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Proteomic Analysis in Human Fibroblasts by Continuous Exposure to Extremely Low-Frequency Electromagnetic Fields

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Abstract: Most people are Exposed to Extremely Low-Frequency Electromagnetic Fields (ELF-EMF). A number of studies have indicated association between exposure to extremely low frequency electromagnetic fields and a variety of cancers. Recently some therapeutic techniques such as repetitive Transcranial Magnetic Stimulation (rTMS) have been used to study localization of brain function, connectively of brain regions and pathophysiology of neuropsychiatric disorders (rTMS utilize low frequency-electromagnetic field). Here, the effect of continuous ELF electromagnetic fields (3 Hz, sinusoidal, 3 h and 4 mT) on the protein expression of human fibroblast cells is investigated via proteomics. The comparison of the 2-DE separated proteins from the exposed and sham (control) cells showed that some protein expressions are affected by radiation. The two proteins that their expression are reduced about 50% are determined as alpha 1 antitrypsin (A1AT) and Transthyretin (TTR). As it is reported that the amounts of these proteins reduced in the pathological conditions it can be concluded that application of ELF-EMF in therapeutic aspects may be to accompanying with their side effects.

Key words: Human fibroblast cells, ELF-EMF, alpha 1 antitrypsin and transthyretin

INTRODUCTION

Electromagnetic fields are non-ionising radiation emitted from household devices, electric power transmission and distribution lines (Gómez *et al*., 2000; Narita *et al*., 1997). Then most people are exposed to extremely low-frequency electromagnetic fields produced by power lines and electrical appliances (Mairs *et al*., 2007). Therefore, ELF-EMF has been considered as a possible human carcinogen by International Agency for Research on Cancer (IARC) while credible mechanisms of its carcinogenicity remain unknown (Li *et al*., 2005).

Therefore, several comprehensive reviews regarding *in vivo* and *in vitro* laboratory studies on ELF-EMF have been published to date (McCann *et al*., 1993; Moulder, 1998; Winker *et al*., 2005). Contradicting results have been reported for cells of different species and tissues (Winker *et al*., 2005). In fact, *in vitro* studies with well-defined exposure conditions and end points may provide a more reliable means of estimating the possible carcinogenic potential of ELF-EMF than investigations based upon cancer risk estimates in human populations (Crumpton and Collins, 2004; Mairs *et al*., 2006; Vijayalaxmi and Obe, 2005).

Human fibroblast is one of the responder cell lines to ELF-EMFs. ELF-EMFs produce Micronuclei (MN), chromosome aberrations (CA) and DNA strand breaks in human fibroblasts (Ivancsits *et al*., 2003a, b). Some data showed that extremely low frequency magnetic field changes the protein profile of various cells like fibroblast cells and may affect many physiological functions of normal cells (Gottwald *et al*., 2007; Löschinger, 1998).

As a consequence, ELF-EMF can affect on gene and protein expression. Proteomics is one of the technologies that tied to the diagnostic and therapeutic aspects of medicine. The conventional proteomics has been the high resolution 2-D gel electrophoresis (Hahn and Van Kersen, 1988) followed by computational image analysis and

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protein identification using mass spectrometry. The proteomic studies could lead to the molecular characterization of cellular events associated with cancer progression, signaling and developmental stages (Moscow and Cowan, 1988; Nooter and Stoter, 1996; Ryu *et al*., 2003).

Repetitive Transcranial Magnetic Stimulation is a therapeutic technique that utilize low frequency-electromagnetic field. It has been used to study localization of brain function, connectively of brain regions and pathophysiology of neuropsychiatric disorders (Mantovani *et al*., 2005). We hypothesise that 3Hz ELF-EMF is produced via rTMS treatment may have side effects on patients. In the present study, therefore, we examined the effects of this frequency on the protein expression in human fibroblast cell with use of proteomic techniques.

MATERIALS AND METHODS

Cell cultures: The human fibroblast were cultured in RPMI-1640 medium supplemented with 10% heat inactivated Fetal Calf Serum (FCS), 2 mM glutamine, penicillin (100 IU mL^{-1}) and streptomycin (100 μg mL^{-1}) at 37°C in an incubator containing 5% CO_2. Twenty four hours before start of the ELF experiments, fibroblasts were seeded into 35 mm Petri dishes at the density of 5×10^4 cells 3 mL^{-1}.

ELF-EMF exposure conditions: EMF exposure systems produced a homogenous vertical ELF–EMF (sinusoidal, 3 Hz, 4 mT) generated by a solenoid. For the analysis of protein expression cell were exposed to EMF for a 3 h.

Temperature was continuously monitored. The temperature difference did not exceed 0.3°C. Control cells were sham exposed in the same exposure chamber but switched-off exposure system.

