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Analysis of Gene Expression in a Human-derived Glial Cell Line Exposed to 2.45 GHz Continuous Radiofrequency Electromagnetic Fields

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Radiofrequency electromagnetic fields/Human-derived cells/DNA microarray/Polymerase chain reaction.

The increasing use of mobile phones has aroused public concern regarding the potential health risks of radiofrequency (RF) fields. We investigated the effects of exposure to RF fields (2.45 GHz, continuous wave) at specific absorption rate (SAR) of 1, 5, and 10 W/kg for 1, 4, and 24 h on gene expression in a normal human glial cell line, SVGp12, using DNA microarray. Microarray analysis revealed 23 assigned gene spots and 5 non-assigned gene spots as prospective altered gene spots. Twenty-two genes out of the 23 assigned gene spots were further analyzed by reverse transcription-polymerase chain reaction to validate the results of microarray, and no significant alterations in gene expression were observed. Under the experimental conditions used in this study, we found no evidence that exposure to RF fields affected gene expression in SVGp12 cells.

INTRODUCTION

The increasing use of mobile phones has aroused public concerns regarding potential health risks of radiofrequency (RF) fields. Many studies have been conducted to elucidate the effects of RF fields at frequencies around 900 MHz and 1.8 GHz, which are typical of Global System for Mobile Telecommunications (GSM) signals, and at around 2.1 GHz, which is typical of the third generation systems.[1)] In cellular investigations, while a few studies have demonstrated adverse effects of RF exposure,[2,3)] most studies have not provided consistent evidence of adverse biological effects under non-thermal RF exposure conditions.[4–6)] Overall, it is critical to resolve these discrepancies.

Studies evaluating the effects of exposure to RF fields on the expression of specific genes, such as heat shock proteins (HSPs) and proto-oncogenes, have also been performed as a basis for understanding the molecular changes. The expression of HSP70, HSP27, HSP90, c-fos, c-jun and c-myc mRNA and/or protein has been investigated using many kinds of cells and animals under various RF exposure conditions.[7)] The majority of these investigations reported no effects,[8–11)] however some reports demonstrated increases in HSP and c-fos expression following RF exposure.[12–15)]

In the past decade, powerful high-throughput screening techniques of transcriptomic and/or proteomic have been used to study the effects of exposure to RF fields. These techniques allow for the simultaneous screening of the expression of thousands of genes and/or proteins.[7,16)] Some studies demonstrated differences of gene expression following exposure to RF fields.[17,18)] However, some studies, that have attempted to validate the results of microarray experiments using reverse transcription polymerase chain reaction (RT-PCR), demonstrated no such changes of gene expression.[19–21)] Validation has not yet been performed in most studies, although the results of microarrays contain serious uncertainties and chances for false-positive findings. Therefore, more microarray studies validated by other methods are necessary to evaluate the certainties of such investigations.

In this study, we investigated the effects of exposure to RF fields on gene expression in a normal human glial cell line, SVGp12,[22)] using DNA microarray and the results were validated by RT-PCR. The aim of this study was to evaluate the effects of the RF fields generated by mobile phones, which are used near the brain. Therefore, brain-derived cells

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were suitable for our study. In addition, the possibility of differences among species supports the necessity of studies using not only animal but also human-derived cells, and the possibility of differences of gene expression between tumor and normal cells supports the necessity of studies using normal cells. However, human brain-derived normal cells are hard to obtain and grow. To solve this problem, we used SVGp12 cells.

MATERIALS AND METHODS

Cell culture

The human fetus-derived astroglia cell line, SVGp12 (ATCC® CRL-8261™; American Type Culture Collection, Manassas, VA, USA), was cultured with Eagle's minimum essential medium (Nikken Bio Medical Laboratory, Kyoto, Japan) supplemented with 10% fetal bovine serum (FBS; BioWest, Miami, FL, USA), 0.1 mM non-essential amino acids and 1 mM sodium pyruvate.

RF exposure

Cells were seeded at a density of 1.5×10^4 cells/cm^2, and cultured for 24 h before exposure. Cells were then exposed to RF fields at 1, 5, and 10 W/kg specific absorption rates (SARs) for 1, 4, and 24 h in a specially designed exposure apparatus based on a cylindrical waveguide using TM_{01} mode (Fig. 1), as reported previously.[23] Briefly, the apparatus consists of a cylindrical waveguide, the end of which is terminated by a short circuiting metallic plate to generate standing waves, and a signal generator (E4438C ESG, Agilent Technologies, Santa Clara, CA, USA). A culture dish of inside diameter 90 mm is placed on the short circuiting metallic plate inside the waveguide, where the conditions of atmosphere are controlled appropriately for cell culture by introducing 5% CO_2 and 95% humidified air.

