Effects of Radiofrequency-Modulated Electromagnetic Fields on Proteome

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Abstract

Proteomics, the science that examines the repertoire of proteins present in an organism using both high-throughput and low-throughput techniques, might give a better understanding of the functional processes ongoing in cells than genomics or transcriptomics, because proteins are the molecules that directly regulate physiological processes. Not all changes in gene expression are necessarily reflected in the proteome. Therefore, using proteomics approaches to study the effects of RF-EMF might provide information about potential biological and health effects. Especially that the RF-EMF used in wireless communication devices has very low energy and is unable to directly induce gene mutations.

Keywords

Proteome • Protein expression • Protein activity • Non-ionizing radiation • RF-EMF • Radiofrequency-modulated • Electromagnetic fields • Two-dimensional gel electrophoresis • 2DE • 2DE-DIGE • Phosphorylation • Signaling pathways • Stress response • Stress proteins • Heat-shock proteins

Proteomics approaches, especially these using HTSTs, seem to be particularly suited for elucidation of the effects of RF-EMF because they could reveal effects that are not possible to predict, based on the present, still very limited, knowledge about the biological effects of RF-EMF. The proposed usefulness of proteomics and transcriptomics in search for molecular targets of RF-EMF [1] has been subsequently demonstrated in a pilot 5-step feasibility study [2].

In spite of the potential to discover molecular targets of RF-EM, the progress in research using proteomics is slow, hampered predominantly by the lack of funding and a very limited number of research groups involved.

6.1 Human Volunteer Study

To date, only one study examined effects of RF-EMF on proteome in humans [3]. As the authors stated, this was only a pilot study aimed

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at demonstrating the feasibility of the proteomics approach to study effects of RF-EMF in human skin. Because of the variability of proteomes between individual volunteers the authors have used samples of skin from the same person as the unexposed sham controls. This way they were able to perform pilot study using only ten volunteers. However, use of the 2-DE and silver staining method (only method available at that time in authors' laboratory) and the low number of volunteers in this pilot study diminish the reliability of the changes found in the proteome. In total there were found changes in expression of eight proteins, out of which changes in two of them were observed in all exposed volunteers. Unfortunately, due to technical difficulties, the authors were unable to identify the affected proteins. This study should be repeated with larger number of volunteers and using 2DE-DIGE method to obtain more reliable information whether mobile phone radiation affects proteome of exposed tissues in human body. Interestingly, in spite of the continuing discussion whether RF-EMF radiation can cause any health effects to people, it is still not known whether human body responds to mobile phone radiation on molecular level.

6.2 Animal Model Studies

6.2.1 *Drosophila melanogaster*

Three studies were executed using *Drosophila melanogaster* biological model. In the first one, Weisbrot and co-workers have determined that RF-EMF radiation increases expression of hsp70.1, phosphorylation of ELK-1 kinase and binding activity of the serum response element (SRE) [4]. However, the reliability of the results of this study is weakened because the exposure was performed using antenna of a regular mobile phone, what makes exposure dosimetry unreliable. Lee and co-workers [5] have shown that exposures activate stress response kinases ERK (at 1.6 W/kg) and JNK (at 4.0 W/kg) but not the p38 MAPK. What strengthens the results of this study is that the protein activity results are supported by the gene expression results determined in the same study. Finally, Chavdoula and co-workers [6] have examined the organization of the actin network and found that exposures cause increase in disorganization of the network. However, also in this study exposure dosimetry is unreliable because the authors have used the actual mobile phone. There are no studies using HTST performed in *Drosophila melanogaster* model.

6.2.2 Mouse Models

Several studies were performed in mouse model, using mice of different age (fetus or adult 6–8 weeks of age) and of different strains (C57BL/6N, C57BL/6NTac, hsp70.1 deficient, Balb/c, ICR). In two studies mice strain and age was not specified [7, 8]. Detection of protein expression changes was done by immunocytochemistry using both monoclonal and polyclonal antibodies. Six of the published studies came from the research group of John Finnie in Australia [7–12]. Most of them are based on the same biological material that was separately stained in order to detect different proteins. All-in-all studies from Finnie and co-workers have shown that mobile phone radiation has no effect on the expression of the following proteins: c-fos in adult and in fetal mice brain, stress proteins in fetal brain (Hsp25, Hsp32, Hsp70) and on aquaporin-4 in adult brain and on ionized calcium binding adaptor molecule Iba1. These studies, however, have some problem of reliability because in number of them is missing numerical and statistical analysis and the conclusions are based on only a brief verbal description of immunocytochemistry staining. Lee and co-workers [13] have determined that mobile phone radiation has no effect on the expression of stress proteins (HSP90, HSP70, HSP25) or phosphorylation of stress kinases (ERK, JNK, p38MAPK) in a hsp70.1 deficient mice. The same research group has also examined expression of PCNA, GFAP and NeuN proteins in C57BL/6N mice and found no effects of radiation exposure. However, the results are difficult to