Two dimensional SDS-PAGE: After 24 h of radiation, exposed and sham (control) cells on 25 mm^2 dishes were washed three times in PBS. 300 μL lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS, 0.2-0.3% DTT, 1-2% ampholin 3-10) was added to cell culture and shaking is done in room temperature about 1 h. The lysate was centrifuged at 10000 g for 10 min at room temperature. The supernatant was saved at -20°C until used. Linear pH 3-10 Immobilized Dry Strip (17 cm) were rehydrated overnight at 20°C in rehydration buffer (8.5 M urea, 2% CHAPS, 40 mM DTT, 0.1% ampholin, 0.001% bromophenol blue). Sample (400 μg) was applied during rehydration. The first dimension of 2D electrophoresis was performed on the PROTEAN IEF Cell system (Bio-Rad). Next, gels were equilibrated for 15 min in equilibration buffer I (6 M urea, 2% SDS, 0.375 M Tris HCl pH 8.8, 20% glycerol, 130 mM DTT). A 12% SDS-Polyacrylamide slab gel was used for the second dimension gel electrophoresis. Equilibrated IPG strips were placed on the surface of the second dimension gels and then sealed with 0.5% agarose in SDS electrophoresis buffer (25 mM Tris base, 192 mM glycine, 0.1% SDS) and were run vertically.

After electrophoresis, the gels were fixed with 50% methanol/12% acetic acid/0.5 mL L^{-1} formaldehyde for 60 min. At the end of fixation, the gels were rinsed 3 times with 50% ethanol (each time 20 min). The gels were sensitized by incubating in 0.2 g L^{-1} sodium thiosulfate for 1 min. After 3 time rinsing with distilled water (each time 20 sec), the gels were incubated in 1.9 g L^{-1} silver nitrate/0.8 mL L^{-1} formaldehyde for 20 min. After 3 times rinsing with distilled water (each time 20 sec) the gels were developed in 60 g L^{-1} sodium thiosulfate. The development was terminated with 12% acetic acid/50% methanol.

Protein identification: Two selected spots were analyzed by bionumeric software and their expressions were determined. The proteins are identified by flicker software.

RESULTS AND DISCUSSION

After exposure of human fibroblast cells to the EMF field of 4 mT and 3 Hz for 3 h, the morphology of the cells were examined (Fig. 1).

For comparing the pattern of protein expression of the exposed and control cells, the 2DE gels of the cells are shown in the Fig. 2. The comparison of the separated proteins from the exposed and sham (control) cells indicates that expression level of some protein spots changes in response to the ELF-EMF exposure (the two desired spots are marked in the gels).

Figure 1 shows the human fibroblast cells in the media culture. Comparing of these cells with the exposed cells indicates that there is not morphological difference between them. For better resolution the effect of radiation on the cell respects investigated by proteomics technique. Recently, proteomics as a powerful method was applied widely for detection of variation of protein expression during desired processes such as some diseases, stress conditions and etc (Rezaei-Tavirani *et al*., 2006). As it is 2DE gels of human fibroblast cells show the separated spots (correspond to the proteins) with high resolution (Fig. 2). The proteins are separated by IEF in the horizontal dimension (first dimension) and by molecular weight in the vertical dimension (as the 2nd dimension).

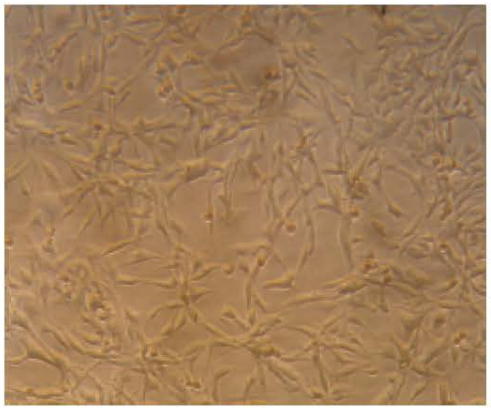

Fig. 1: Exposed and sham human fibroblast cells have the same morphology

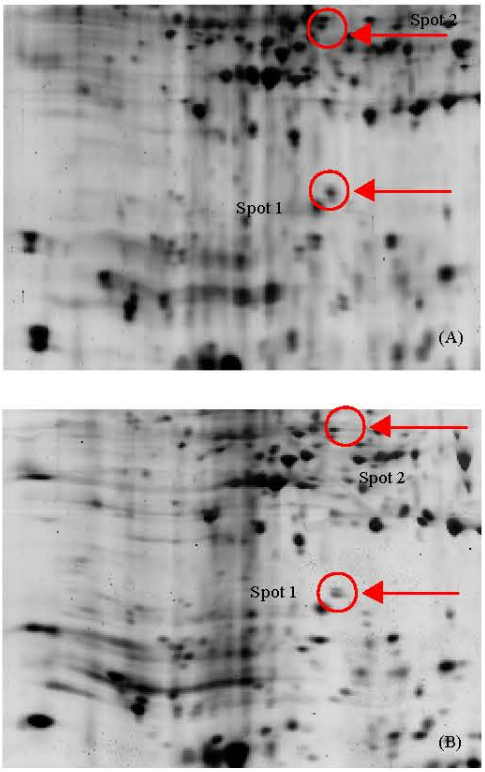

Fig. 2: 2DE gel patterns of A: Human fibroblast cells (as control cells) and B: The exposed cells to the 3 Hz EMF. The two spots (1, 2) are selected and analyzed

