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2.45 GHz radiofrequency fields alter gene expression in cultured human cells

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Abstract The biological effect of radiofrequency (RF) fields remains controversial. We address this issue by examining whether RF fields can cause changes in gene expression. We used the pulsed RF fields at a frequency of 2.45 GHz that is commonly used in telecommunication to expose cultured human HL-60 cells. We used the serial analysis of gene expression (SAGE) method to measure the RF effect on gene expression at the genome level. We observed that 221 genes altered their expression after a 2-h exposure. The number of affected genes increased to 759 after a 6-h exposure. Functional classification of the affected genes reveals that apoptosis-related genes were among the upregulated ones and the cell cycle genes among the downregulated ones. We observed no significant increase in the expression of heat shock genes. These results indicate that the RF fields at 2.45 GHz can alter gene expression in cultured human cells through non-thermal mechanism.

Keywords: Radiofrequency; Biological effect; SAGE; Gene expression

1. Introduction

Radiofrequency (RF) refers to the electromagnetic waves ranging between 10 MHz and 300 GHz. RF have been widely used as a signal carrier in telecommunications. Recent advances in mobile phone technology have resulted in the exponential use of mobile phone communication around the world. The increasing exposure of humans to RF fields has raised wide concerns for potential adverse effects of RF fields on human health (http://www.fcc.gov/oet/rfsafety, http://www.fda.gov/cdrh/phones/index.html, http://www.who.int/emf, http://www.iegmp.org.uk/, http://www.verum-foundation.de/).

While it is clear that high energy-electromagnetic waves, such as X-rays have strong biological effects through ionizing damage, it is uncertain whether the low energy, non-ionizing RF fields could have effects on biological systems. Several epidemiological studies suggest a link between long-term RF exposures and pathological consequences such as cancer [1–7]. Molecular studies also suggest the possible influence of RF fields on various aspects of biological activities [8–13]. Although these studies have provided many clues to the issue of RF biological effects, the results are inconclusive and even controversial.

In this study, we used genome-wide gene expression as the indicator to address the issue of biological effects of RF. We used a 2.45 GHz waveguide system to expose human HL-60 cells. We used the serial analysis of gene expression (SAGE) technique to analyze the RF effect on gene expression at the genome level [14]. Although gene expression has been used as an indicator in previous RF studies, those studies focused only on a handful number of genes pre-selected with defined functions. We aim to provide genome-wide coverage of the expressed genes regardless their functional categories in the RF treated cells to address if RF has biological effects [15,16]. We consider it particularly important to use this approach for the subject that there is limited biological information available. Our study shows that under the conditions used in our experimental system, the 2.45 GHz RF fields caused the expression changes of a number of genes.

2. Materials and methods

2.1. Cell culture

Human HL-60 cell line was purchased from ATCC. Cells were cultured in the RPMI 1640 medium + 10% fetal bovine serum (FBS) in an incubator at 37 °C with 5% CO_2. Cells used for experiments were at the exponential growth phase. Prior to RF exposure, cells were spanned down and re-suspended in 10 ml of fresh medium at the density of 10^6/ml. The cells were then transferred to a 25 ml culture flask for RF exposure.

2.2. RF exposure system

The RF exposure system used for experiments was described in detail (Gerber et al. manuscript in preparation). Briefly, the RF source was a pulsed magnetron (Cober Muegge). It was pulsed at duration

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Abbreviations: RF, radiofrequency; SAGE, serial analysis of gene expression

