

RESEARCH ARTICLE

Mobile phone radiation causes changes in gene and protein expression in human endothelial cell lines and the response seems to be genome- and proteome-dependent

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We have examined *in vitro* cell response to mobile phone radiation (900 MHz GSM signal) using two variants of human endothelial cell line: EA.hy926 and EA.hy926v1. Gene expression changes were examined in three experiments using cDNA Expression Arrays and protein expression changes were examined in ten experiments using 2-DE and PDQuest software. Obtained results show that gene and protein expression were altered, in both examined cell lines, in response to one hour mobile phone radiation exposure at an average specific absorption rate of 2.8 W/kg. However, the same genes and proteins were differently affected by the exposure in each of the cell lines. This suggests that the cell response to mobile phone radiation might be genome- and proteome-dependent. Therefore, it is likely that different types of cells and from different species might respond differently to mobile phone radiation or might have different sensitivity to this weak stimulus. Our findings might also explain, at least in part, the origin of discrepancies in replication studies between different laboratories.

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1 Introduction

Induction of biological and health effects by mobile phone radiation remains a controversial issue. In spite of years of research, there is still ongoing discussion whether low-energy mobile phone radiation could induce any detrimental health effects, especially in long-time avid users and in children (Stewart Report, National Radiation Protection Board, London, UK, 2000 (<http://www.iegmp.org.uk>)).

We have proposed earlier [1, 2] and subsequently experimentally demonstrated [3] that the research approach using genome-wide and proteome-wide screening techniques of

transcriptomics and proteomics will speed up the identification of genes and proteins that are responding to mobile phone radiation. Using this approach we have shown that mobile phone radiation activates cellular stress response by increasing the expression and phosphorylation (activity) of small stress protein Hsp27 [4]. We also have shown that mobile phone radiation-induced phosphorylation of Hsp27 has a physiological impact on cells. It induces phospho-Hsp27-dependent stabilization of stress fibers [3]. We have also shown that mobile phone radiation affects expression of the cytoskeletal protein vimentin, an intermediate filament protein [5].

In the present study, we have examined the effects of 900 MHz GSM mobile phone radiation on the gene and protein expression in two closely related human endothelial cell lines, EA.hy926 and EA.hy926v1. Our results show that gene and protein expression are altered in both cell lines in response to mobile phone radiation exposure. Furthermore, different genes and different proteins were affected by the same exposure in both cell lines. It suggests that, at least in

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Abbreviation: RF-EMF, radio-frequency electromagnetic field

the laboratory, the cell responses to mobile phone radiation seem to depend on the repertoire of genes and proteins that are expressed, and are also most likely active, in the cells at the time of the exposure.

2 Material and methods

2.1 Cells and culture conditions

Human endothelial cell line, EA.hy926 [6], was obtained from the laboratory of Dr. Cora-Jean S. Edgell (North Carolina University at Chapel Hill, NC, USA) in 1987 and has since been maintained at the University of Helsinki and at STUK. In 2000, the second batch of human endothelial cell line was obtained by STUK from Dr. Edgell's laboratory. This new batch of endothelial cells was generated in Dr. Edgell's laboratory by sub-cloning of the EA.hy926 cell line. To distinguish between these two different batches of cells, the cell line that has been obtained and maintained in Helsinki since 1987 is called EA.hy926. The batch of cells that was obtained in the year 2000, is called EA.hy926v1.

Both cell lines were grown in Dulbecco's MEM, supplemented with antibiotics, 10% fetal bovine serum, L-glutamine and HAT-supplement (Sigma, USA). For mobile phone radiation experiments, cells were removed from culture flasks by brief trypsinization, washed in cell culture medium and seeded at a density of 1.2×10^6 cells per 55 mm-diameter glass Petri dish (DURAN, Germany). After overnight culturing, the semi-confluent monolayers of EA.hy926 and EA.hy926v1 cells were exposed either to sham or to RF-EMF radiation. Cell cultures for sham and irradiation were prepared in similar glass dishes, derived from the same passage of cells, were seeded at the same cell density, and were grown for the same period of time before the exposure experiment. The only difference between sham and RF-EMF irradiated samples was that the irradiated cells have resided for 1 h in the irradiation chamber with RF-EMF radiation turned on whereas the sham cells resided in the irradiation chamber for the same period of time but with radiation turned off.

2.2 Cell cycle analysis

Cell cycle analysis was performed by determining the DNA content of cells with the standard propidium iodide staining method as described previously [7]. Briefly, cells were harvested with trypsin, washed with PBS and fixed in 90% alcohol v/v for 10 min. on ice. After fixation the cells were washed in PBS, suspended in RNase solution in PBS (100 units/mL), and incubated for 30 min. at 37°C. Afterwards, the propidium iodide solution (10 mg/mL) was added to the cells and the cells were incubated for 18 h at 4°C. After propidium iodide staining, cells were washed with PBS and analyzed with FACScan using the CellFit Cell-Cycle analysis software (Becton Dickinson, USA). The growth pattern and cell cycle distribution of both cell lines is shown in Fig. 1.

2.3 RF-EMF exposure system and exposure protocol

Cells were irradiated with a simulated mobile phone microwave radiation as previously described [4]. In all experiments reported here, cells were exposed for 1 h to a 900 MHz GSM signal at an average specific absorption rate of 2.8 W/kg. The specific absorption rate level for cell exposure was originally set at 2.0 W/kg because it is the safety limit for mobile phone emissions set by the International Commission on Non-Ionizing Radiation Protection. However, the improved measurements and simulations since our first experimental publication in 2002 [4], have shown that the specific absorption rate level to which cells were exposed was 2.8 W/kg. Importantly, the temperature of the cells and the culture medium had remained relatively constant throughout the one hour exposure period at $37 \pm 0.2^\circ\text{C}$ and therefore the effects observed by us are not caused by bulk heating of the cells.

2.4 Gene expression analysis by cDNA Expression Arrays

The total RNA was isolated from sham and RF-EMF exposed cells using the NucleoSpin RNA II kit according to the manufacturer's instructions (Clontech, USA). Briefly, semi-confluent cell cultures were lysed directly in the glass Petri dishes. RNA from cleared cell lysates was immobilized in spin columns provided by the NucleoSpin RNA II kit. After DNase treatment, the total RNA was eluted from the columns and analyzed for possible remaining DNA contamination by PCR using β -actin primers against genomic DNA [8]. The PCR products were run by agarose gel electrophoresis and only DNA free samples were used in Atlas™ Microarray procedure.

For the synthesis of cDNA probes and differential analysis of gene expression we used Atlas Pure Total RNA Labelling System (Clontech) and Atlas cDNA Expression Arrays (Clontech), respectively. The poly A⁺ RNA enrichment of 50 μg total RNA and ³²P-labelled cDNA probe synthesis made by reverse transcription were performed according to Atlas™ Pure Total RNA Labeling System (Clontech). The precisely same amount of ³²P-labelled cDNA from sham and RF-EMF exposed cells were used as a probe in Atlas™ Human 1.2 cDNA Expression Array, containing probes for 1167 genes immobilized on a nylon membrane. Hybridization and washing procedures were performed according to the recommendations of the membrane manufacturer. Briefly, the array membranes were prehybridized for 30 min at 68°C in ExpressHyb hybridization solution containing 0.1 mg/mL salmon testis DNA. The ³²P-labelled cDNA was added to the hybridization solution, the array membrane was hybridized overnight at 68°C and then next day array membranes were washed four times with SSC/1% SDS solutions. The filters were exposed at -70°C for 7–21 days in a manner such that similar signal intensity was produced from filters containing hybridized cDNA from both sham and RF-EMF exposed cells.