In this experiment, we made three improvements on the apparatus previously used; i) addition of a Peltier effect device on the metallic plate to control the temperature of the culture medium, ii) increasing the length of the waveguide to improve the precision of electromagnetic distribution in the waveguide, iii) changing the structure of the end of the waveguide to allow a cell culture dish to be easily inserted and removed. The Peltier effect device, a 31 mm diameter disk, was controlled by a Peltier controller (TDC-1550; Cell Sytem Co. Ltd, Kanagawa, Japan), and maintained the temperature at 36.8 ± 0.4°C at the bottom of a culture dish. The length of the waveguide was extended from 230 mm to 490 mm, which improved the purity of transmission mode of TM_{01} in the waveguide to allow the better-defined electromafnetic field distribution in the medium.

A continuous microwave signal of 2.45 GHz from a signal generator was provided through a power amplifier (A0825-5050-R; R & K Co. Ltd., Shizuoka, Japan). A directional power meter (Power Reflection Meter NRT with power sensor NRT-Z44; Rohde & Schwarz, München, Germany) monitored the input power and the reflected power. A 3-stub tuner (MS-N-808, Nihon Koshuha Co. Ltd., Yokohama, Japan) was inserted to establish impedance matching.

The dosimetry of RF fields is performed with both numerical and experimental approaches. The results agreed fairly well. The SAR distribution is shown in Fig. 2, which was obtained from temperature elevation measured by fiber optic temperature probes (Fluoroptic Thermometer 790, Luxtron, Santa Clara, CA, USA). The SAR values are defined by spacially averaged values on the bottom of the medium, where cells were located. The relationship between input power and the average SARs are shown in Table 1. The input power was determined by the forward transmission power minus reflected power measured with the directional power meter. Sham exposures were performed using the same unit except for RF signal.

RNA extraction, amplification, and hybridization

After exposure to the RF fields, total RNA was extracted

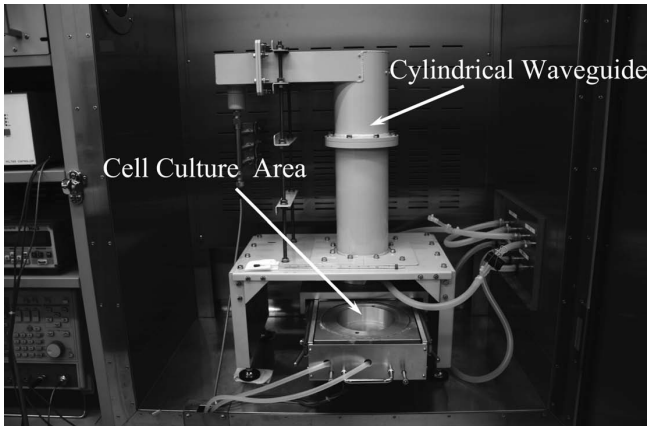

Fig. 1. A picture of the experimental system used for exposure to radiofrequency electromagnetic fields.

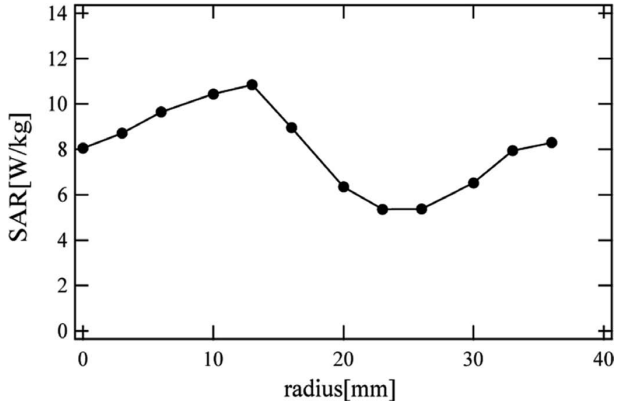

Fig. 2. Measured specific absorption rate (SAR) value at the bottom of the culture medium. SAR value is calculated by using temperature elevation at each point.

Table 1. Relationship between specific absorption rate (SAR) and input power.

SAR (W/kg)[a)]	Input Power (W)[b)]
1	0.11
5	0.54
10	1.1

[a)] Spatially averaged SAR value at the bottom of the culture medium.
[b)] Input power is defined by Pf minus Pr. Here, Pf and Pr indicate forward power and reverse power measured by the directional power sensor, respectively.

using an RNeasy Mini Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instruction. The concentration of extracted RNA was measured using a UV-visible spectrometer (SmartSpec™3000; Bio-Rad, Hercules, CA, USA) by absorption at a wavelength of 260 nm, and the purity of the RNA was assessed using an Agilent 2100 bioanalyzer (Agilent Technologies) and an RNA Nano Chips Kit (Agilent Technologies). Only RNA samples in which the ratio of ribosomal RNA 23S to 18S was more than 1.8, were used for the subsequent microarray analysis.

