



# Proteomic Analysis of the Effect of Extremely Low-Frequency Electromagnetic Fields (ELF-EMF) With Different Intensities in SH-SY5Y Neuroblastoma Cell Line

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## Abstract

**Introduction:** During the last 3 decades, human is exposed to extremely low frequency electromagnetic fields (ELF-EMF) emitted by power lines and electronic devices. It is now well accepted that ELF-EMF are able to produce a variety of biological effects, although the molecular mechanism is unclear and controversial. Investigation of different intensities effects of 50 Hz ELF-EMF on cell morphology and protein expression is the aim of this study.

**Methods:** SH-SY5Y human neuroblastoma cell line was exposed to 0.5 and 1 mT 50 Hz (ELF-EMF) for 3 hours. Proteomics techniques were used to determine the effects of these fields on protein expression. Bioinformatic and statistical analysis of proteomes were performed using Progenesis SameSpots software.

**Results:** Our results showed that exposure to ELF-EMF changes cell morphology and induces a dose-dependent decrease in the proliferation rate of the cells. The proteomic studies and bioinformatic analysis indicate that exposure to 50 Hz ELF-EMF leads to alteration of cell protein expression in both dose-dependent and intensity dependent manner, but the later is more pronounced.

**Conclusion:** Our data suggests that increased intensity of ELF-EMF may be associated with more alteration in cell protein expression, as well as effect on cell morphology and proliferation.

**Keywords:** ELF-EMF; Proteomics; Neuroblastoma; Protein expression; Intensity.

## Introduction

During the last 3 decades, human has been exposed to many new physical and chemical agents. A major physical agent among other agents is electromagnetic field (EMF), which is widespread and ubiquitous in modern daily life. Therefore, most people are exposed to ELF-EMF that are emitted by power lines, electrical panels, transformers, domestic electrical and electronic devices.<sup>1-3</sup>

Risks of exposure to ELF-EMF for human health have been investigated in several epidemiological and experimental studies.<sup>4</sup> Several epidemiological studies reported that exposure to ELF-EMF have increased incidence of certain types of cancer, especially acute childhood leukemia.<sup>5-7</sup>

Furthermore, several in vitro and in vivo studies related

to the biological effects of ELF-EMF have been published, and some hypothetical mechanisms have been presented.<sup>8</sup> A limited number of these studies have demonstrated that ELF-EMF promotes carcinogenic effects. Some available evidence showed that exposure to ELF-EMF can either enhance or inhibit cell proliferation and apoptosis.<sup>9,10</sup> Several studies indicated that ELF-EMF exposure leads to alteration of chromosomal regulation and structure.<sup>11-13</sup> Moreover, other in vitro studies suggested that ELF-EMF can change the expression of some protein involved in control of cell proliferation processes,<sup>14,15</sup> while others demonstrated that exposure to ELF-EMF has no effects on cell proliferation, DNA replication and regulation.<sup>16</sup> The results of these studies are different and may correspond to the opposite effects. Some of these conflicting

data might be due to differences in frequency, intensity, duration and also certain type of cell lines.<sup>17</sup> Generally, despite profound studies, there has not been any accepted molecular mechanism to explain the carcinogenic effects of ELF-EMF.<sup>18</sup> Proteomics is a powerful tool to elucidate underlying mechanism of exposed cells.

In this study, we investigated the possible effects of 50 Hz ELF-EMF, with different intensities (0.5 and 1 mT) on cell protein expression, proliferation and morphology, using proteomics in SH-SY5Y- human neuroblastoma cell line.

## Methods

### Cell Lines and Cultures

SHSY5Y cells (human neuroblastoma cell line) were purchased from the national cell bank of Iran (NCBI, number: C611) and cultured in RPMI: Ham's F12 (1:1), 2 mM glutamine, 1% non-essential amino acids (NEAA), 15% fetal bovine serum (FBS), 100 units/mL penicillin and 100 µg/mL streptomycin. The cells were incubated at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> and were seeded into T75 flasks. When the cells grew into approximately 70% of confluence (in exponential growth phase) at a density 3 × 10<sup>5</sup>, they were chosen for the experiments.