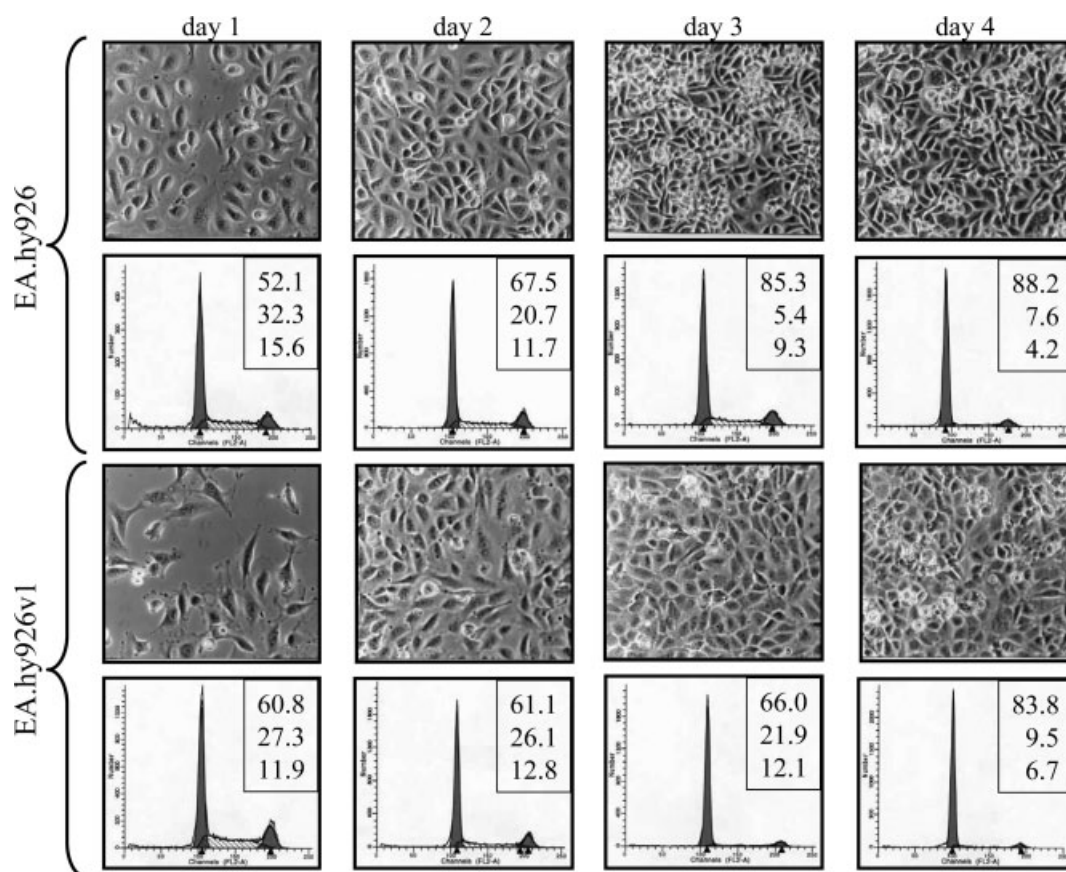


Figure 1. Growth pattern (cobble stone-like growth pattern of endothelial cells) and cell cycle distribution of EA.hy926 and EA.hy926v1 cell lines grown for 1–4 days. Cells were used for experiments on day one of the culture.

Hybridization signals on the autoradiograms were scanned by GS-710 Calibrating Imaging Densitometer (Bio-Rad, USA) and the intensity of gene spots was analyzed by Atlas-Image 2.0 Software (Clontech). After orientation and alignment of the two array membranes, array background was subtracted, and the intensity of the detected gene spots were adjusted by the normalization coefficient, which was calculated by software based on the intensity of total genes (the global normalization, sum method).

Each cDNA expression array experiment was repeated three times ($n = 3$) for each cell line using three different cultures of cells. As per cDNA Expression Array manufacturer's instructions, only genes whose adjusted intensity was higher than 3000 were selected for further analysis. After excluding genes that had adjusted intensity lower than 3000, the expression levels of the remaining genes were normalized to the average expression level of the nine housekeeping genes. Thereafter, the average $\pm$ SD for three replicates of sham and exposed genes was calculated for each cell line. Then the ratio between average exposed and average sham sample was calculated. For each cell line, as specified in the cDNA Expression Array manufacturer's instructions, only the genes which had a ratio

with at least 2-fold increase or 2-fold decline in expression level were included in the final table of the irradiation affected genes.

2.5 Protein expression analysis by 2-DE

Protein extraction was performed immediately after the end of the exposure. Petri dishes with cell cultures were placed on ice, washed with ice-cold PBS and lysed with buffer consisting of: 7 M urea, 2 M thiourea, 4% chaps, 2% IPG buffer pH 3–10 NL, 1% DTT, 1 mM sodium orthovanadate, and 1 mM PMSF. Protein concentration in lysates was measured using the Bradford method and 175 μ g of total protein was used for 2-DE.

Proteins were separated by 2-DE. The IEF was performed using an IPGphor apparatus and non-linear pH 3–10 18 cm long IEF strips (Amersham Biosciences, Sweden). The samples were loaded using in-gel rehydration in a buffer containing 9 M urea, 2% chaps, 0.2% DTT, 0.5% IPG buffer pH 3–10 NL for 12 h. IEF was run at 20°C using step-and-hold with gradient methods as follows: 30 V, 2 h; 100 V, 0.5 h; 300 V, 0.5 h; 600 V, 0.5 h; 1500 V, 0.5 h; 8000 V gradient 4 h; 8000 V, until the 65000 V·h were achieved. For SDS-PAGE the IEF strips were

equilibrated for 15 min with 6 M urea, 30% glycerol, 50 mM Tris-HCl, 2% SDS, and 10 mg/mL DTT for 15 min and then for another 15 min in the same buffer, but where 25 mg/mL iodoacetamide replaced DTT. SDS-PAGE was run in 8% gel using Protean IIXi Multicell apparatus (Bio-Rad) and a constant current of 40 mA/gel at 10°C. After electrophoresis, gels were fixed overnight with a mixture of 30% ethanol and 0.5% acetic acid, then washed with 20% ethanol in ddH₂O, sensitized with sodium thiosulfate (0.2 g/L), incubated in silver nitrate solution (2 g/L) and developed in a solution of potassium anhydride (30 g/L), 37% formaldehyde (0.7 mL/L) and sodium thiosulfate (0.01 g/L). The development was stopped with Tris (50 g/L) and acetic acid (0.5%) solution. Silver stained gels were stored in ddH₂O at 4°C. The gels were scanned using GS-710 densitometer (Bio-Rad) and analyzed using PDQuest 6.2 software (Bio-Rad). 2-DE artificial gels were generated from ten independent protein samples from ten independent replicates of sham and irradiated cell cultures. The protein expression pattern in ten replicate sham samples was then compared with the protein pattern in ten replicate irradiated samples. The normalized spot volumes of the proteins from sham and exposed sample gels were statistically analyzed using student *t*-test at the confidence level of 95%.