An amplification reaction with simultaneous introduction of amino-allyl groups to the amplified antisense RNA (aRNA) was performed using an amino-allyl RNA amplification kit (Sigma-Aldrich, St Louis, MO, USA) according to the manufacturer's instruction, starting with 2 μg of total RNA. Purified aRNA was labeled using the Cy3 Mono-Reactive Dye pack (Amersham Biosciences UK Limited, Little Chalfont, Buckinghamshire, UK) for aRNA derived from sham-exposed cells, and Cy5 Mono-Reactive Dye pack (Amersham Biosciences) for aRNA derived from RF exposed cells according to the manufacturer's instructions. Briefly, 15 μg of aRNA was precipitated with 70% ethanol/78 mM sodium acetate solution and reprecipitated with 70% ethanol. After removal of the 70% ethanol, aRNA was reacted with Cy3 or Cy5 Mono-Reactive Dye at 40°C for 1 h, and purified using an aRNA Filter Cartridge, which was provided as a component of an Amino Allyl MessageAmp™ aRNA kit (Ambion Inc., Austin, TX, USA). The dye incorporation efficiency and concentration of dye-labeled aRNA were analyzed spectrophotometrically at 260 nm for aRNA concentration, 550 nm for Cy3 incorporation, and 650 nm for Cy5 incorporation (SmartSpec™3000). After fragmentation of the dye-labeled aRNA was performed at 94°C for 15 min, samples were hybridized on a DNA chip (AceGene Premium® Human; DNA chip laboratory, Yokohama, Japan) at 50°C for 18 h using a hybridization chamber (CHBIO™; Hitachi Software Engineering Co., Ltd., Tokyo, Japan).

Three independent experiments were performed under each experimental condition, for a total of nine experimental conditions, consisting of three SARs at three time points.

Table 2. Primer sequences for RT-PCR analysis.

Gene Symbol[a)]		Sequence
RPL37A	Forward	5'-GGGATCTGGCACTGTGGTTC-3'
	Reverse	5'-GATGGCGGACTTTACCGTGA-3'
CCDC86	Forward	5'-GTGGAAGGACCGCTCCAAGA-3'
	Reverse	5'-CTCCAGGTGACGGGCAAAGT-3'
HMG20B	Forward	5'-GCTCTGGGCTCATGAACACTCTC-3'
	Reverse	5'-TCCTCGAAGGCCACATTCATC-3'
FARSA	Forward	5'-CCGAGATGCCGACTGATAACTTC-3'
	Reverse	5'-GGACATAGTCCATTGGGAGCTG-3'
GNAI2	Forward	5'-TGGTTCACAGACACGTCCATCA-3'
	Reverse	5'-GATGTAGCTGGCTGCCTCATCA-3'
TUBA1A	Forward	5'-GCCTAAGAGTCGCGCTGTAAGAA-3'
	Reverse	5'-AGGCATTGCCAATCTGGACAC-3'
SLC25A1	Forward	5'-CGCAGCCAGTGTCTTTGGAA-3'
	Reverse	5'-GACAAACACTATGGCCACATCCAG-3'
NUP188	Forward	5'-ACGCAGTGAGGACAGTGCAGA-3'
	Reverse	5'-GAGGTACATGCCTGGCACAAGTAA-3'
EPHA8	Forward	5'-CCTATGGAAGTCGGAAACATGGTC-3'
	Reverse	5'-AGAGCCCAGAAATTGGGTAAGAGTG-3'
BNIP3	Forward	5'-GAGTCTGGACGGAGTAGCTCCAA-3'
	Reverse	5'-TCCAATGCTATGGGTATCTGTTTCA-3'
KLF16	Forward	5'-GCCTACTACAAGTCCTCGCACCTAA-3'
	Reverse	5'-GTGAAGCGCTTGGAGCACAG-3'
BTBD3	Forward	5'-TCAGGTGCAGTGCGTTCATTC-3'
	Reverse	5'-TTTCACACGACTTTCACCAGCAG-3'
FEN1	Forward	5'-CTGTGGACCTCATCCAGAAGCA-3'
	Reverse	5'-CCAGCACCTCAGGTTCCAAGA-3'
ANKRD36B	Forward	5'-AAACCGGCTCTCAAATCAGCA-3'
	Reverse	5'-CCTGAGCCTGGCAATTTCATC-3'
ANKRD57	Forward	5'-AACCCAAGTATGTCCATGTGTTTC-3'
	Reverse	5'-GACAAGGCAACCTAGACTCCAAG-3'
ADI1	Forward	5'-CTGGAAGCTGGATGCTGACAAATA-3'
	Reverse	5'-GGATCTCATCGTCCAAGTGCAA-3'
PSPH	Forward	5'-TTCCTGCCTTTGAGCGGACT-3'
	Reverse	5'-CTGCAAGAGCAAGAGCCCTGT-3'
C1orf63	Forward	5'-CTACGGCTTTGGTCGCACAG-3'
	Reverse	5'-TGGCAAGTCAATGTTGGTTGTTC-3'
NAP5	Forward	5'-TTCGTTGCCAGAGATTCAGGTG-3'
	Reverse	5'-TGGTATTGGGAATAGAATTCGGTCA-3'
ITM2B	Forward	5'-GCCTGTCCCAGAGTTTGCAGATA-3'
	Reverse	5'-TGGCATAACAATGGAAGTGTTCAGA-3'