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of 155 μs and a duty cycle of 7.5%, producing a peak power of 3 W into the waveguide. The measured average power was 225 mW, of which 100 mW was absorbed by the 10 ml cell suspension to provide the average SAR value of 10 W/kg. Using the measured 2.61 S/m conductivity of the medium at 2.45 GHz with the 133 W/kg SAR during the pulse, the calculated electric field is 320 V/m. A control waveguide, identical to the experimental waveguide was used for a sham exposure. Restricted by the cost of SAGE experiment, only the 2-h sham exposed cells were used as the control for the 2 and 6 h RF exposed cells. A flask containing a 10 ml HL-60 cell suspension at 10^6/ml was placed inside a WR340 brass waveguide having inside dimensions of 86.36×43.18 mm. The cells were allowed to settle down to the bottom of the flask to form a monolayer before exposure. The bottom of the flask is ground flat and coated with mineral oil to obtain good thermal conduction between the cell monolayer and brass waveguide. The bottom of the waveguide has an exterior plastic water channel glued to it such that the turbulent flowing water is in direct contact with the brass surface. A 5% air–CO_2 mixture was introduced into the waveguide through a hole in its top surface. The brass surface was maintained at 37 °C through the use of a temperature-controlled water circulator. Two temperature probes (Luxtron) were inserted into the bottom surface of the flask to monitor the temperature. The temperature was maintained at 37.2 ± 0.2 °C during the exposure period.

2.3. SAGE process

The SAGE process followed the standard procedures [14,17]. Briefly, it includes the following steps: mRNA isolation from the cells, cDNA synthesis, *Nla*III digestion of cDNAs, 3′cDNA collection, tag releasing from 3′ cDNA, ditags formation, ditag concatemerization, cloning, and DNA sequencing. SAGE tag sequences were extracted from the raw sequences using SAGE300 software. The SAGE data is deposited in NCBI with accession number GSE3025 (www.ncbi.nlm.nih.gov/projects/geo).

2.4. SAGE data analysis

To determine the gene origin of SAGE tags, the experimental SAGE tags were matched to the SAGEmap database (www.ncbi.nlm.nih/SAGEmap). A SAGE tag is assigned to a gene if it has a match in the reference database; and a SAGE tags is defined as a novel tag if it has no match in the SAGEmap database. To identify a specific gene for the SAGE tags shared by multiple genes in SAGEmap database, these tags were matched to a tissue-specific SAGE annotation database under the cell type "HL-60" (www.basic.northwestern.edu/SAGE/). By using the microarray expression data from the specific tissue type to annotate the SAGE tags collected from the same tissue type, this database provides high accuracy of gene prediction for SAGE tags shared by multiple genes (Ge et al., manuscript in preparation). To identify the differences in SAGE tags between the control and exposed cells, the method of Audic and Claverie ([18]; http://telethon.bio.unipd.it/bioinfo/IDEG6_form/), a statistical method designed for SAGE analysis, was used for the comparison under $P < 0.05$ as the cut-off. Greater than 4-fold differences between samples was set as the second cut-off threshold to provide high confidence for the identification of alternatively expressed genes between different samples. To visualize the changes of gene expression, the "Cluster" and "Treeview" programs were used to generate the average linkage hierarchical clustering using Pearson's correlation coefficient as a distance metrics [19]. The Gene Ontology "biological process" terms were used to identify the functional categories of RF-response genes at $P < 0.05$ ([20]; http://www.geneontology.org).

3. Results

3.1. Collection of SAGE tags

Three samples were used for the analysis: a control with 2 h sham exposure, 2-h exposed and 6-h exposed HL-60 cells. A total of 155696 SAGE tags were collected from these three

Table 1
Summary of SAGE tags from the control and RF-exposed HL-60 cells

Items	Total copy of SAGE tags	Unique SAGE tags
Control	52171	17300
RF for 2 h	51923	15490
RF for 6 h	51602	17816
Total	155696	38871 (100)
Match to known gene	126852	24179 (62)
No match to known gene	28844	14692 (38)