3 Results

3.1 Effect of RF-EMF on gene expression

The expression of 1167 genes was analyzed in EA.hy926 and EA.hy926v1 cells. After disregarding all genes that had expression levels below 3000, we compared effects of RF-EMF exposure on the expression of 136 genes in both cell lines.

Mobile phone radiation exposure had caused changes in the expression level of numerous genes in both cell lines. The comparison between the genes affected in each of the used cell lines revealed that both cell lines responded differently to the same RF-EMF exposure. It appeared that RF-EMF exposure had caused a predominant decline in gene expression in EA.hy926 cells whereas in EA.hy926v1 cell line it caused a predominant increase in gene expression. Most strikingly, the majority of genes that were found to be down-regulated in EA.hy926 cell line were either up-regulated or not affected in the EA.hy926v1 cell line (Table 1).

In EA.hy926 cell line, four genes were found that had increased expression levels by more than 2-fold (ratio ≥ 2.0 ; red color bold numbers in Table 1). Of these, one gene (cell surface glycoprotein MUC18; code A08g) had also increased expression level in EA.hy926v1 but the increase was much more dramatic (≥ 10 -fold). The other three genes had too low expression levels in the EA.hy926v1 cell line to be considered.

In the EA.hy926v1 cell line, 61 genes were found that had increased expression levels by more than 2-fold (ratio ≥ 2.0 ; red color bold numbers in Table 1). Of these, only one gene displayed up-regulated expression levels in EA.hy926 cells—

i.e. the cell surface glycoprotein MUC18. The 19 genes, that were up-regulated 2-fold or more in EA.hy926v1 cells, were down-regulated 2-fold or more in EA.hy926 cells. For 41 genes that were up-regulated in EA.hy926v1, the expression level of the same genes in EA.hy926 cells was either not accounted for *i.e.* too low expression level to be considered; < 3000 , or their up- or down-regulation was less than 2-fold.

In the EA.hy926 cell line, 89 genes were found where expression levels had been down-regulated by the exposure by 2-fold or more (ratio ≤ 0.5 ; blue color bold numbers in Table 1). Of these, the only expression level of one gene that encodes homeobox B7 protein had declined in both cell lines (Table 1; code E04a). The expression levels of 19 genes, which had declined in EA.hy926 cells, were found to increase in EA.hy926v1 cells. For 69 genes, that were down-regulated in EA.hy926 cell line, the expression levels of the same genes in EA.hy926v1 cells were either not accounted for *i.e.* too low expression level to be considered; < 3000 , or their up- or down-regulation was less than 2-fold.

Statistical significance of the changes in gene expression levels in three experiments was analyzed using student's *t*-test (Table 1; purple color italic numbers). Only one gene expression change (serine/threonine protein phosphatase; Table 1; code C12a) was statistically significant in the EA.hy926 cell line. In the EA.hy926v1 cell line there were 13 genes with statistically significant change in expression (Table 1; codes: A07d, A09b, A12l, A13l, B08m, B10m, C05k, D08i, D14j, E12n, F07b, F07e, and F10e). For clarity, the summary of the statistically significantly affected genes in both cell lines is shown in Table 2.

3.2 Effect of RF-EMF on protein expression

Protein expression in EA.hy926 and EA.hy926v1 cells was analyzed using 2-DE. The effect of mobile phone radiation exposure on the EA.hy926 cell line was shown by us previously [5]. Here we have made the comparison between this cell line and the EA.hy926v1 cell line. The comparison has revealed that the protein expression profiles in both cell lines are different, in spite of the closely related origin of the cell lines (Fig. 2). Only approximately half of all of the protein spots could be matched confidently between the cell lines. This indicates that, in each cell line different proteins were active at the time of irradiation. Since mobile phone radiation induced changes in gene expression, it was expected that also protein expression would be affected. Indeed, the comparison of the sham and mobile phone radiation-exposed samples (ten experiments for each cell line for each treatment) had shown that several tens of protein spots had altered their expression level ($p < 0.05$). In the EA.hy926 cell line there were 38 protein spots with altered expression level due to radiation exposure (Fig. 2A–B) whereas in the EA.hy926v1 cell line there were 45 differentially expressed protein spots (Fig. 2C–D). Similarly, as was the case with gene expression changes, also different proteins were affected by the exposure in both cell lines.

Table 1. List of genes that in response to mobile phone radiation, increased (red) or decreased (blue) expression in EA.hy 926 cells. The effect of the exposure on the same genes in EA.hy926v1 cells is shown in parallel. Empty fields indicate that for one of the cell lines, the data were not valid for the analysis (the difference between gene expression in sham and exposed cells was less than 3000 density units).