[a)] Further information related to the gene symbol is shown in Tables 4 and 5.

Table 3. Cell number (cells/cm^2) during the RF exposure and sham exposure.

	Sham	1 W/kg	5 W/kg	10 W/kg
0 h	$3.1 \times 10^4 \pm 9.7 \times 10^2$	$3.0 \times 10^4 \pm 1.2 \times 10^3$	$3.0 \times 10^4 \pm 9.6 \times 10^2$	$3.0 \times 10^4 \pm 1.1 \times 10^3$
1 h	$3.0 \times 10^4 \pm 8.7 \times 10^2$	$3.0 \times 10^4 \pm 9.7 \times 10^2$	$3.0 \times 10^4 \pm 9.9 \times 10^2$	$3.0 \times 10^4 \pm 1.2 \times 10^3$
4 h	$3.4 \times 10^4 \pm 7.4 \times 10^2$	$3.4 \times 10^4 \pm 1.3 \times 10^3$	$3.3 \times 10^4 \pm 1.1 \times 10^3$	$3.4 \times 10^4 \pm 1.7 \times 10^3$
24 h	$6.6 \times 10^4 \pm 3.1 \times 10^3$	$6.5 \times 10^4 \pm 2.3 \times 10^3$	$6.4 \times 10^4 \pm 2.5 \times 10^3$	$6.4 \times 10^4 \pm 3.0 \times 10^3$

Consequently, 27 independent hybridizations were performed on non-pooled RNA.

Semi-quantitative RT-PCR analysis

The RNA samples, which were used for microarray analysis, were also used for semi-quantitative RT-PCR analysis. cDNA was synthesized from the extracted RNA using a PrimeScript™ RT reagent Kit (TaKaRa Bio, Shiga, Japan) according to the manufacturer's instructions using random 6mers and oligo dT as primers. Semi-quantitative PCR was performed using a SYBR® Premix Ex Taq™ II Kit (TaKaRa Bio) and a Smart Cycler® II System (Cepheid, Sunnyvale, CA, USA) or RotorGene RG-3000 (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. In this system, PCR products were quantified using SYBR® Green I as an intercalating dye. Cycle conditions were as follows: after an initial incubation at 95°C for 10 s, 40 cycles of denaturation at 95°C for 5 s and primer annealing at 60°C for 20 s were performed. Fluorescence was measured at 60°C. The threshold cycle (Ct) value was determined by the 2nd derivative maximum method. The primers used in the study are summarized in Table 2. Expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and ribosomal protein S18 (RPS18) was used to standardize the amount of template cDNA.

Oligonucleotide microarray data acquisition

The hybridized AceGene Premium® Human slides were scanned using a ChipReader™ (Virtek Vision Corp., Waterloo, ON, Canada) with a 10 μm resolution and excitation wavelengths of 532 nm for Cy3 and 635 nm for Cy5, simultaneously. Each chip contained 50 mer oligonucleotide probes for 30,000 features. For about 10,000 out of the 30,000 features, gene names and gene functions had been assigned. For about another 10,000 features, only gene names had been assigned (their functions remain unknown). Of the remaining 10,000 genes, neither gene name nor gene function had been assigned, because these 10,000 genes were selected by the DNA chip laboratory as predicted expression genes. The 16 bit grayscale image files obtained were quantified using DNASIS Array version 2.6 (Hitachi Software Engineering Co., Ltd.), which used an adaptive spot finding method to acquire spot intensities from the mean pixel value and automatically flagged poor quality spots.