appreciate because only visual evaluation was performed in the second study and without any statistics [14]. Maskey and co-workers [15] have shown that mobile phone radiation might affect expression of calbindin and calretinin in different areas of brain.

Only a single study in mouse model used HTST to examine effects of RF-EMF on proteome. Research team from Greece [16] published a study suggesting that RF-EMF alters expression of over 100 proteins in mice brain. The authors speculated that some of the affected proteins are important in regulation of learning, memory and in regulation of processes leading to Alzheimer's disease and suggested far reaching health hazard implications of their observation. However, the only effect that was somewhat shown by Fragopoulou and co-workers is the possibility that RF-EMF might alter expression levels of some brain proteins [16]. The indication of the processes that might be affected was only an unconfirmed speculation because the data concerning the affected proteins, obtained from the proteomic analysis, were insufficiently confirmed. Certain proteins were named as affected based only on identification of protein spots in 2D-gel analyses but the confirmation experiments, using e.g. western blot, that the change is real were not performed.

6.2.3 Rat Models

Several studies were performed using rats of different age (newborn to adult) and of different strains (Wistar, Fisher 344, hairless rat, Sprague–Dawley) and different tissues were examined (brain, skin, kidney, testis, thyroid). Detection of protein expression changes was done mostly by immunocytochemistry using both monoclonal and polyclonal antibodies and in some studies by western blot.

Four studies looked at the effects in rat brain. In three of them was observed no effect on protein expression [17–19] whereas in two of them was observed an effect [19, 20]. Unfortunately, in some of the studies samples were analyzed only visually and without calculating statistical significance and therefore diminishing reliability of the obtained results. In three studies effects of mobile phone radiation on skin of hairless mice were analyzed [21–23]. No effects were observed on any of the analyzed proteins, however, the reliability of some analyzes is questionable. Study by Pyrpasopoulou and co-workers [24] examined effects of mobile phone radiation on kidneys of newborn rats and found by using two methods (immunocytochemistry and in situ hybridization) that exposure affected expression of bone morphogenic protein (BMP-4) and bone morphogenic protein receptors (BMPR-II, BMPR-IA). These observations are strengthened by the similar changes observed by the authors in the expression of the corresponding genes. Esmekaya and co-workers [25] have observed changes in expression of apoptosis-regulating proteins cacpase-3 and caspase-9. However, the results are unreliable because the expression changes were evaluated only by visual examination. Lee and co-workers [26] have examined effects of mobile phone radiation on rat testis and found lack of statistically significant effect on several tested apoptosis associated proteins (p21, p53, bcl-2, caspase-3, PARP).To date, no rat model proteome studies were performed with the use of HTST.

6.3 In Vitro Studies in Human Primary Cells and in Cell Lines

Small number of in vitro studies used HTST to examine effects of RF-EMF. However, because of the variety of limiting factors, caused by the differences in the study designs and/or methods used, the conclusions of all studies should be looked at with caution. As of now it appears that all the studies can be considered rather as "feasibility studies", paving the way for further, more thorough and better designed, proteomics studies.

In all studies was used variety of RF-EMF signals and exposure conditions. Some of the exposure conditions caused changes in protein expression and some not. However, because of

very limited number of studies, it is not possible to determine whether any of the observed effects can be in any particular way correlated, or not, with certain exposure conditions.

In all of the studies, proteome changes were determined by separating proteins with two-dimensional electrophoresis (2-DE). The protein spot detection was done in most of the studies by silver staining [27–35]. The silver-based staining procedure for protein visualization, although a well established and widely accepted method, might be not the best option for differential proteomics. This is because of the poor reproducibility of this procedure and the reduced linear dynamic protein concentration range that makes it difficult to draw clear conclusions about changes in protein expression. Nevertheless, the data from 2D-PAGE analyses show that proteome changes caused by RF-EMF do not result in major changes of high abundant proteins, which should be taken into account for designing future proteome studies on RF-EMF effects.