Code ^{a)}	GENE NAME	EA.hy 926 cells				EA.hy 926v1 cells			
		Sham ^{b)} average ± SD	Exposed ^{b)} average ± SD	Ratio ^{c)} exposed/sham	t-test ^{d)}	Sham ^{b)} average ± SD	Exposed ^{b)} average ± SD	Ratio ^{c)} exposed/sham	t-test ^{d)}
A01g	Transforming protein rhoA H12	4.16	4.66	1.32	0.05	0.32	0.402	0.32	0.402
A01f	Vascular endothelial growth factor receptor 1					0.03	0.11	0.13	0.404
A02h	G2/mitotic-specific cyclin B1 (CCNB1)	1.09	0.57	0.83	0.24	0.04	0.71	0.62	0.201
A02i	Cell division control protein 2 homolog (CDC2)					0.02	0.49	0.47	0.225
A02j	Serine/threonine-protein kinase PLK1 (STPK13)	0.52	0.22	0.22	0.38	0.06	0.04	0.41	0.196
A03b	EB1 protein					0.14	0.13	0.21	0.130
A03i	Prothymosin alpha (ProT-alpha; PTMA)	6.78	9.04	1.38	0.06	1.67	1.54	0.07	0.593
A04b	Ezrin; cyto villin 2; villin 2 (VIL2)	0.52	0.30	0.43	0.08	0.10	0.34	0.17	0.126
A04i	Cell division protein kinase 4	0.18	0.14	0.14	0.24	0.18	0.08	0.14	0.536
A05e	Tyrosine-protein kinase receptor UFO precursor					0.34	0.50	0.72	0.354
A06e	fms proto-oncogene (c-fms)					0.03	0.13	0.15	0.359
A07d	fos-related antigen (FRA1)	3.25	3.06	1.33	0.04	0.41	0.21	1.48	0.07
A07k	Cyclin-dependent kinase inhibitor 1 (CDKN1A)	0.39	0.35	0.00	0.00	0.23	0.24	0.21	0.103
A07l	40S ribosomal protein S19 (RPS19)	4.92	5.86	1.34	0.07	0.76	1.47	1.12	0.074
A08d	v-erbA related protein (EAR2)					0.03	0.04	0.11	0.334
A08g	Cell surface glycoprotein MUC18	0.24	0.22	0.49	0.02	0.04	0.03	0.41	0.236
A08k	Cyclin-dependent kinase inhibitor 3	1.05	0.44	0.23	0.40	0.06	0.02	0.33	0.226
A08n	Chloride conductance regulatory protein ICLN	0.36	0.13	0.74	0.18	0.09	0.25	1.54	0.07
A09b	c-myc purine-binding transcription factor puf	6.81	9.10	1.37	0.06	0.20	0.39	1.28	0.21
A09h	fte-1	4.46	5.06	1.34	0.04	0.30	0.39	1.28	0.21
A09i	Proliferating cell nuclear antigen P120	0.52	0.38	0.88	0.14	1.69	0.02	0.30	0.316
A10b	Nucleoside diphosphate kinase A	2.96	3.13	1.18	0.11	0.40	0.41	0.42	0.273
A10g	T-lymphoma invasion and metastasis inducing					0.05	0.04	0.41	0.42
A12e	ERBB-3 receptor protein-tyrosine kinase precursor	0.65	0.61	0.24	0.10	0.36	0.60	0.62	0.022
A12i	Transducer of erbB2 (TOB)	0.00	0.00	0.15	0.13	DIV/0	0.189	0.19	0.312
A13g	Cyclin K					0.07	0.09	0.62	0.021
A13j	Cyclin-dependent kinase regulatory subunit 1	1.83	1.45	0.70	0.41	0.38	0.309	0.13	0.021
A13l	p55CDC	3.80	4.35	1.08	0.18	0.28	0.21	1.00	0.014
A14j	Cyclin-dependent kinase regulatory subunit 2					0.08	0.10	0.63	0.151
B02n	ras-related protein RAP-1B	0.68	0.37	0.47	0.21	0.68	0.440	0.43	0.163
B03n	ras-related protein RAB2	0.97	0.39	0.88	0.26	0.90	0.741	0.13	0.102
B05j	c-jun N-terminal kinase 2 (JNK2)	1.93	2.15	0.35	0.39	0.18	0.329	0.21	0.021
B06d	Thrombin receptor	1.80	1.93	0.66	0.10	0.36	0.413	0.27	0.014
B06h	MAPKAP kinase	0.09	0.15	0.01	0.01	0.07	0.450	0.24	0.151
B06k	Serine kinase	0.13	0.13	0.01	0.01	0.04	0.241	0.13	0.163
B07h	Mitogen-activated protein kinase p38 (p38 MAP Kinase)	2.01	2.57	0.47	0.23	0.24	0.409	0.23	0.102

Table 1. Continued

Code ^{a)}	GENE NAME	EA.hy 926 cells				EA.hy 926v1 cells			
		Sham ^{b)} average ± SD	Exposed ^{b)} average ± SD	Ratio ^{c)} exposed/sham	t-test ^{d)} sham	Sham ^{b)} average ± SD	Exposed ^{b)} average ± SD	Ratio ^{c)} exposed/sham	t-test ^{d)}
B07m	Ral A; GTP-binding protein	0.33	0.30	0.64	0.26	1.95	0.243		
B08m	Transforming protein rhoB								
B10m	ras-related protein RAB-7	2.43	1.88	0.73	0.53	0.30	0.253	0.30	0.243
B13m	ras-related C3 botulinum toxin substrate 1	1.07	1.18	0.23	0.31	0.21	0.343	0.21	0.343
B14d	Ephrin type-A receptor 1 precursor	3.38	4.30	0.68	0.34	0.20	0.391	0.20	0.391
C01e	PKC inhibitor protein-1 (KCIP-1)	1.03	1.05	0.67	0.33	0.65	0.624	0.65	0.624
C02e	PKC substrate 80-kDa protein heavy chain	4.61	6.42	1.05	0.49	0.23	0.437	0.23	0.437
C02j	CAD; DNA fragmentation factor 40-kDa subunit	0.20	0.25	0.00	0.00	0.00	0.295	0.00	0.295
C02k	Inducible nitric oxide synthase (iNOS)	0.28	0.34	0.00	0.00	0.01	0.300	0.01	0.300
C03f	SH3P18 SH3 domain-containing protein								
C03k	Defender against cell death 1 (DAD1)								
C04e	Hint protein; PKC inhibitor 1 (PKCI1)	2.73	3.77	0.78	0.30	0.29	0.466	0.29	0.466
C05a	Leukocyte surface CD47 antigen precursor	0.52	0.42	0.06	0.05	0.11	0.193	0.11	0.193
C05e	macMARCKS; MARCKS-related protein	0.47	0.54	0.07	0.12	0.15	0.326	0.15	0.326
C05h	Caspase-3 (CASP3)	0.74	0.99	0.14	0.13	0.19	0.404	0.19	0.404
C05k	Cytoplasmic dynein light chain 1 (HDLC1)								
C06h	Caspase-4 precursor (CASP4)	5.52	7.25	1.09	0.38	0.20	0.401	0.20	0.401
C06k	Cytochrome P450 reductase	0.79	1.17	0.18	0.30	0.22	0.465	0.22	0.465
C06l	Proliferating cyclic nuclear antigen (PCNA); cyclin	6.02	8.90	1.26	0.16	0.21	0.452	0.21	0.452
C06n	mutL protein homolog; DNA mismatch repair protein	6.66	9.26	1.37	0.06	0.21	0.427	0.21	0.427
C08e	Tuberlin	1.18	1.13	0.48	0.43	0.41	0.402	0.41	0.402
C09d	rho GDP dissociation inhibitor 1	3.02	3.91	0.64	0.58	0.21	0.404	0.21	0.404
C09g	Adenosine A1 receptor (ADORA1)	0.06	0.10	0.27	0.29	4.66	0.334		
C09h	Caspase-8 precursor (CASP8)	0.20	0.26	0.04	0.06	0.19	0.397	0.19	0.397
C11h	Caspase-10 precursor (CASP10)								
C11k	ALG-2 calcium-binding protein	1.15	1.34	0.81	0.24	0.70	0.706	0.70	0.706
C12a	Serine/threonine protein phosphatase PPI-alpha 1 catalytic subunit	1.16	0.18	0.46	0.19	0.40	0.010	0.40	0.010
C12d	Cortactin; amplexin; ems-1 oncogene	3.20	3.92	0.89	0.23	0.28	0.416	0.28	0.416
C13a	Dual-specificity protein phosphatase 2	0.05	0.09	0.01	0.01	0.12	0.472	0.12	0.472
C14h	Calpain 2 large (catalytic) subunit	1.72	2.16	0.49	0.43	0.28	0.428	0.28	0.428
D02g	Glial growth factor 2 precursor	0.95	0.49	0.85	0.33	0.89	0.786	0.89	0.786
D02j	Hepatic leukemia factor (HLF)	0.46	0.69	0.01	0.01	0.01	0.375	0.01	0.375
D02n	60S ribosomal protein L6 (RPL6)	6.82	9.12	1.36	0.07	0.20	0.408	0.20	0.408
D03i	Myelin-oligodendrocyte glycoprotein precursor (MOG)	0.66	0.86	0.08	0.14	0.12	0.361	0.12	0.361
D03k	YL-1 protein	0.37	0.48	0.12	0.21	0.33	0.482	0.33	0.482
D04a	HHR23A; UV excision repair prot. RAD23A	0.91	0.08	0.96	0.10	1.05	0.538	1.05	0.538
D05a	Ubiquitin-conjugating enzyme E2 17-kDa (UBE2A)	2.85	2.53	1.26	0.05	0.44	0.389	0.44	0.389
D05l	ets-related gene transforming protein (ERG1)	0.31	0.37	0.07	0.12	0.22	0.371	0.22	0.371