Table 2
Summary for the changes of gene expression in 2 and 6 h radiated cells

Changes	2 h radiation			6 h radiation		
	SAGE tags	Corresponding genes		SAGE tags	Corresponding genes	
		Known genes	Novel transcripts		Known genes	Novel transcripts
A. The number of genes changed in 2 and 6 h radiated cells						
Turn-on	28	24	4	147	112	35
Turn-off	36	30	6	188	157	31
Increase	86	80	6	195	166	29
Decrease	71	51	20	229	193	36
Total	221(100)	185(84)	36(16)	759(100)	628(83)	131(17)
B. The number of genes changed only in the 2 or 6 h radiated cells						
Turn-on	23	20	3	142	108	34
Turn-off	13	10	3	155	127	28
Increase	67	61	6	175	146	29
Decrease	34	23	11	203	176	27
Total	137(100)	114(83)	23(17)	675(100)	557(83)	118(17)
C. The number of genes commonly changed in both 2 and 6 h radiated cells						
Turn-on	5	4	1			
Turn-off	23	20	3			
Increase	19	19	0			
Decrease	37	28	9			
Total	84(100)	71(85)	13(15)			

Table 3
Genes alternatively expressed in both 2 and 6 h radiated cells

SAGE tag	Tag copy			UniGene ID	Gene
	Control	2 h	6 h		
Known genes					
Turn-on and increase					
TGCACGTTCT	0	8	15	Hs.265174	Ribosomal protein L32
TGGCTTGCTC	0	8	6	Hs.130293	Cisplatin resistance-associated overexpressed protein
GGCTGGGGTC	0	6	5	Hs.185235	Similar to nitric oxide synthase 2A, clone IMAGE:5168672, mRNA
TGAATGGCCT	0	5	5	Hs.415236	Kelch domain containing 2
GGCCTTTTTT	1	14	19	Hs.75307	H1 histone family, member X
TTTCTGTCTG	1	13	6	Hs.426967	Phosphoinositide-3-kinase, catalytic, delta polypeptide
CGATTCTGGA	1	10	9	Hs.301412	Ufm1-conjugating enzyme 1
TGCTGGTGTG	1	10	9	Hs.430725	Myosin phosphatase-Rho interacting protein
TTGATGCCCG	1	7	5	Hs.381167	Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 1
CTCTCACTCT	1	7	6	Hs.53066	hsp70-interacting protein
ATGCTGCGAT	1	6	77	Hs.27413	Adaptor protein containing pH domain, PTB domain and leucine zipper motif 1
GCCTGCTCCC	1	6	9	Hs.355929	Chromosome 10 open reading frame 137
GGGGATGGGG	1	6	5	Hs.321231	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 3
TTGGACTGAG	1	6	6	Hs.6518	GABA(A) receptor-associated protein-like 2
TTGGTCTCTG	1	6	8	Hs.181551	Cross-immune reaction antigen PCIA1
CTGCATTTGT	1	5	8	Hs.443227	Replication factor C (activator 1) 5, 36.5kDa
GATGGCTGCC	1	5	5	Hs.356729	Beta 5-tubulin
GATTACCTGT	1	5	5	Hs.411157	Hexosaminidase A (alpha polypeptide)
GCTGCACCGG	1	5	16	Hs.203581	DEAD (Asp-Glu-Ala-Asp) box polypeptide 54
GCTGTTCATT	2	15	11	Hs.288986	Survival of motor neuron 2, centromeric
GTGGGGCTAG	2	10	18	Hs.431861	Protein phosphatase 5, catalytic subunit
Turn-off and decrease					