Supply of stress conditions on the desired cell line usually affects on the expression of related proteins, so it may suppress expression of a certain group of proteins or inversely the new proteins may be express. The amount of expression of certain proteins also affects by stress conditions (Hamdan and Righetti, 2005). Comparing of the gels reveals that the amounts of expression of many proteins are affected by radiation. Here the two desired spots are selected, marked and analyzed by Bionumerics software (Fig. 2). Bionumerics software is a suitable tool for detection of density of the spots in compare to the similar ones on the other gels. By using Bionumerics software it was confirmed that the amounts volume of the first spot (it is marked as spot 1 in the Fig. 2A and B) in the sham and exposed cells are equal to 1720 ±17 and 820±8, respectively. Similarly for the spot 2 the calculated volumes are 1317±12 and 832±8 correspond to the control and exposed cells, respectively. The findings indicate that the amounts of expression of these spots after exposed to the radiation are reduced (the fitting error was 0.9%) approximately 50%. Identification of the proteins that their expression are affected by supply of stress conditions is a suitable process for determination of protein, metabolite and also gene targets in the cells or living organism for pharmacological and clinical trial (Hamdan and Righetti, 2005). Flicker is a suitable tool for determining of the protein corresponds to the desired spots, the problem in front of this method is restriction of the known proteins that are introduced to the data banks. Fortunately these two spots were determined by flicker as transthyretin (TTR) and alpha1 antitrypsin correspond to the spots 1 and 2 (Fig. 2A and B).

Alpha 1-Antitrypsin or α_1-antitrypsin (A1AT), also known as serum trypsin inhibitor, is a serine protease inhibitor (serpin) and trypsin inhibitor. It protects tissue from enzymes of inflammatory cells, especially elastase. A1AT is a 52 kDa serine protease inhibitor and in medicine it is considered the most prominent one, given the fact that the words α_1-antitrypsin and *Protease inhibitor* (P_i) are often used interchangeably. Most serpins inactivate enzymes by binding to them covalently, requiring very high levels to perform their function. In the acute phase reaction, a further elevation is required to limit the damage caused by activated neutrophil granulocytes and their enzyme elastase, which breaks down the connective tissue fiber elastin (Axelsson *et al*., 1965; Gettins, 2002; DeMeo and Silverman, 2004). Today, various studies show that the expression of A1AT reduces in gastric and ovarian cancers. Consequently, A1AT is used as a biomarker in to distinguish these cancers (El-Akawi *et al*., 2005; Ryu *et al*., 2003). Transthyretin, a carrier protein for thyroxine and retinol in plasma and cerebrospinal fluid, has been shown to bind

the amyloid beta peptide. Accordingly, TTR has been suggested to protect against amyloid beta deposition, a key pathological feature in Alzheimer's Disease (AD) (Sousa *et al*., 2007). Lowered concentrations of TTR could therefore be associated with Alzheimer's disease (Castaño *et al*., 2006; Serot, 1997). Many researchers indicated that the expression of α_1-Antitrypsin reduced in some cancers like ovarian cancer and Primary Hepatic Cancer (PHC) (Gu *et al*., 1991; Schweigert and Sehouli, 2005). As it is depicted, the under-expression of α_1-antitrypsin and Transthyretin are accompanied some diseases like gastric, ovarian cancer and Alzheimer's disease.

The data of the present study indicate that exposure of fibroblast cells to extremely low frequency (3 Hz) electromagnetic field lead to decreased amounts of α_1-antitrypsin and transthyretin. So exposure of cells to ELF-EMF radiation resulted in an increased risk of some cancers and Alzheimer's disease.

Therefore, some therapeutic technique such as rTMS, which use for treatment of neurodegenerative disease (Aare *et al*., 2003; Berman *et al*., 2000; Loo and Mitchell, 2005; Saba *et al*., 2004) may lead to the side effects in these patients. These results suggest to the need of further studies about environmental threshold values for ELF exposure. The important aspects of the future studies should be focused on the stability of the induced changes in the regulation of gene expression and also protein interactions that may concern the cell and organism life.

CONCLUSIONS

In conclusion, our data indicate that exposure of fibroblast cells to extremely low frequency (3 Hz) electromagnetic field lead to under-expression of α_1-antitrypsin and Transthyretin. Decreased amounts of these proteins are reported in the certain pathological condition such as gastric, ovarian, primary hepatic cancer and Alzheimer's disease. Therefore, if rTMS, that utilize low frequency-electromagnetic field, is used for treatment neurodegenerative disease like depression and schizophrenia, it requires further study about side effects of this technique in patients.

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