Table 1. Continued

Code ^{a)}	GENE NAME	EA.hy 926 cells				EA.hy 926v1 cells			
		Sham ^{b)} average ± SD	Exposed ^{b)} average ± SD	Ratio ^{c)} exposed/sham	t-test ^{d)}	Sham ^{b)} average ± SD	Exposed ^{b)} average ± SD	Ratio ^{c)} exposed/sham	t-test ^{d)}
				↑	↓			↑	↓
D07n	cAMP-responsive element-binding protein (CREB1)	0.34	0.22	0.13	0.22	0.16	0.28	0.18	0.357
D08j	Major prion protein precursor (PRP)	3.05	2.86	1.32	0.04	1.10	1.44	0.07	0.016
D09f	acyl-CoA-binding protein	4.67	5.47	1.36	0.05	0.86	1.50	0.05	0.061
D09i	Alzheimer's disease amyloid A4 protein precursor	3.24	3.39	1.20	0.12				
D09n	Transcription factor E4TF1-60	0.26	0.43	0.09	0.16				
D14a	Deoxyribonuclease II (DNase II)	0.46	0.17	0.56	0.16	0.14	0.16	0.18	0.357
D14j	Activated RNA polymerase II transcriptional coactivator p15	6.01	7.79	1.35	0.08	0.30	0.34	0.45	0.044
E01l	Interleukin-2 receptor alpha subunit precursor	2.24	2.64	0.81	0.11	0.11	0.09	0.11	0.710
E02e	cAMP-dependent transcription factor ATF-4	5.27	6.48	1.36	0.05	0.40	0.47	0.18	0.061
E03d	Transcription initiation factor TFIIID	1.04	0.62	0.70	0.17	0.13	0.19	0.33	0.207
E03f	DNA-binding protein A	3.97	4.44	1.09	0.17	0.27	0.377		
E03g	Cadherin 5 (CDH5); vascular endothelial cadherin (VE-cadherin)	2.78	2.74	1.23	0.12	0.60	0.37	0.38	0.278
E03k	Neurexin B receptor (NMBR)	2.10	2.01	0.70	0.31	0.25	0.25	0.00	0.229
E04a	Homeobox B7 protein HOXB7	1.56	1.93	0.77	0.67	1.58	0.30	0.07	0.844
E04b	Nuclease-sensitive element DNA-binding protein (NSEP)	6.61	8.76	1.37	0.04	0.07	0.03	0.25	0.380
E05h	NADH-ubiquinone oxidoreductase B18	0.32	0.31	0.06	0.11	0.17	0.26	0.84	0.114
E06f	Polyadenylate binding protein-interacting protein 1 (PAIP1)	1.75	1.01	1.10	0.09	0.63	0.380		
E07b	fli-1 oncogene; ergB transcription factor	0.31	0.30	0.05	0.09	0.17	0.272		
E08b	Paired box protein PAX-5; B-cell specific transcription factor	6.08	7.91	1.36	0.05	1.25	0.23	1.54	0.149
E08e	Guanine nucleotide-binding protein Gα-subunit	3.68	4.06	1.31	0.04	0.22	0.410		
E08f	High mobility group protein (HMG-I)					0.36	0.418		
E09b	Special AT-rich sequence binding protein 1	2.01	2.07	0.63	0.36	0.13	0.21	0.36	0.421
E09i	Fibronectin receptor beta subunit (FNBR)	0.13	0.16	0.62	0.56				
E10a	Early growth response protein 1 (hEGRI)	0.89	0.87	0.43	0.11				
E10i	Integrin alpha 6 precursor								
E11a	Transcription factor ETR101					0.32	0.371		
E11d	Purine-rich single-stranded DNA-binding-α					4.66			
E11i	Integrin beta 4 (ITGB4); CD104 antigen					0.48			
E11n	Microsomal glutathione S-transferase 12	6.40	8.44	1.31	0.08	0.21	0.406		
E12e	Nucleobindin precursor (NUC)	1.18	1.56	0.31	0.09	0.27	0.435		
E12n	Glutathione S-transferase pi (GSTP1; GSTT3)	6.53	8.63	1.37	0.05	0.21	0.410		
E13g	alpha1 catenin (CTNNA1);								
E14a	Transcriptional repressor prot. yin & yang 1					0.11	0.08	0.27	0.10
E14b	FUSE binding protein					0.05	0.05	0.28	0.22
E14i	Leukocyte adhesion glycoprotein LFA-11α	0.74	0.76	0.56	0.21	0.01	0.01	0.18	0.280
F01c	MPV17 protein	0.37	0.38	0.04	0.08	0.21	0.30	1.43	0.006
F01f	Insulin-like growth factor binding protein 1	0.91	1.19	0.22	0.02				
F01l	Proteasome component C2	1.72	1.68	0.86	0.16				

Table 1. Continued

Code ^{a)}	GENE NAME	EA.hy 926 cells				EA.hy 926v1 cells			
		Sham ^{b)} average ± SD	Exposed ^{b)} average ± SD	Ratio ^{c)} exposed/sham	t-test ^{d)}	Sham ^{b)} average ± SD	Exposed ^{b)} average ± SD	Ratio ^{c)} exposed/sham	t-test ^{d)}
F02a	Heat shock cognate 71-kDa protein					0.06	0.10	0.94	0.55
F02b	Heat-shock protein 40 (HSP40)					0.13	0.20	0.49	0.44
F02f	Vascular endothelial growth factor precursor								
F02f	Proteasome component C3;	0.45	0.59	0.00	0.01				
F03b	Heat shock protein 60 (HSP-60)	3.25	3.67	1.14	0.17				
F03e	Macrophage-specific colony-stimulating factor (CSF-1; MCSF)					0.03	0.04	0.52	0.51
F03f	Proteasome component C5	4.29	5.30	0.83	0.25				
F04b	Heat shock 90-kDa protein A (HSP90A)	3.17	3.22	1.05	0.25				
F04k	Heregulin-beta3; glial growth factor; neuregulin	6.06	7.98	1.33	0.08	0.39	0.68	1.53	0.07
F04f	Proteasome component C8	1.38	1.28	0.36	0.63				
F05b	27-kDa heat-shock protein (HSP27)	4.99	6.25	1.28	0.11	0.12	0.17	1.15	0.58
F05e	Hepatoma-derived growth factor (HDGF)	6.82	9.11	1.38	0.05	1.68	0.37	1.55	0.07
F05k	alpha-1-antitrypsin precursor	1.35	1.42	0.61	0.26				
F06c	Eosinophil granule major basic protein precursor	0.32	0.47	0.00	0.01	0.02	0.02	0.11	0.17
F07b	Cytosolic superoxide dismutase 1 (SOD1)	5.38	7.02	0.90	0.40				
F07e	Neuroleukin (NLK)	3.00	3.40	1.09	0.17	0.16	0.20	1.15	0.18
F08a	Thioredoxin peroxidase 2 (TDPX2)	4.94	6.77	0.81	0.24	0.15	0.20	0.92	0.29
F10e	T-cell-specific rantes protein precursor	0.36	0.37	0.12	0.08				
F10f	Embryonic growth/differentiation factor 1					0.15	0.15	1.24	0.49
F10i	Interleukin-1 beta precursor (IL-1; IL1B); catabolin	2.06	2.21	0.88	0.09	0.04	0.02	0.11	0.13
F13b	Glutathione S-transferase mu1 (GSTM1; GST1)	0.53	0.70	0.09	0.08	0.10	0.01	0.19	0.22
F13f	Thymosin beta-10	6.83	9.12	1.38	0.06	1.69	0.39	1.54	0.07
F13j	Thymosin beta 4	6.82	9.10	1.38	0.06				
F14b	Glutathione S-transferase A1	0.69	0.85	0.12	0.15				