AAACAGTGGC	6	0	0	Hs.180062	Proteasome (prosome, macropain) subunit, beta type, 8
CCAGCTGCCA	6	0	0	Hs.406693	Ubiquitin-activating enzyme E1
CTCCCCCAAG	6	0	0	Hs.497707	Immunoglobulin heavy constant alpha 2 (A2m marker)
GGGGCACCCG	6	0	0	Hs.334521	Hypothetical protein MGC16037
GTGGCCACGG	6	0	1	Hs.112405	S100 calcium binding protein A9 (calgranulin B)
AACCAGAATG	5	0	0	Hs.433278	Emopamil binding protein-like
AAGTGGAGGA	5	0	0	Hs.337766	Ribosomal protein L18a
CCAGTTCCTT	5	0	1	Hs.106620	Nicotinamide nucleotide transhydrogenase
GGGCTGCTTT	5	0	1	Hs.77436	Pleckstrin
GGTGAGCTAC	5	0	0	Hs.300684	Calcitonin gene-related peptide-receptor component protein
GTCAGACTGT	5	0	1	Hs.442223	Lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)
GTGAAACGCC	5	0	0	Hs.109052	Chromosome 14 open reading frame 2
GTTTTTCATT	5	0	0	Hs.539	Ribosomal protein S29
TGACCAAATG	5	0	0	Hs.289112	Chromosome 7 open reading frame 28B
TGCTTGACAA	5	0	1	Hs.222061	Hypothetical protein MGC9850
TGGTCCTCTG	5	0	0	Hs.387725	Integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)
TGTTTGACTG	5	0	0	Hs.435065	Ring finger protein 3
ACTTTCCAAA	20	4	2	Hs.78921	A kinase (PRKA) anchor protein 1
TAAGACTTCA	14	3	1	Hs.135052	Zinc finger protein 30 (KOX 28)
GCCAGGCACT	9	2	1	Hs.99863	Elastase 2, neutrophil
CCTGTAATGC	8	1	0	Hs.7179	RAD1 homolog (*S. pombe*)
CGCCGCCGGT	8	1	1	Hs.182825	Ribosomal protein L35
AAGCGCTCTC	6	1	1	Hs.168913	Serine/threonine kinase 24 (STE20 homolog, yeast)
ATAGAGGCAA	6	1	1	Hs.411358	Mortality factor 4 like 2
GTGTTCCCCT	6	1	0	Hs.117176	Poly(A) binding protein, nuclear 1
TCACGGGTTT	6	1	0	Hs.200022	Clone IMAGE:5285814, mRNA
TCTTGTAACT	6	1	1	Hs.256549	Nucleotide binding protein 2 (MinD homolog, *E. coli*)
AAGACACGTG	5	1	0	Hs.3352	Histone deacetylase 2
AGTCAGTGGG	5	1	1	Hs.21943	NGG1 interacting factor 3-like 1 (*S. pombe*)
CCCAGGAAGG	5	1	0	Hs.194714	Synaptosomal-associated protein, 29 kDa
CTAGACGTTG	5	1	1	Hs.306307	Rho-associated, coiled-coil containing protein kinase 1
CTGAGAGATT	5	1	0	Hs.277445	Diacylglycerol kinase, zeta 104 kDa
CTGTTTATGA	5	1	0	Hs.110713	DEK oncogene (DNA binding)
GCTGCCCTGA	5	1	1	Hs.19400	Mitotic arrest deficient-like 2 (yeast)
GGAGGAGCTG	5	1	1	Hs.182579	Leucine aminopeptidase 3
GTGAAACCTA	5	1	0	Hs.325081	SVAP1 protein
GTGGCATATG	5	1	0	Hs.63984	Cadherin 13, H-cadherin (heart)
GTTCACATTA	5	1	0	Hs.446471	CD74 antigen
TGGCACTTCA	5	1	0	Hs.32217	RAB32, member RAS oncogene family
TTGACAGCCT	5	1	0	Hs.78885	Biotinidase
GACAGTCGGT	5	1	1	Hs.523181	*Homo sapiens* T84 colon carcinoma cell IL-1beta regulated HSCC1 mRNA