### Exposure System

EMF exposure system produced homogenous sinusoidal ELF-EMF, with intensity of 0.5–2.0 mT and frequency of 50 Hz, which was generated with a Helmholtz coil. The system was designed to be embed inside the incubator and guarantee standard cell culture condition during exposure (37°C and 5% CO<sub>2</sub>). Control and exposed Human SH-SY5Y cells were placed in the incubator outside and inside the coil, respectively. Cells were exposed to 50-Hz EMF at 0.5 and 1 mT for 3 hours in a day.

### Preparation

After 3 hours of exposure to 50 Hz ELF-EMF with different intensities, SH-SY5Y cells were harvested by centrifugation at 3000 rpm for 10 minutes, washed 3 times with washing buffer (10 mM Tris pH 7.0 and 250 mM D-sorbitol) and then resuspended in standard lysis buffer (8M urea, 4% CHAPS, 40 mM DTT, 2% pharmalyte (pH 3–10 NL), 1 mM PMSF and 1 mM EDTA). Samples were sonicated with a sonicator probe for 5 minutes and centrifuged at 40 000 g for 30 minutes at 4°C. Protein concentration in collected supernatants was determined using Bradford's method. After quantification of proteins, the supernatants were kept at -20°C until electrophoresis.

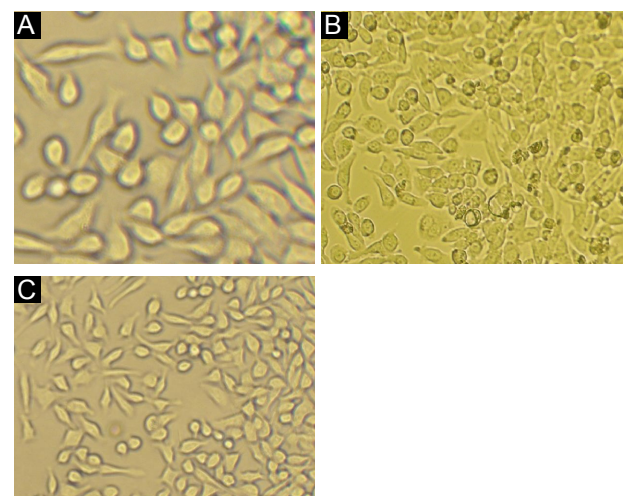
### Two-Dimensional Gel Electrophoresis

The extracted protein was separately mixed with rehydration buffer and applied to a 17 cm immobilized pH gradient (IPG) strip, pH 3-10, and was passively rehydrated with the above sample solution overnight at room temperature. Isoelectric focusing (IEF) was performed in 5 steps including 150 V, 1 hour, 300 V, 1 hour, 1000 V, 1 hour, 5000 V, 2 hours (in a gradient pattern) and 5000 V, 2 hours. Following IEF, IPG strips were incubated in

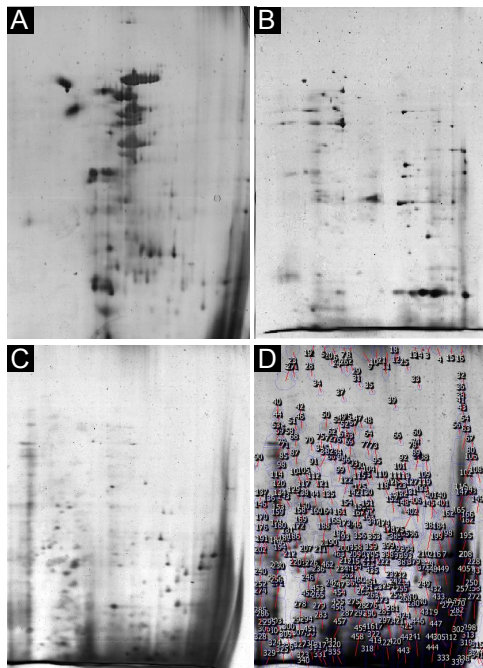
equilibration buffer containing 6M urea, 30% glycerol, 2% SDS, 2% DTT and then alkylated for 20 minutes in the same buffer with 2.5% iodoacetamide instead of DTT. In the second dimension, the treated strips were transferred onto 12% SDS-polyacrylamide slab gel and sealed with 1% agarose. The gels ran in 2.5 W for 30 minutes and then 15 W until the bromophenol blue reached the end of the gel. Analytical gels were stained with Coomassie brilliant blue staining. Gels were scanned using Bio Rad Image Scanner and Spot detection, matching; and quantitative gel analysis was carried out with Nonlinear Progenesis software.