a) Gene code according to the location on cDNA Array

b) Genes in bold were considered as down- or up-regulated as their ratio of exposed/sham was either ≥ 2 or ≤ 0.5 c) Ratios in bold were either ≥ 2 (red; increase in expression) or ≤ 0.5 (blue; decline in expression)d) Purple figures in bold were statistically significant changes ≤ 0.05

Table 2. List of genes that in response to mobile phone radiation, have statistically significant altered expression.

Cell Line	Code ^{a)}	GENE NAME	Sham average $\pm$ SD		Exposed average $\pm$ SD		Ratio exposed/sham $\uparrow$ $\downarrow$		<i>t</i> -test	Potential targets of RF-EMF
EA.hy926	C12a	Serine/threonine protein phosphatase	1.16	0.18	0.46	0.19		0.40	0.010	yes
EA.hy926v1	A07d	fos-related antigen (FRA1)	0.99	0.21	1.48	0.07	1.49		0.043	?
	A09b	c-myc purine-binding transcription factor puf	0.97	0.25	1.54	0.07	1.59		0.052	?
	A12l	Transducer of erbB2 (TOB)	0.07	0.09	0.62	0.19	8.52		0.022	yes
	A13l	p55CDC	0.15	0.21	1.00	0.27	6.57		0.014	yes
	B08m	Transforming protein rhoB	0.07	0.13	0.45	0.17	6.19		0.040	yes
	B10m	ras-related protein RAB-7	0.16	0.26	1.25	0.26	7.63		0.007	yes
	C05k	Cytoplasmic dynein light chain 1 (HDLC1)	0.32	0.24	1.23	0.25	3.88		0.010	yes
	D08i	Major prion protein precursor (PRP)	1.10	0.11	1.44	0.07	1.30		0.016	?
	D14j	RNA polymerase II transcriptional coactivator p15	0.30	0.34	1.28	0.45	4.25		0.044	yes
	E12n	Glutathione S-transferase pi (GSTP1; GST3)	1.23	0.15	1.55	0.07	1.25		0.050	?
	F07b	Cytosolic superoxide dismutase 1 (SOD1)	0.16	0.20	1.15	0.18	7.23		0.003	yes
	F07e	Neuroleukin (NLK)	0.15	0.20	0.92	0.29	5.96		0.024	yes
	F10e	T-cell-specific rantes protein precursor	0.15	0.15	1.24	0.49	8.46		0.050	yes

a) Gene code according to the location on cDNA array

4 Discussion

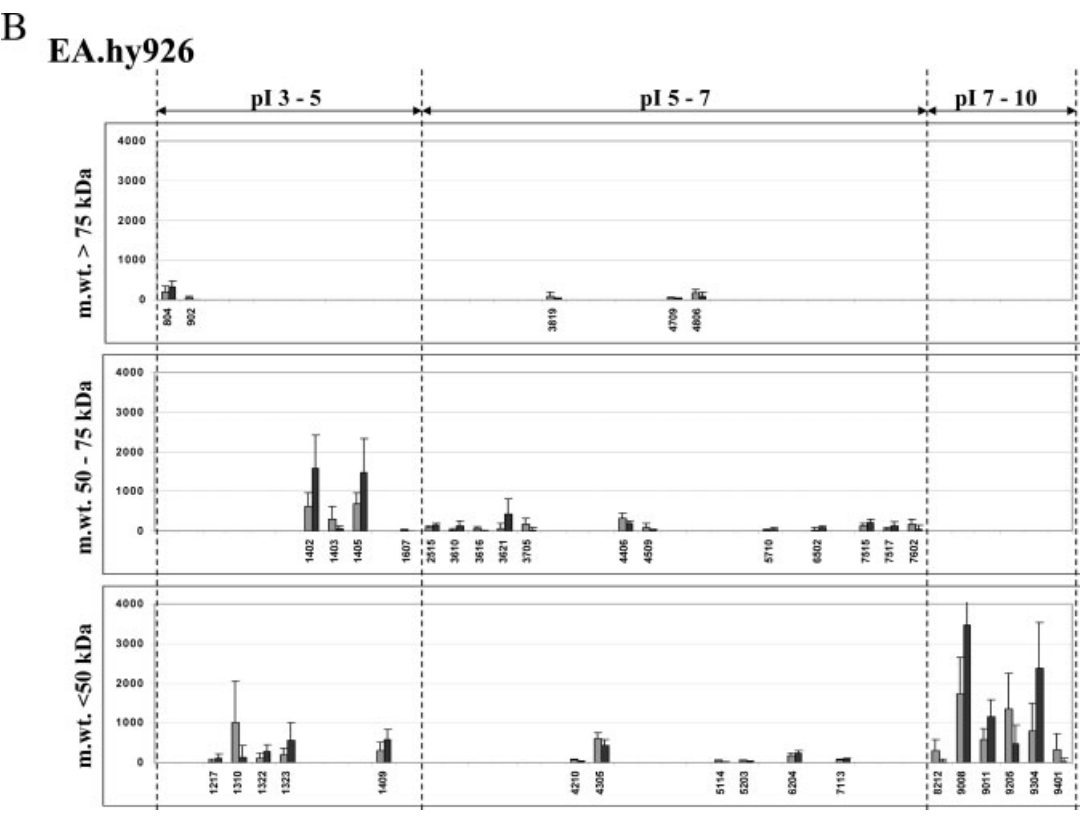
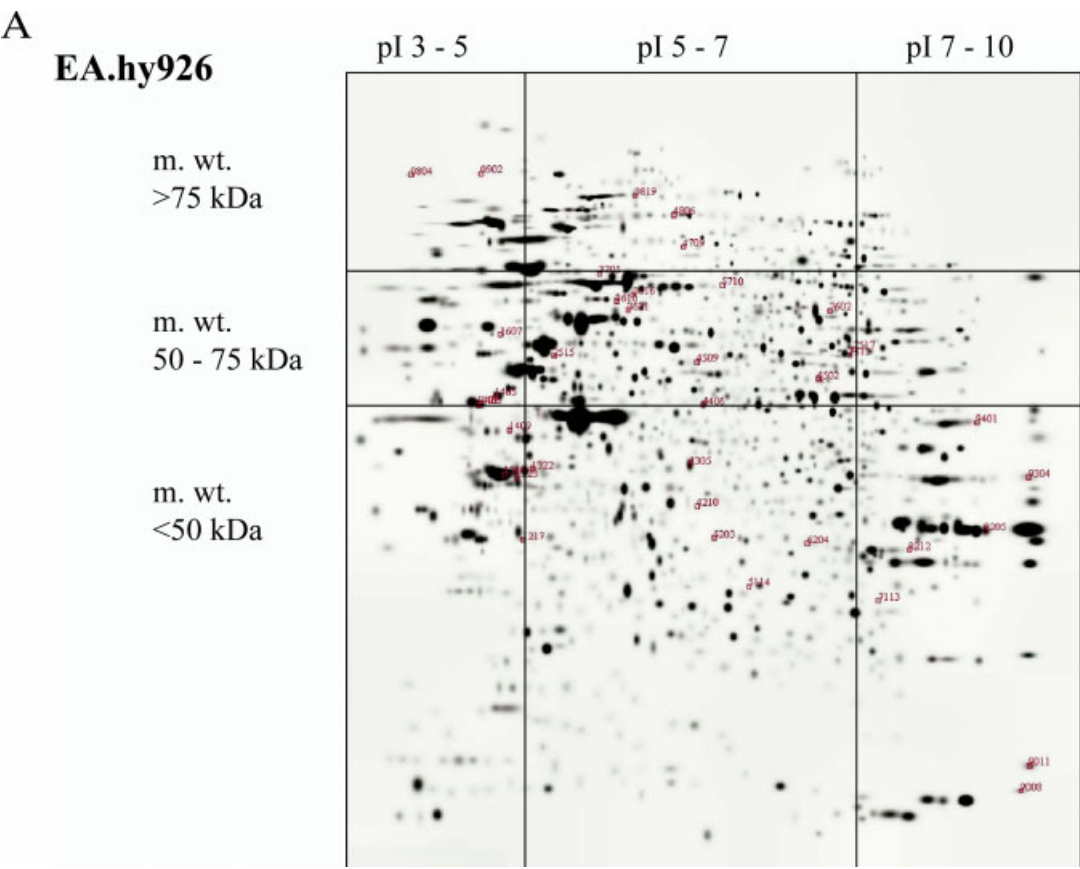
In spite of years of research, there is still an uncertainty about whether low-energy EMF can induce biological effects that would be able to alter cell physiology. Importantly, even when biological effects have been reported, the biophysical mechanisms behind their occurrence are still unknown. We have postulated that the combined use of the genome-wide and proteome-wide screening techniques of proteomics and transcriptomics (the so-called “Discovery Science” approach; [9]) will be a useful approach to determine a broad variety of biological targets of RF-EMF on the sub-cellular level, both *in vitro* and *in vivo*. These targets could then be used to formulate new health-related hypotheses for testing in animal and human-volunteer studies.