Microarray data preprocessing and normalization and statistical analysis

The intensity data were transferred to GeneSpring GX version 7.3.1 (Agilent Technologies) and normalized using the LOWESS method. The data were filtered by fold increase with more than 2-fold and less than 0.5 (1/2), or more than 1.5-fold and less than 0.67 (1/1.5). The filtered data were again filtered on the basis of reproducibility; the fold changes were altered beyond the threshold as noted above, in more than 2 out of 3 experiments. Statistical analysis of the remaining genes after filtration was performed by *t*-test corrected with the Benjamini and Hochberg false discovery rate.

RESULTS

Differential gene expression

There was no difference in cell growth between RF and sham exposures (Table 3). No morphological changes were observed between RF and sham exposures and between before and after exposure (data not shown).

The results of differential gene expression are shown in Table 4. The number of significantly altered genes was obtained after filtration of flags, cutoff by fold-change

Table 4. The number of genes that were significantly differentially expressed by microarray.

Exposure Conditions	Up-regulation		Down-regulation	
	>1.5	>2.0	<1/1.5	<1/2.0
1 W/kg-1 h	2	1	1	0
1 W/kg-4 h	9	0	1	0
1 W/kg-24 h	1	0	0	2
5 W/kg-1 h	1	0	0	0
5 W/kg-4 h	0	0	0	0
5 W/kg-24 h	3	0	8	1
10 W/kg-1 h	0	0	1	0
10 W/kg-4 h	1	0	0	1
10 W/kg-24 h	0	0	0	0
Total	17	1	11	4

threshold, shown in Table 4, and statistical analysis using *t*-test with correction for multiple comparisons. After filtration of the 2-fold cutoff, only one gene was detected as being significantly up-regulated by exposure to RF fields, and a total of 4 genes were detected as being significantly down-regulated by exposure to RF fields. After filtration of the 1.5-fold cutoff, a total of 17 genes were detected as being significantly up-regulated by exposure to RF fields, and a total of 11 genes were detected as being significantly down-regulated by exposure to RF fields. Overall, we concluded that very few genes were altered by RF exposure, since only 0.28% of about 10,000 genes, which were judged as "present", were found to be altered significantly. The gene names that were judged as being significantly altered are shown in Tables 5 and 6 for up-regulated and down-regulated genes, respectively.

Validation of significantly altered genes by RT-PCR

Although the technologies for DNA microarray have progressed, the potential for miss-leading results still remain because this technology is designed to evaluate the expressions of many genes simultaneously. Some genes do not exhibit correct expression profiles because the hybridization conditions are not appropriate for all 30,000 genes. To

Table 5. Genes that were up-regulated relative to sham.

Exposure Conditions	AceGene ID	Accession No.	Gene Title	Gene Symbol	Biological Process
1 W/kg	S010603	NM_000998	ribosomal protein l37a	RPL37A	translation
1 h	B100215	not assigned	–	–	–
1 W/kg 4 h	A080724	NM_024098	coiled-coil domain containing 86	CCDC86	
	A131524	NM_006339	high-mobility group 20B	HMG20B	regulation of transcription
	A140419	NM_004461	phenylalanyl-tRNA synthetase, alpha subunit	FARSA	phenylalanyl-tRNA aminoacylation
	A221414	NM_002070	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 2	GNAI2	signal transduction
	B130224	NM_006009	tubulin, alpha 1a	TUBA1A	microtubule-based movement
	B211222	NM_005984	solute carrier family 25, member 1	SLC25A1	
	B220910	NM_015354	nucleoporin 188kDa	NUP188	intracellular protein transport across a membrane
	C211219	not assigned	–	–	–
	S010809	NM_000998	ribosomal protein l37a	RPL37A	translation
1 W/kg 24 h	S010810	NM_000998	ribosomal protein l37a	RPL37A	translation
5 W/kg 1 h	B091202	NM_020526	EPH receptor A8	EPHA8	transmembrane receptor protein tyrosine kinase signaling pathway
5 W/kg 24 h	A041109	NM_004052	BCL2/adenovirus E1B 19kDa interacting protein 3	BNIP3	
	B160211	NM_031918	Kruppel-like factor 16	KLF16	regulation of transcription from RNA polymerase II promoter
	C211219	not assigned	–	–	–
10 W/kg 4 h	A130517	NM_181443, NM_014962	BTB (POZ) domain containing 3	BTBD3	

Table 6. Genes that were down-regulated relative to sham.

