Overview of the present status and future directions of research into biological effects of EMF using high-throughput screening techniques

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ABSTRACT

In spite of years of research there is still an uncertainty whether low-energy EMF can induce any biological effects and whether such effects would be able to alter cell physiology and pose any risk of future health hazard. So far, the vast majority of research effort has focused on the possibility of induction of cancer. At the same time there continues discussion whether EMF could induce some weak effects that, although would not induce any life-threatening disease, however, they could induce effects detrimental to the quality of life, causing such symptoms as sleep-disorders, headaches etc. The so far observed biological effects are uncertain as the biophysical mechanisms behind their occurrence are unknown. Thus far used research approach is too slow to determine all the possible effects of EMF. The use of the high-throughput screening techniques (HTST) of proteomics and transcriptomics ("Discovery Science") has been proposed as a useful approach to determine all possible biological targets of EMF on cellular level. However, it is necessary to keep in mind that the high-throughput screening is just a starting point. The newly discovered molecular targets need to be confirmed by other methods as modulators of cell physiology. The overview of the most recent data from several laboratories will present the current status of the research using HTST and will examine the future needs and directions for this research where the final goal is evaluation of the health risks associated with the use of EMF.

BRIEF OVERVIEW

The vast majority of the biological research that has examined the effects of RF-EMF has focused on the possibility of induction of genotoxicity, mutations, cancer or impairments in embryonic development [1, 2, 3, 4]. At the same time, there continues discussion on whether RF-EMF could induce some weak effects that, although not able to induce life-threatening disease, could induce effects that could be detrimental to the quality of life. These life-non-threatening effects could include such ailments as e.g. sleep-disorders, headaches, allergy-like symptoms etc. There is also the possibility that the biological effects could lead to health benefits.

Epidemiological studies are often expected to provide the answer whether RF-EMF exposure might be hazardous to human population. However, finding and scientific validation of any potential health hazard using epidemiological approach alone might be not possible. This, because the "low sensitivity" of epidemiological methodology might be insufficient to reliably detect health impact of the weak biological effects induced by low energy RF-EMF. Furthermore, the presently conducted epidemiological studies are focused on the induction of cancer. Therefore, independently of their outcome, these studies will provide information only about the cancer. These studies will not provide information about the other potential effects of RF-EMF exposure. Thus, the presently conducted epidemiological studies will be unable, because of their focus on only one aspect of the health issue, to give RF-EMF "a clean bill of health". Therefore, we need systematic in vitro studies of RF-EMF effects to determine whether it indeed induces biological effects and, if so, then what is the biophysical and biochemical mechanism of these effects. Such information could then be used to design new epidemiological studies that would consider end-points other than cancer alone.

The so far executed classical, hypothesis-driven, research did not produce convincing evidence about the

possibility of induction of biological effects by mobile phone radiation. We have proposed that in order to formulate testable hypotheses we need to know the molecular targets of the radiation in cells [5]. This information is possible to obtain relatively rapidly using modern molecular biology high-throughput screening techniques (HTST). This approach seems to be particularly suited for studying biological effects of RF-EMF because it might reveal effects that are not possible to predict based on the presently available knowledge. It is our opinion that the systematic screening of the transcriptome (gene expression), proteome (protein expression) and phospho-proteome (protein activity) will generate data that will allow us to formulate valid hypotheses concerning the potential health hazard of mobile phone radiation. It is also our opinion, that we need simultaneously information from transcriptome, proteome and phospho-proteome. The combination of the three above mentioned sets of data is necessary because, as observed in our studies, it is possible to find target of RF-EMF exposure that, for example, will not be detectable on gene or protein expression level but that will be detectable on phospho-proteome level.