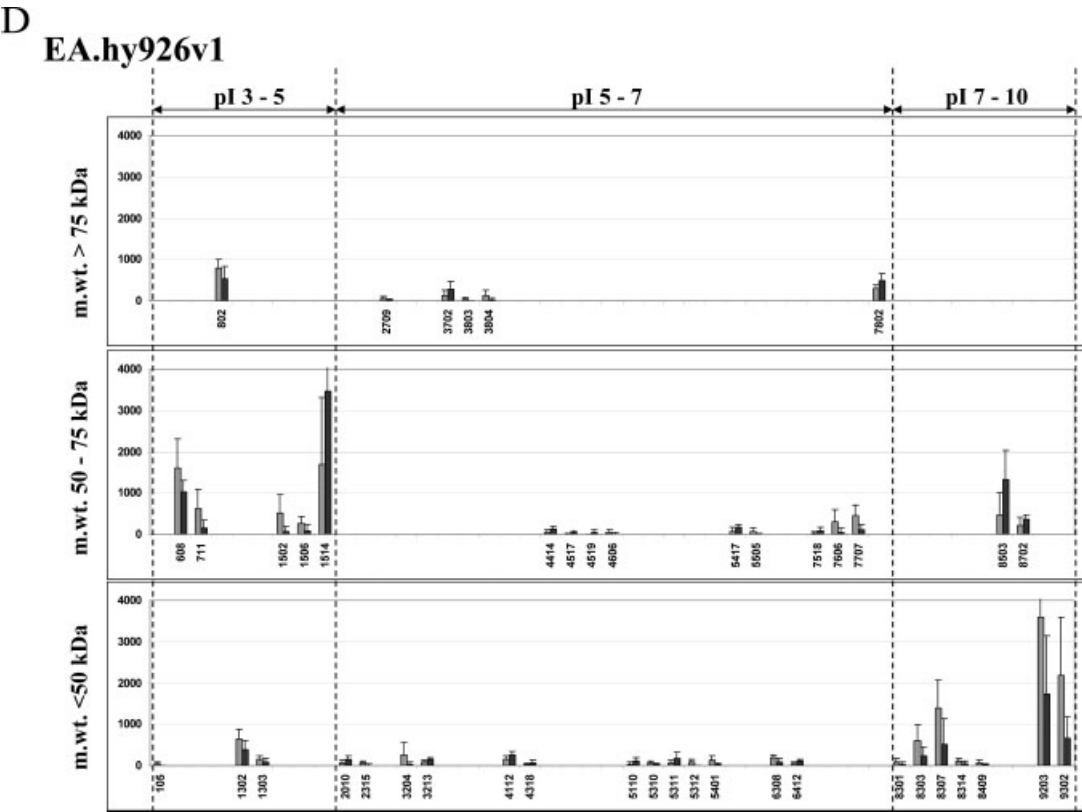
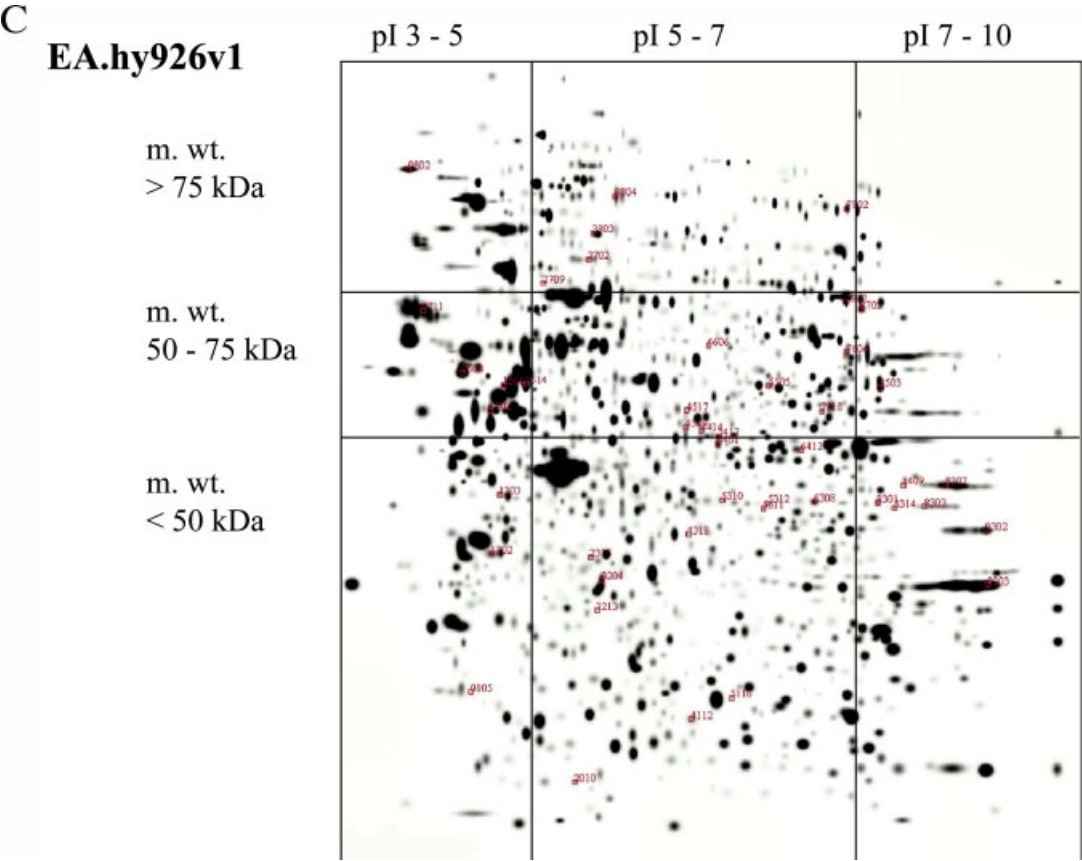
Search through publication databases shows that only very few studies have used methods of transcriptomics and proteomics to determine gene or protein responses to EMF exposure. Only four studies from two research groups have used this approach to examine effects of mobile phone radiation exposure [3–5, 12, 13]. Few of the studies that examined effects of electromagnetic fields have presented data obtained using the transcriptomics examined changes in the protein approach [10–15] and few have used the proteomics approach [3–5, 14,