## Results

Since cell morphology and proliferation can be affected by ELF-EMF exposure, the SH-SY5Y cells were exposed to 50 Hz ELF-EMF (0.5 and 1 mT) for 3 hours. Cell morphology alteration was analyzed using microscopic assessments (Figure 1). The findings corresponded to an inhibition of cellular proliferation rate (data not shown). Cell morphology changes and cell proliferation alteration may be associated with changes in gene expression regulation, therefore proteomes of neuroblastoma cells exposed to 50 Hz ELF-EMF with 2 different intensities (0.5 and 1 mT) for 3 hours were provided. In the 2-DE technique, proteins were separated according to the pI and molecular weight in the first and second dimensions, respectively. The gels of the exposed (0.5 and 1 mT) and control cells are shown in Figure 2. The protein patterns on the gels were analyzed using Progenesis SameSpots software. With this software, the control gel was compared to ELF-EMF exposed gels. The findings indicate that gene expression is affected by exposure and it is a dose dependent process. Sixty-four and 40 genes were up regulated and down regulated respectively, after the cells were exposed to 0.5 mT of 50 Hz radiation ( $P < 0.05$ ). The expression of 151 genes significantly differed when the cells were exposed to 50 Hz, 1 mT for 4 hours ( $P \leq 0.05$ ), including 86 upregulated and 65 downregulated genes. Up and down regulated proteins can be grouped based on their expression



**Figure 1.** Morphological Responses of (A) Exposed SH-SY5Y Cells to 50 Hz, 0.5 mT, (B) 50 Hz, 1 mT and (C) Control Cells.



**Figure 2.** Electrophoresis Feature of Exposed SH-SY5Y Cells to (A) 50 Hz, 0.5 mT, (B) 50 Hz, 1 mT and (C) Control Cells. The gel of normal cells is selected as reference gel (D).

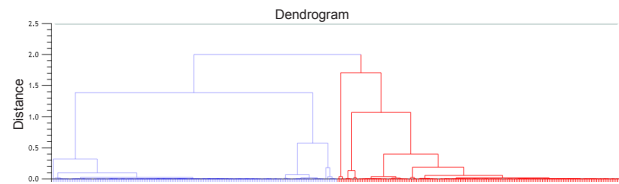
pattern via hierarchical clustering. The results can be presented in a dendrogram tree in which similar expression patterns cluster together. Statistical analysis by Progenesis SameSpot was performed via hierarchical cluster analysis and principal components analysis (PCA) (Figures 3-6).

**Discussion**

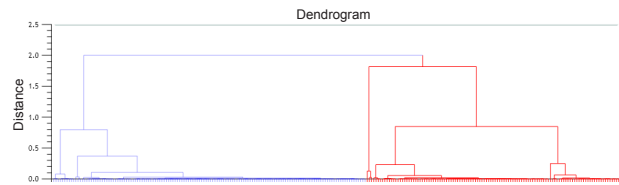
Dendrograms of clustered proteins are represented in Figure 3. As depicted in this figure, the range changes of expression in down regulated proteins is wider than in up regulated proteins. After exposure (0.5 and 1 mT), the number of up regulated proteins was higher than the down regulated proteins (about 1.3 and 1.6 fold). As showed by the results we reported, exposure leads to up regulation relative to down regulation. However, the number of proteins which regulation are changed might be not as current work as the differences may be related to the fact that responses to exposure can be irregular.

In order to determine if there are any outliers in the data and verify the quality of the replicates, PCA was used. The results showed that there are no outliers in the protein set of both groups.