HTST are able to pick-up small changes in protein or gene expression which changes might be of insufficient magnitude to alter cell physiology. Thus, although using HTST it might be possible to find biological effects, induced by RF-EMF, these effects might be of limited or no significance at all for the cell physiology. Therefore, to determine the usefulness of HTST approach to the issue of bio-effects induced by RF-EMF, we have performed a 5-step feasibility study (Leszczynski et al. 2004) where we have shown that use of HTST indeed helps to identify molecular targets of RF-EMF that may have down-stream impact on cell physiology. Only such physiologically relevant targets, which have impact on cell physiology, are worth pursuing in health hazard examining studies.

Search through databases shows that only very few of the so far published studies have been using HTST methods to determine gene or protein responses to electromagnetic radiation exposures (Table 1). Part of the studies has presented data obtained using transcriptomics approach [6, 7, 8, 9, 10] and part of the studies has used proteomics approach [9, 11, 12, 13, 14]. Interestingly, in two proteomics studies, by Pipkin et al. [11] and by Leszczynski et al. [12], besides examining protein expression changes there were examined changes in the phospho-proteome, by examining the changes in protein phosphorylation that correspond with the activity of proteins.

Table 1. Published studies that have examined effects of electromagnetic radiation using HTST approach.

Biological model	**EMF exposure**	**Examined gene/protein expression**	**Response**	**Reference**
Human HL60 cells	60 Hz magnetic field	gene expression	no	[6]
Human mammary epithelium & HL60	60 Hz magnetic field	gene expression	no	[7]
Human fibroblasts	GSM	gene expression	yes	[8]
S. cerevisiae	50 Hz magnetic field	gene & protein expression	no	[9]
E. coli	static magnetic field	gene expression	yes	[10]
Human HL60 cells	60 Hz magnetic field	protein expression & phosphorylation	yes	[11]
Human EA.hy926 endothelial cell line	900 MHz GSM	protein expression & phosphorylation	yes	[12]
Human EA.hy926 endothelial cell line	900 MHz GSM	gene & protein expression & protein phosphorylation	yes	[13]
Human EA.hy926 endothelial cell line	900 MHz GSM	protein expression	yes	[14]

However, our approach of using HTST in EMF research is gaining support within the scientific community. The first workshop on the use of HTST in studies of RF-EMF is being organized in collaboration with the World Health Organization and other organizations and it will take place at STUK, Helsinki, Finland, in October 2005 (http://www.who.int/peh-emf/meetings/proteomics_helsinki05/en/). During this workshop scientists will discuss the following issues:

1. We have an uncertain effect - induction of a biological response by EMF, and we have a new technology

that has very many limitations and uncertainties of its own. Will the combination of the uncertainty of the effect and the uncertainty of the method bring:

   a. more uncertainty to the studies on the possible biological effects of EMF, or
   b. will it speed up the discovery of new biological end-points that could be used in generating new hypotheses for further health-related studies?

2. Which of the presently available HTST are the most suitable for studying EMF-induced biological effects?
3. How can the data obtained with the use of HTST help in discovering the biophysical mechanism(s) behind any biological effects of EMF?
4. Telecommunications are continuously developing and new EMF frequencies and modulations will be continuously introduced. Is there is a need for a relatively simple screening test that would determine whether new EMF frequency or modulation will induce unpredicted/unexpected biological effects, and what might it be? Such a rapid screening technique could be:
   a. DNA chip/array with selected genes screening gene expression changes
   b. protein array/chip with selected proteins – screening of protein expression changes
   c. protein array/chip for screening of the changes in the activity of proteins (e.g. protein phosphorylation)
   d. combination of all of the above?
5. Are we ready, and is it possible at this time, to develop a standardized test for screening future EMF frequencies in order to compare their effects with the effects of EMF frequencies already in use?

In conclusion, it appears that cells can recognize mobile phone radiation as an external stress factor and launch protective response in form of activation of stress proteins. Simultaneously occurring broad changes in protein phosphorylation indicate that a large variety of cellular signaling pathways are activated in response to this radiation. Identification of these signaling pathways is possible only by using modern high-throughput screening techniques of transcriptomics and proteomics. This will allow formulating of much better, knowledge-based, hypotheses to determine whether there exists a possibility of induction of health hazard by mobile phone radiation.

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