Exposure Conditions	AceGene ID	Accession No.	Gene Title	Gene Symbol	Biological Process
1 W/kg 1 h	A180513	NM_004111	flap structure-specific endonuclease 1	FEN1	double-strand break repair
1 W/kg 4 h	B140218	NM_025190	ankyrin repeat domain 36B	ANKRD36B	
5 W/kg	A051306	NM_023016	ankyrin repeat domain 57	ANKRD57	
24 h	A080513	NM_024055	solute carrier family 30 (zinc transporter), member 5	SLC30A5	regulation of proton transport
	B061322	NM_018269	acireductone dioxygenase 1	ADI1	amino acid biosynthetic process
	B161602	NM_004577	phosphoserine phosphatase	PSPH	amino acid biosynthetic process
	C021114	NM_020317	chromosome 1 open reading frame 63	C1orf63	
	C040809	NM_207481 NM_207363	Nck-associated protein 5	NAP5	biologica process
	C120417	not assigned	–	–	–
	C261019	not assigned	–	–	–
10 W/kg 1 h	A091310	NM_021999	integral membrane protein 2B	ITM2B	nervous system development

validate the results of the microarray analysis, we performed semi-quantitative RT-PCR analysis for all genes that were judged as being significantly altered after filtration of the 1.5-fold cutoff and were linked to Gene Accession Numbers. Although a total of 28 genes passed as prospective altered genes after filtration of the 1.5-fold cutoff, 23 genes out of the 28 could be linked to Gene Accession Numbers. One out of the 23 genes, suitable primer set could not be designed. As a result, we performed RT-PCR experiment to validate the expression of these 22 genes. In PCR analysis, target gene expression was standardized to both GAPDH and RPS18 mRNA expression because, to date, no genes have been sufficiently consistent to be considered as a suitable house-keeping gene for standardization of the results of PCR. The results of PCR standardized to GAPDH were similar to those standardized to RPS18. For all tested genes, there was no significant difference between the gene expression in the RF exposed cells and that in sham-exposed cells (Table 7).

DISCUSSION

In this experiment, we evaluated the effects of exposure to RF fields on gene expression in human fetus-derived SVGp12 cells using a high throughput analysis method. Under all conditions investigated in this study, we could not detect any alteration in the expression of genes by exposure to RF fields.

We performed 3 independent hybridizations on non-pooled RNA under 9 different conditions (27 in total) to obtain sufficient biological replicates and to allow us to conduct statistical analysis. When we selected a cutoff value of 2-fold, which is a common cutoff value in microarray experiments,[24–26] only 5 genes appeared to be statistically significantly altered throughout the 9 conditions (Table 4). This represents too few genes to allow us to understand the tendencies of the RF exposure effects. Therefore, we again performed statistical analysis using a cutoff value of 1.5-fold, which is another common cutoff value in microarray experiments.[27,28] This change led to an increase in the number of genes; however, the difficulty of understanding the tendencies of the RF exposure effects remained. Increasing the SAR did not lead to any increase in altered genes, and increasing exposure duration did not lead to any increase or decrease in altered genes.

The number of genes that were judged as statistically significantly altered changed depending upon the number of genes that were used for the statistical analysis. The number of genes used for the statistical analysis changed depending upon the cutoff threshold value. For these reasons, the number of the statistically significantly down-regulated genes that met the 1.5-fold cutoff might be smaller than that of the 2-fold cutoff in 1 W/kg-24 h and 10 W/kg-4 h experimental groups (Table 4). In statistical analysis of 1 W/kg-24 h, 3 genes were used for statistical analysis after filtration of the 2.0-fold cutoff although 16 genes were used after fil-

Table 7. Comparison of changes in the expression of genes in the RF exposed group relative to the sham group.

Gene	Microarray	RT-PCR	
		GAPDH	RPS18
Up-regulation			
1 W/kg-1 h			
RPL37A	1.82	1.04 ± 0.11	1.02 ± 0.05
1 W/kg-4 h			
CCDC86	1.55	0.93 ± 0.09	0.96 ± 0.23
HMG20B	1.74	1.07 ± 0.26	1.07 ± 0.12
FARSA	1.64	1.01 ± 0.10	1.05 ± 0.29
GNAI2	1.55	0.94 ± 0.08	0.96 ± 0.10
TUBA1A	1.61	1.00 ± 0.00	1.03 ± 0.18
SLC25A1	1.55	0.96 ± 0.03	0.91 ± 0.16
NUP188	1.52	0.80 ± 0.12	0.86 ± 0.33
RPL37A	1.67	0.85 ± 0.04	0.90 ± 0.25
1 W/kg-24 h			
RPL37A	1.71	1.08 ± 0.12	0.98 ± 0.05
5 W/kg-1 h			
EPHA8	1.53	not detected	not detected
5 W/kg-24 h			
BNIP3	1.54	1.34 ± 0.17	1.57 ± 0.48
KLF16	1.59	0.97 ± 0.16	0.98 ± 0.06
10 W/kg-4 h			
BTBD3	1.53	0.87 ± 0.10	0.95 ± 0.10
Down-regulation			
1 W/kg-1 h			
FEN1	0.56	0.99 ± 0.16	1.01 ± 0.14
1 W/kg-4 h			
ANKRD36B	0.64	0.99 ± 0.11	0.92 ± 0.04
5 W/kg-24 h			
ANKRD57	0.64	0.95 ± 0.19	1.09 ± 0.21
ADI1	0.63	0.88 ± 0.06	0.92 ± 0.10
PSPH	0.62	not detected	not detected
C1orf63	0.47	1.04 ± 0.55	1.13 ± 0.60
NAP5	0.66	0.91 ± 0.17	1.05 ± 0.27
10 W/kg-1 h			
ITM2B	0.59	1.18 ± 0.23	1.02 ± 0.07