(*continued on next page*)

Table 3 (*continued*)

SAGE tag	Tag copy			UniGene ID	Gene
	Control	2 h	6 h		
Different change					
GGGATTTGGC	7	1	41	Hs.437594	Ribosomal protein, large P2
Un-assigned SAGE tags[*]					
CTGAACTGTG	1	11	7	Hs.96901; Hs.242947	
GCTCACACCT	1	6	5	Hs.161582; Hs.491107	
GAAAACCCCT	6	0	0	Hs.154133; Hs.347474;	Hs.446350
CTGTAATCCC	5	0	0	Hs.170915; Hs.406300	
CTTCTATGTA	5	0	0	Hs.102648; Hs.437959	
AACCTCGAGT	6	1	0	Hs.247478; Hs.323502	
GCCTGGACCA	5	1	0	Hs.10964; Hs.413494	
GTGGCGCACA	5	1	0	Hs.188661; Hs.334788	

[*]The gene identity for these SAGE tags are unable to be identified in both SAGEmap and tissue-specific SAGEmap database.

samples, with the identification of 38 871 unique SAGE tags. Matching the SAGE tags to SAGE reference databases shows that 62% of the SAGE tags represent known genes, and 38% are novel tags without matches to known genes (Table 1).

3.2. Dynamic changes of gene expression during RF exposure

Expressions of many genes were altered at the 2- and 6-h exposure conditions. In the 2-h exposed cells, 221 affected SAGE tags were identified, representing 185 known genes, and 36 novel transcripts; in the 6-h exposed cells, the number of affected SAGE tags increased to 759, representing 628 known genes, and 131 novel transcripts (Table 2). Comparing the 2-h data with the 6-h data shows that the changes of gene expression was in a dynamic manner: Of the 185 2-h RF-response known genes, 114 were only present in the 2-h data set (Supplementary Table 1) that went back to the control level; for the 628 6-h RF-response genes, 557 were only present in the 6-h data set (Supplementary Table 2). For the 71 genes present in both 2- and 6-h data sets, all except one had the same patterns of changes regarding up-or down-expression (Table 3). In all, a total of 896 SAGE tags were identified to be different between the control and the exposed cells. These SAGE tags represent 742 known genes, and 154 novel transcripts with unknown gene origins (Supplementary Table 3). Fig. 1 shows the distribution patterns of the altered genes in the 2- and 6-h exposed cells.

3.3. Functional classification of RF-response genes

The altered gene expression observed in the exposed cells includes early and late RF response genes. There were 114 known genes, of which the expression changes occurred only in the 2-h exposed cells (Table 2B, Supplementary Table 1). These genes represent the early RF response genes. They are functionally diversified widely, such as the genes involved in DNA replication, transcriptional and translational regulation. Under the Gene Ontology terms, individual 2-h RF-response genes cannot be grouped into specific functional categories.

There were 71 known genes that were affected in both 2- and 6-h exposed cells (Table 2C, Table 3). These genes represent early RF-response genes but they maintain their altered expression levels throughout the prolonged RF exposure. Among them, the number of downregulated genes doubled that of upregulated genes (47 vs. 23). The ribosome protein large P2 gene is exceptional in that its SAGE tag decreased

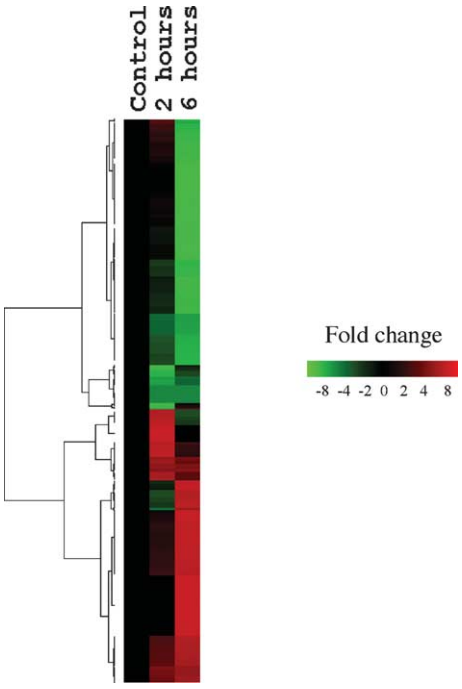

Fig. 1. Hierarchical clustering for the RF-response genes. The RF-response genes identified in the 2- and 6-h RF radiated HL60 cells were clustered using the "Treeview" program for visual comparison. The red color represents the genes increasingly expressed, the green color represents the genes decreasingly expressed.

from seven copies in the control to a single copy under a 2-h exposure but increased to 41 copies after a 6-h exposure. These genes are involved in various functions but they cannot be grouped into specific functional categories under the Gene Ontology terms.