A further limitation of the reliability of some studies is the low number of gel replicates. Normally, the silver-based approach requires high numbers (ten or more) of gel replicates. However, in three of the studies the authors rely on only three gel replicates [30, 31, 35], which is not sufficient to draw any reliable conclusions and may lead to false negative or false positive results. In three studies with sufficient number of replicates were obtained two opposite results suggesting existence of effect of 900 MHz GSM exposure on protein expression [28, 29] but indicating lack of effect when exposing to 1,800 MHz GSM signal [32].

The use of relatively novel DIGE system for 2-DE (2-D Fluorescence Difference Gel Electrophoresis), currently considered to be the gold standard in 2-DE, might have been limited in mobile phone radiation proteomics studies by the availability of specialized hardware in the laboratories performing the studies and the high costs for the reagents. There is only a single study [33] where changes in protein expression were examined using 2-DE-DIGE system and with a sufficiently high number of ten replicate gels. This study has shown that the exposure of human primary endothelial cells at SAR of 2.0 W/kg for 1 h using an 1,800 MHz GSM signal does not cause changes in protein expression. Because of the techniques used, this is the most reliable differential proteome analysis to date.

An interesting hypothesis has been presented in the study by Gerner and co-workers [34]. The authors suggest that the protein expression changes might be not the best end-point to show effects of RF-EMF. They have demonstrated that in the cells exposed to mobile phone radiation changes de novo synthesis of some proteins, without statistically significant change in the overall protein expression. However, these conclusions are weakened by only three gel replicates performed in the study, what is not sufficient for reliable determination of changes in protein expression.

The observations of Gerner and co-workers [34] and Leszczynski and co-workers [27] suggest that examining of only protein expression is not sufficient to detect effects of mobile phone radiation. Experiments that examine de novo protein synthesis as well as post-translational modifications of proteins (e.g. phosphorylation) are necessary in order to determine impact low-energy RF-EMF exposures on cell proteome.

The numbers of proteins shown to be affected by RF-EMF, in all proteomics studies that show an effect, are not only low but they are lower than the number of expected false positives. This argument is often used to suggest that there is no effect of RF-EMF on cell proteome. However, the low number of responding proteins, even when it is below the expected rate of false positives, does not mean automatically that the every protein appearing as affected by RF-EMF is a sure false positive finding. The calculation of the number of expected false positives shows only the probability that the affected proteins might be false positives, but it does not prove that all of them are indeed false positives. The proof can be only obtained by biological experiment. This has been demonstrated in study where the number of detected statistically significantly affected

proteins was lower than the expected number of false positives [28]. However, further western blot analysis of the changes in the expression of one of the affected proteins, vimentin, has shown that this protein did respond to RF-EMF.

6.4 Conclusions

It is necessary to keep in mind that the execution of a screening study using proteomics, or other high-throughput screening approach, is just the beginning of the process of finding out the biological effects of RF-EMF. The validity of expression changes detected in identified protein targets needs to be confirmed by other, non-high-throughput screening methods. Such validation experiments were performed only in four of the nine published proteomics studies. In two of them [27, 28] the authors were able to confirm that the proteomics identified targets were correct. Whereas in two other studies, were not [32, 35]. However, the success of the validation experiments depends e.g. on the availability of antibodies directed against the identified protein targets [32]. Once the affected proteins are confirmed, this information can be used for determining what physiological functions of the cell might be altered by the observed protein expression change and whether the observed change is of sufficient magnitude to change cell physiology. Only when the altered protein expression is able to alter cell physiology we can suspect that the effect might have some potential to cause biological or health-related effects but if the cell physiology will not be affected then there will not be any risk of health effects.

In summary, because of the small number of executed proteomics studies, with the variety of shortcomings caused by study design and by the availability of methods and reagents, the information provided by them is very limited, at the best. In the future, proteomics studies should be continued [36] in order to get sufficient amount of information that will permit to draw valid conclusions about the impact of RF-EMF on cell proteome and, consequently, on cell physiology.

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