tration of the 1.5-fold cutoff. Two out of 3 genes were judged as statistically significantly altered after filtration of the 2.0-fold cutoff although 0 out of 16 genes were judged as statistically significantly altered after filtration of the 1.5-fold cutoff. In statistical analysis of the 10 W/kg-4 h group, 1 gene was used for statistical analysis after filtration of the 2.0-fold cutoff, whereas 83 genes were used after filtration of the 1.5-fold cutoff. This suggests that increasing the number of candidate genes by declining the cutoff threshold does not always increase the number of statistically significantly altered genes.

The results of microarray studies should be validated by other methods although microarray technology is advancing. In this experiment, we tried to validate the results of microarray by RT-PCR. The results of RT-PCR showed that exposure to RF fields did not affect gene expression (Table 7). The most plausible reason for the false-positive results of microarray is thought to be the low expression level of genes that have been judged as being significantly altered by microarray. For example, in microarray analysis of exposure to RF fields at 1 W/kg of SAR for 4 h, the most intensely expressed gene was β-actin; with an average intensity from 3 independent experiments of 22,008 fluorescence unit under our scanning conditions of the microarray chips. However, the fluorescence intensity of almost all genes that were judged as being significantly altered was around 100–500 units. These intensities were less than 1/40 than that of β-actin, and were almost at the lower limit of the microarray accuracy. The larger standard deviation of lower intensity signals compared with higher intensity signals has been reported previously, which further supports our suggestion.[29,30)]

In this experiment, we detect 23 assigned gene spots and 5 non-assigned gene spots as prospective altered gene spots by microarray analysis. However, no significant alterations in gene expression were observed in 22 genes out of the 23 assigned genes analyzed by RT-PCR. Compared with microarray, RT-PCR is more accurate for investigating the levels gene expression. Therefore, our results suggested that there were no effects of exposure to RF fields on gene expression. Our results are consistent with previous studies showing that there were no detectable biological effects of exposure to non-thermal RF fields.[19–21)] In contrast, our results are not consistent with previous studies showing the biological effects of exposure to RF fields.[17,18)] This inconsistency thought to be due to non-validation of experiments[17)] or different species, different exposure systems, different cell homogeneity compared with our experiments.[18)] In conclusion, we found no evidence to suggest that exposure to RF fields affected gene expression in SVGp12 cells under the current experimental conditions.