The 628 known genes affected in 6-h exposed cells represent the late RF response genes (Table 2A, Supplementary Tables 2 and 4). This number far exceeds early response genes, indicating that more genes were altered upon prolonged RF exposure. Under the Gene Ontology "biological process" terms, 138 of the 628 genes can be grouped into specific functional categories (Table 4). Among the upregulated genes were those related to apoptosis, metabolism, polysaccharide biosynthesis, RNA processing and translation. Among the downregulated genes were those involved in transport, metabolism, RNA process-

ing, and cell cycle. Table 5 shows the six genes grouped into "apoptosis" and the twenty-three genes grouped into "Cell cycle" categories.

3.4. The novel transcripts detected in the exposed cells

There are 36 and 131 novel SAGE tags detected in the 2- and 6-h exposed cells, respectively. The patterns of changes of these novel SAGE tags are similar to those representing known genes. Of the 36 SAGE tags detected in the 2-h exposed cells, 23 occurred only in 2-h cells; of the 131 novel SAGE tags detected in 6-h, 118 occurred only in 6-h cells; and 13 novel SAGE tags were common in both 2- and 6-h data set (Table 6 and Supplementary Table 3). These SAGE tags represent novel transcripts expressed from known genes or unknown genes response to RF irradiation.

Table 4
Functional catagories of the RF-response genes in the 6-h radiated cells

Genes turned on or increased expression
Apotosis
Induction of apoptosis
Induction of programmed cell death
Positive regulation of apoptosis
Positive regulation of programmed cell death
Metabolism
Carbohydrate metabolism
DNA metabolism
RNA metabolism
Polysaccharide metabolism
Polysaccharide biosynthesis
RNA processing
Translation
Genes truned off or decreased expression
Transport
ATP synthesis coupled electron transport
ATP synthesis coupled electron transport (sensu Eukaryota)
Electron transport
Intracellular transport
Intracellular protein transport
Mitochondrial electron transport NADH to ubiquinone
Protein transport
Metabolism
DNA metabolism
mRNA metabolism
Peptide metabolism
RNA processing
mRNA processing
Nuclear mRNA splicing via spliceosome
RNA processing
RNA splicing\,via transesterification reactions
RNA splicing\,via transesterification reactions with bulged adenosine as nucleophile
Cell cycle
Cell cycle
Regulation of cell cycle
Homeostasis
Oxidative phosphorylation
Protein localization
Regulation of biosynthesis
Rho protein signal transduction
Translational initiation

The "biological process" in Gene Ontology database was used for the classification.
The probability of the assigned gene in each functional group was at $P < 0.05$.

3.5. Stable status of heat shock genes in RF exposed cells

To investigate if there were thermal effects caused by the RF exposure to the cells, we compared the expression levels of classical heat-shock genes between the control and the exposed cells. We identified all SAGE tags representing not only the dominant transcripts but also the alternatively spliced/polyadenylated transcripts for these heat shock genes (Table 7, and Supplementary Table 5). Most of the heat shock genes were expressed at stable levels between the control and the exposed cells. Minor increases (less then 1-fold) upon a 2-h expose for heat shock protein 5 gene, heat shock 90 kDa protein 1 alpha and beta were seen. In the 6-h exposed cells, certain genes, including those for heat shock protein 8, heat shock 90 kDa protein 1 alpha and heat shock 90 kDa protein 1 beta, dramatically decreased their levels of expression. Most notably, the SAGE tag for heat shock 90 kDa protein 1 beta gene decreased from 64 copies in the control cells to eight copies in the 6-h exposed cells. The expression status of the heat shock genes confirms the stable thermal condition during the RF experiment.