16, 17]. Interestingly, only in two proteomics studies [4, 16] activity by determining changes in phospho-proteome. Altogether, the number of published studies is still very low and the data is insufficient to make any generalizing conclusions as to the biophysical and cell-wide biological impact of EMF radiation (including mobile phones radiation) on living organisms. It is also necessary to remember that some of the published studies, most likely due to limited funding, either suffer from technical problems or are based on a single experiment, which significantly diminishes their scientific value.

We have previously shown that exposure of EA.hy926 human endothelial cell line to 900 MHz mobile phone radiation induces activation of the p38 MAP kinase stress response pathway and leads to an increase in expression and phosphorylation of the small stress response protein Hsp27 [4]. We have also shown that cells' response to the mobile phone radiation affects components of the cytoskeleton, the F-actin stress fibers [3–5], that are stabilized in the presence of phosphorylated Hsp27. In other studies, it has been shown that the phosphorylated form of Hsp27 has the ability to translocate to the nucleus and to induce changes in gene expression [18, 19]. Thus, we examined whether the mobile phone radiation exposure, that induces Hsp27 phosphorylation, will cause changes in gene expression and, subsequently, changes in protein expression.

Figure 2. Effect of mobile phone radiation on protein expression in EA.hy926 and EA.hy926v1 cell lines. Each result is a compilation of ten different experiments. (A): PDQuest-generated 2-DE artificial gel of proteins extracted from human endothelial cell line EA.hy926. The 1-D IEF pH gradient 3–10 NL, 2-D 8% SDS-PAGE. Statistically significantly (*t*-test $p < 0.05$; $n = 10$) differentially expressed spots are numbered using PDQuest SSP numbers. In EA.hy926 cell line 38 statistically significantly differing spots were detected (numbered). Corresponding bar graphs of the statistically significantly changed protein spots in EA.hy926 cells are shown in (B). (C): PDQuest-generated 2-DE artificial gel of proteins extracted from human endothelial cell line EA.hy926v1. The 1-D IEF pH gradient 3–10 NL, 2-D 8% SDS-PAGE. Statistically significantly (*t*-test $p < 0.05$; $n = 10$) differentially expressed spots are numbered using PDQuest SSP numbers. In EA.hy926v1 cell line were detected 45 statistically significantly differing spots (numbered). Corresponding bar graphs of the statistically significantly changed protein spots in EA.hy926v1 cells are shown in (D).





The results of our study suggest that mobile phone radiation alters expression levels of numerous genes and proteins in both of the examined human endothelial cell lines. The broadness of the response suggests that a variety of cellular functions and cellular signaling pathways might be affected by the mobile phone radiation exposure. This observation agrees with our previously published study showing the changes in phosphorylation levels of hundreds of proteins, which were induced by mobile phone radiation exposure [4]. Also, our results are in agreement with the earlier published study suggesting that mobile phone radiation exposure affects gene expression in human fibroblasts [12].

Another observation of this study is that both human endothelial cell lines have responded differently to the same exposure. This opens the possibility to hypothesize that cells, depending on their origin, functions and metabolic status at the time of exposure, might respond differently to the same mobile phone radiation exposure. As shown here, different genes and different proteins responded to the mobile phone radiation in both endothelial cell lines used in our study. At the present, we do not know which of the genes and proteins might be responsible for the observed diversity of the response. Interestingly, expression of p38 MAP kinase gene is 10-fold higher in EA.hy926 cell line than in the EA.hy926v1 cell line (data not shown). We have shown previously that p38 MAP kinase protein is activated by the RF-EMF exposure [3, 4]. Whether the 10-fold difference in p38 MAP kinase gene expression between both cell lines has any influence on the observed difference in the gene and protein response between the examined human endothelial cell lines remains to be determined.

In conclusion, our results suggest that the mobile phone radiation causes broad changes in gene and protein expression *in vitro*. The difference in response observed between closely related cells suggests the possibility that the genome and proteome expressed by the cells at the time of exposure to mobile phone radiation might play a role in cellular responses. This observation also suggests that the genome- and proteome-dependent differences in cell response to mobile phone radiation might be, at least in part, responsible for the failures of replication experiments in different laboratories. It might be that the same cell lines, when grown in different laboratories for extended periods of time, spontaneously acquire new properties that are responsible for the different behavior of cells under exposure to mobile phone radiation. Finally, the hypothesis of the possible link between cell genome and proteome, and the cellular response to mobile phone radiation supports the possibility that some cell types or cells expressing certain pattern of genes and/or proteins might be in some cases more sensitive and in some cases less sensitive to the mobile phone radiation. Such possibility might not be surprising, as it is well known that genotypically different cells might respond differently to the same stimulus, e.g. ionizing radiation, chemicals, carcinogens, drugs *etc.*, for reviews see [20–23].

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