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REFERENCES

1. ICNIRP (2009) II Review of experimental studies of RF biological effects (100 kHz-300 GHz) In: Exposure to high frequency electromagnetic fields, biological effects and health consequences (100 kHz-300 GHz). ICNIRP 16/2009. pp. 90–303. International Commission on Non-Ionizing Radiation Protection.
2. Tice RR, *et al* (2002) Genotoxicity of radiofrequency signals. I. Investigation of DNA damage and micronuclei induction in cultured human blood cells. Bioelectromagnetics **23**: 113–126.
3. Sun L, *et al* (2006) Effects of 1.8 GHz radiofrequency field on DNA damage and expression of heat shock protein 70 in human lens epithelial cells. Mutat Res **602**: 135–142.
4. Vijayalaxmi, *et al* (2000) Primary DNA damage in human blood lymphocytes exposued *in vitro* to 2450 MHz radiofrequency radiation. Radiat Res **153**: 479–486.
5. McNamee JP, *et al* (2003) No evidence for genotoxic effects from 24 h exposure of human leukocytes to 1.9 GHz radiofrequency fields. Radiat Res **159**: 693–697.
6. Sakuma N, *et al* (2006) DNA strand breaks are not induced in human cells exposed to 2.1425 GHz band CW and W-CDMA modulated radiofrequency fields allocated to mobile radio base stations. Bioelectromagnetics **27**: 51–57.
7. McNamee JP and Chauhan V (2009) Radiofrequency radiation and gene/protein expression: A review. Radiat Res **172**: 265–287.
8. Fritze K, *et al* (1997) Effect of global system for mobile communication microwave exposure on the genomic response of the rat brain. Neuroscience **81**: 627–639.
9. Chauhan V, *et al* (2006) Analysis of proto-oncogene and heat-shock protein gene expression in human derived cell-lines exposed *in vitro* to an intermittent 1.9 GHz pulse-modulated radiofrequency field. Int Radiat Biol **82**: 347–354.
10. Simkó M, *et al* (2006) Hsp70 expression and free radical release after exposure to non-thermal radio-frequency electromagnetic fields and ultrafine particles in human Mono Mac 6 cells. Toxicol Lett **161**: 73–82.
11. Hirose H, *et al* (2007) Mobile phone base station-emitted radiation dose not induce phosphorylation of hsp27. Bioelectromagnetics **28**: 99–108.
12. Goswami PC, *et al* (1999) Proto-oncogene mRNA levels and activities of multiple transcription factors in C3H 10T1/2 murine embryonic fibroblasts exposed to 835.62 and 847.74 MHz cellular phone communication frequency radiation. Radiat Res **151**: 300–309.
13. Leszczynski D, *et al* (2002) Non-thermal activation of the hsp27/p38MAPK stress pathway by mobile phone radiation in human endothelial cells: molecular mechanism for cancer- and blood-brain barrier-related effects. Differentiation **70**: 120–129.
14. Czyz J, *et al* (2004) High frequency electromagnetic fields (GSM signals) affect gene expression levels in tumor suppressor p53-deficient embryonic stem cells. Bioelectromagnetics **25**: 296–307.
15. Whitehead TD, *et al* (2005) Expression of the proto-oncogene *Fos* after exposure to radiofrequency radiation relevant to wireless communications. Radiat Res **164**: 420–430.
16. Vanderstraeten J and Verschaeve L (2008) Gene and protein expression following exposure to radiofrequency fields from mobile phones. Environ. Health Perspect **116**: 1131–1135.
17. Nylund R and Leszczynski D (2006) 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. Proteomics **6**: 4769–4780.
18. Zhao R, *et al* (2007) Studying gene expression profile of rat neuron exposed to 1800 MHz radiofrequency electromagnetic fields with cDNA microarray. Toxicology **235**: 167–175.
19. Grurislk E, *et al* (2006) An *in vitro* study of the effects of exposure to a GSM signal in two human cell line: monocytic U937 and neuroblastoma SK-N-SH. Cell Biol Int **30**: 793–799.
20. Zeng Q, *et al* (2006) Effects of global system for mobile communications 1800 MHz radiofrequency electromagnetic fields on gene and protein expression in MCF-7 cells. Proteomics **6**: 4732–4738.
21. Chauhan V, *et al* (2007) Analysis of gene expression in two human-derived cell lines exposed *in vitro* to a 1.9 GHz pulse-modulated radiofrequency field. Proteomics **7**: 3896–3905.
22. Major EO, *et al* (1885) Establishment of line of human fetal glial cells the supports JC virus multiplication. Proc Natl Acad Sci USA **82**: 1257–1261.
23. Sonoda T, *et al* (2005) Electromagnetic and thermal dosimetry of a cylindrical waveguide-type in vitro exposure apparatus. IEICE Trans Commun **E88-B**: 3287–3293.
24. Bennett L, *et al* (2003) Interferon and granulopoiesis in systemic lupus erythematosus blood. J Exp Med **197**: 711–723.
25. Ponnampalam AP, *et al* (2004) Molecular classification of human endometrial cycle stages by transcriptional profiling. Mol Hum Reprod **10**: 879–893.
26. Yin H-Q, *et al* (2007) Differential gene expression and lipid metabolism in fatty liver induced by acute ethanol treatment in mice. Toxicol Appl Pharmacol **223**: 225–233.
27. Pacini S, *et al* (2002) Exposure to global system for mobile communication (GSM) cellular phone radiofrequency alters gene expression, proliferation, and morphology of human skin fibroblasts. Oncol Res **13**: 19–24.
28. Chilakapati J, *et al* (2010) Genome-wide analysis of BEAS-2B cells exposed to trivalent arsenicals and dimethylthioarsinic acid. Toxicology **268**: 31–39.
29. Martin-Requena V, *et al* (2009) PreP+07: improvements of a user friendly tool to preprocess and analyse microarray data. BMC Bioinformatics **10**: 16.
30. Kim JH, Shin DM and Lee YS (2002) Effect of local background intensities in the normalization of cDNA microarray data with a skewed expression profiles. Exp Mol Med **34**: 224–232.

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