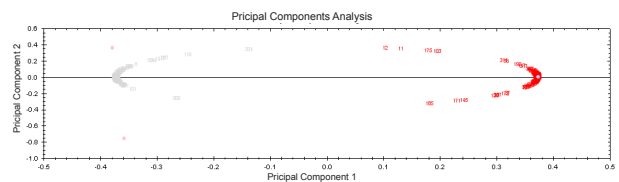
Since cell morphology and function are closely related, in this study, the potential effect of 50 Hz electromagnetic wave on SHSY5Y cell morphology and relevant proteomes' profile was investigated. As it is depicted in Figure 1, exposure to 50 Hz ELF-EMF can affect cell morphology and increasing intensity causes a complete decrease in cell proliferation. It can be considered that 50 Hz exposure affects cell function and survival in a dose dependent manner. Moreover, we determined possible changes in the expression levels of proteins at 2 intensi-



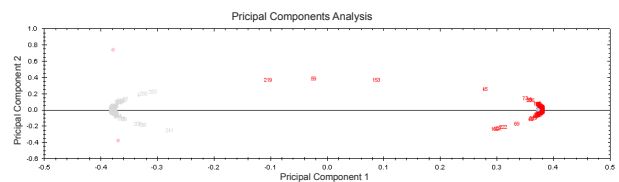
**Figure 3.** Clustered Proteins Exposed SH-SY5Y Cells to 50 Hz, 0.5 mT. The up regulated proteins are presented in red color and the down regulated proteins are shown in blue color.



**Figure 4.** Clustered Proteins Exposed SH-SY5Y Cells to 50 Hz, 1 mT. The upregulated proteins are presented in red color and the down regulated proteins are shown in blue color.



**Figure 5.** Principal Components Analysis in Exposed Cells to 50 Hz, 0.5 mT. The up regulated proteins are shown in red color and the down regulated proteins are represented in gray color.



**Figure 6.** Principal Components Analysis in Exposed Cells to 50 Hz, 1 mT. The upregulated proteins are shown in red color and the down regulated proteins are represented in gray color.

ties (0.5 and 1 mT) of 50 Hz ELF-EMF. As depicted in Figure 2, our data indicated that protein expression changes (proteome profiles) are highly dependent to the intensity of exposure. Gel analysis was performed with Progenesis SameSpot software. Proteomics is a powerful technique that can help determine the molecular mechanism of exposure.<sup>19-21</sup> Clustering, as an analytical tool in proteomics, can provide an informative view of molecular categories.<sup>19</sup> In spite of different studies, there are a few researches that have investigated the biological effects and underlying mechanism of exposure to ELF-EMF via proteomics.<sup>22-24</sup> For more than 30 years several epidemiological and experimental studies have been performed in order to detect the potential effect of exposure to ELF EMF.<sup>1,2,5</sup> Controversial data might be due to different exposure frequencies, intensities, timing and also certain types of cell line.<sup>17</sup> As it is shown in Figures 3 and 4, clustering of the changed expression proteins indicates that

the clustering pattern is highly dependent to the dose intensity. It seems that variation in the number of clusters is reduced (specially for down regulated proteins), which corresponds to the increment of intensity. This finding refers to the nearly equal response of the genes to high intensity doses. Neuronal cells are responsive and sensitive to environmental exposure, therefore some experimental studies were carried out on gene expression of these cell types. There are a few studies on neuroblastoma cells using proteomics.<sup>23,25</sup> However, the findings cannot be compatible with our results; this is because of the various cell responses which are related to several parameters such as intensity and pattern of exposure. As mentioned in the results, 2 important points are to be considered; first, the number of affected genes in higher intensity are 45% higher than in low intensity; and second, the ratio of up regulated genes to down regulated genes are 1.6 and 1.3 in 0.5 and 1 mT, respectively. It can be concluded that radiation effects as an undesired stress and higher intensity level of ELF-EMF exposure correspond to higher stress strength. In conclusion, the results of this study indicate that cell morphology and gene expression can be affected by ELF-EMF exposure and depend on exposure intensity. The other finding corresponds to the more stimulatory role relative to the inhibitory effect of ELF-EMF exposure on gene expression.

#### Ethical Consideration

This project was confirmed by Ethic Committee of Shahid Beheshti University of Medical Sciences (Code No. IR.SBMU.RETECH.1395.671).

#### Conflict of interest

The authors report no conflict of interest.

#### Acknowledgment

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