4. Discussion

In our study, we aim to use gene expression as the indicator to determine if there is any biological effect of radiofrequency fields. We designed the experiment based on the following considerations: (a) use an RF exposure system at 2.45 GHz, the frequency commonly used in telecommunication; (b) maintain a tight control of the thermal environment during RF exposure to minimize thermal effects on the irradiated cells; (c) survey gene expression at the genome-level without pre-selection of any particular genes to provide a genome-wide picture of gene expression in the irradiated cells; (d) provide sequences and quantitative information for the detected transcripts to identify potential RF-response genes; (e) detect both known genes and novel transcripts of unknown genes. Under the experimental conditions used in this study, we observed that the RF fields at 2.45 GHz causes expression changes for considerable number of genes in the RF exposed cultural human cells. Although some individual genes identified might not be reliable due to experimental errors, the majority of the genes is unlikely to be artifacts considering that: (1) cells used for the experiment were not synchronized, (2) the cells were aliquots from the same preparation maintained in the same cultural medium and (3) many genes were from the highly specified functional groups, such as apoptosis-related genes.

It is interesting to see the dynamic changes of gene expression during RF exposures. The short term RF irradiation caused the expression changes in a smaller number of genes. These genes represent the early RF-response genes, and most of them later fall back to the control levels. Upon prolong RF irradiation, three times more genes responded. These genes represent the late RF-response genes. The majority of altered genes under the prolonged exposure were different from the early response genes. The temporal patterns of gene expression reflect the dynamic genome response to the RF fields. It is likely that some genes are directly responsive to RF exposure, whereas others might be regulated by the initial RF response genes that are transcriptional and translational regulators. These early response genes are distributed in various functional groups, they cannot be grouped into specific functional

Table 5
Examples of changed genes in 6 h radiated cells with specific functional catagories

Gene	UniGene I.D.	SAGE tags	Copy number		
			Control	6 h	*P* value
Increasingly expressed genes related with apoptosis					
Beta 5-tubulin	Hs.356729	GGTCCCCTTT	0	5	0.015
		GATGGCTGCC*	1	5	0.046
		TGTTTTCAGC	8	0	0.002
Tubulin, beta, 2	Hs.433615	CTGTACAGAC	6	31	0.000
Death-associated protein	Hs.75189	CATCTGTGAG	4	20	0.000
Programmed cell death 8 (apoptosis-inducing factor)	Hs.18720	CGACCTCCTC	1	6	0.027
Protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), alpha isoform	Hs.173902	TCCAGATCTT	0	6	0.008
Etoposide induced 2.4 mRNA	Hs.343911	CTCCTTCACC	0	5	0.015
Decreasely expressed genes related with cell cycle					
MCM2 minichromosome maintenance deficient 2, mitotin (*S. cerevisiae*)	HS.57101	CCAGGTGCAG	12	0	0.000
Minichromosome maintenance deficient 5, cell division cycle 46 (*S. cerevisiae*)	HS.77171	GACTCGCCCA	12	1	0.001
TAR DNA binding protein	HS.300624	GACTGAGCTT	12	2	0.003
Transforming growth factor beta regulator 4	HS.231411	GACTGCGTGC	12	0	0.000
Cell division cycle 2, G1 to S and G2 to M	HS.334562	GCAGGAATTG	11	0	0.000
Chaperonin containing TCP1, subunit 7 (eta)	HS.368149	ATGGGCCTGT	10	1	0.003
High-mobility group box 1	HS.434102	TCTGCTAAAG	10	1	0.003
RAD1 homolog (*S. pombe*)	HS.7179	CCTGTAATGC	8	1	0.009
CDC28 protein kinase regulatory subunit 2	HS.83758	AGCTGTATTC	7	1	0.016
SET translocation (myeloid leukemia-associated)	HS.436687	TGAATCTGGG	7	0	0.004
CDC28 protein kinase regulatory subunit 2	HS.83758	AGCTGTATTC	7	1	0.016
High-mobility group box 2	HS.434953	TCTGCAAAGG	6	1	0.028
Kinesin family member 2C	HS.69360	GGACACTCCT	6	0	0.008
RAP1A, member of RAS oncogene family	HS.865	ATCCTCCCTA	6	1	0.028
Transcription factor Dp-1	HS.79353	GATGTGGTTG	6	1	0.028
Ubiquitin-activating enzyme E1	HS.406693	CCAGCTGCCA	6	0	0.008
Cyclin D3	HS.83173	CCCTCCTCTC	5	1	0.048
Extra spindle poles like 1 (*S. cerevisiae*)	HS.153479	CCCAGGCTCC	5	0	0.016
MAD2 mitotic arrest deficient-like 2 (yeast)	HS.19400	GCTGCCCTGA	5	1	0.047
Microtubule-associated protein, RP/EB family, member 1	HS.408754	CTCTGTGTGG	5	0	0.016
Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide	HS.74405	GCGGGAGCGG	5	0	0.016
v-myc myelocytomatosis viral oncogene homolog (avian)	HS.202453	ATCAAATGCA	5	0	0.016

