by MF exposure in SF767 human glioma cells [11]. The

cells were exposed to 1.2 μT of 60 Hz MF or epidermal growth factor (EGF). SF767 cells were exposed for 3 h to sham conditions ($<0.2 \mu\text{T}$ ambient field strength) or 1.2 μT of MF with or without combination of 10 ng/ml of EGF. The solubilized proteins from four groups of cells (sham; 1.2 μT MF; sham + EGF; 1.2 μT MF + EGF) were loaded for electrophoresis by 2D-PAGE and stained using a colloidal Coomassie blue technique to resolve and characterize the proteins. The spots with significant alterations in the densities were excised and subjected to peptide mass fingerprinting. After exposure to 1.2 μT of MF for 3 h, the mean abundances of ten identified proteins were significantly altered, including three proteins with increased expression level and seven decreased. In the presence of EGF, the MF exposure changed protein expression profile in SF767 cells, and four proteins were identified with increased expression level and two decreased. The authors suggested that differentially expressed proteins in SF767 cells may be useful as biomarkers for biological changes caused by exposure to magnetic fields. However, these candidate MF responsive proteins should be validated and the biological functions need further elucidated.

In the recent study by Sulpizio et al., human SH-SY5Y neuroblastoma cells were exposed to a 50 Hz, 1 mT sinusoidal MF at three different times i.e. 5 days (T5), 10 days (T10) and 15 days (T15) and then the effects of MF exposure on proteome expression were investigated by 2D-PAGE and MS analyses [12]. Through comparative analysis between treated and control samples, the authors analyzed the proteome changes induced by the MF exposure. Nine new proteins resolved in sample after a 15 day treatment, were involved in a cellular defense 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. The authors also showed that the MF exposure altered the cell proliferation and cell viability. Furthermore, the MF-exposed cells showed a higher and more widespread expression level of alpha tubu-

lin, especially in the periphery of cell clusters, compared to control cells, suggesting that the MF exposure induce a spatial orientation of cells. The authors hypothesized that the MF exposure could trigger a shift toward a more invasive phenotype. However, future studies are needed to address this hypothesis.

7.4 Summary

Generally, recent studies on global protein expression responding to ELF MF have been conducted in different biological systems by applications of different proteomics approaches. The mammalian cells seem more sensitive to ELF MF exposure, however, the bacterial and yeast cells, as model organism, did not react to ELF MF exposure. Some proteome analyses showed that ELF MF exposure could change protein expression in the mammalian cells; there are lacks of confirmations by other assays to determine if they are real ELF MF responsive proteins and future studies are needed to elucidate the biological functions of these candidate ELF MF responsive proteins. The human neuroblastoma cell line SH-SY5Y is suggested as a model to further confirm the effect of ELF MF on global protein expression, and the role of ELF MF responsive proteins in ELF MF induced cell behavior changes.

References

1. Feychting M, Ahlbom A, Kheifets L (2005) EMF and health. *Annu Rev Public Health* 26:165–189
2. Phillips JL, Haggren W, Thomas WJ, Ishida-Jones T, Adey WR (1992) Magnetic field-induced changes in specific gene transcription. *Biochim Biophys Acta* 1132(2):140–144
3. Wei LX, Goodman R, Henderson A (1990) Changes in levels of c-myc and histone H2B following exposure of cells to low-frequency sinusoidal electromagnetic fields: evidence for a window effect. *Bioelectromagnetics* 11(4):269–272
4. Leszczynski D, Joenvaara S, Reivinen J, Kuokka R (2002) Non-thermal activation of the hsp27/p38MAPK stress pathway by mobile phone radiation in human endothelial cells: molecular

- mechanism for cancer- and blood-brain barrier-related effects. *Differentiation* 70(2-3):120-129
5. Leszczynski D (2006) The need for a new approach in studies of the biological effects of electromagnetic fields. *Proteomics* 6(17):4671-4673
 6. Leszczynski D, Meltz ML (2006) Questions and answers concerning applicability of proteomics and transcriptomics in EMF research. *Proteomics* 6(17):4674-4677
 7. Nakasono S, Saiki H (2000) Effect of ELF magnetic fields on protein synthesis in *Escherichia coli* K12. *Radiat Res* 154(2):208-216
 8. Nakasono S, Laramie C, Saiki H, McLeod KJ (2003) Effect of power-frequency magnetic fields on genome-scale gene expression in *Saccharomyces cerevisiae*. *Radiat Res* 160(1):25-37
 9. Sinclair J, Weeks M, Butt A, Worthington JL, Akpan A, Jones N, Waterfield M, Allanand D, Timms JF (2006) Proteomic response of *Schizosaccharomyces pombe* to static and oscillating extremely low-frequency electromagnetic fields. *Proteomics* 6(17):4755-4764
 10. Li H, Zeng Q, Weng Y, Lu D, Jiang H, Xu Z (2005) Effects of ELF magnetic fields on protein expression profile of human breast cancer cell MCF7. *Sci China C Life Sci* 48(5):506-514
 11. Kanitz MH, Witzmann FA, Lotz WG, Conover D, Savage RE (2007) Investigation of protein expression in magnetic field-treated human glioma cells. *Bioelectromagnetics* 28(7):546-552
 12. Sulpizio M, Falone S, Amicarelli F, Marchisio M, Di Giuseppe F, Eleuterio E, Di Ilio C, Angelucci S (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