*The copy number of this tag was already 5 in the 2 h radiated cells.

categories under the Gene Ontology terms. This suggests that no specific functional gene groups are present among the early response genes. Jun and Fos genes were reported to be early RF response genes [21–23]. However, we were not able to confirm this observation. Of the four SAGE tags representing Fos and seven SAGE tags representing Jun, all were present at basal levels in the exposed cells. The discrepancy may be due in part to the differences of the RF systems, cell types, or the techniques used.

Among the functional categories of the upregulated RF response genes during the prolonged exposure, the genes involved in apoptosis are particularly interesting. They include programmed cell death 8, etoposide induced 2.4 mRNA, beta 5-tubulin, tubulin, beta, 2, PPP2R1A, and death-associated protein. Etoposide induced 2.4 mRNA gene is a p53 response gene. It activates apoptosis pathway upon p53 activation [24]. Programmed cell death 8 gene, which was upregulated from 1 to 6 copies upon a 6-h exposure, triggers the release of cytochrome *c* and plays roles in chromosome condensation and fragmentation in the apoptotic cells [25]. Death-associated protein is a positive mediators of apoptosis induced by IFN-gamma [26]. Tubulins are also known to be involved in apoptotic process [27]. Interestingly, the beta 5-tubulin gene responded to RF by use of alternatively spliced transcripts: one represented by the SAGE tag sequence GATGGCTGCC that was increased upon 2-h exposure and maintained at high level after 6-h exposure, the other represented by the SAGE tag sequence TGTTTTCAGC that was expressed in the control cells but became undetectable after 2- and 6-h exposures. PPP2R1A is a phosphatase [28], which influences apoptosis through changing the phosphorous state of apoptosis-related genes. The increased expression of apoptosis-associated genes and the multiple targeting sites in the apoptosis pathway suggests that longer RF irradiation may trigger apoptosis-related activities in the exposed cells. Among the downregulated genes, 23 are classified into the "cell cycle" category. For example, "cell division cycle 2, G1 to S and G2 to M" gene is a Ser/Thr protein kinase. It regulates cell cycles through phosphorylation and dephosphorylation [29]. Cyclin D3 gene is important for cell cycle progression through G2 phase into mitosis [30]. The SET protein regulates G2/M transition by modulating cyclin B-cyclin-dependent kinase 1 activity [31]. It is reasonable to suggest that the cells response to the RF stress by slowing down

Table 6
Examples of novel SAGE tags identified between the control and radiated cells

Items	SAGE tags	Copy number		
		Control	2 h	6 h
Only in 2-h radiated cells				
Turn-on	AGCGGCCGCT	0	5	
	TGTACCTTAA	0	5	
	CCCACCGTCC	0	5	
Turn-off	ATCTGAGTTC	8	0	
	CCCCACCAGT	8	0	
	CCCCCGTGAT	5	0	
Increase	GCAGATCGGG	1	9	
	GCGGCCCTAG	1	9	
	ATCAAGCCAC	1	6	
Decrease	TCGGTTGCAT	13	2	
	CACACTACTA	7	1	
	AGTTGCGAAC	6	1	
Only in 6-h radiated cells				
Turn-on	TGCCACGTTT	0		27
	CCCATCCGTC	0		18
	TGCAACGTTT	0		13
Turn-off	CACCTATTGG	28		0
	AGCTCTGTAG	21		0
	AAGACGTGGC	16		0
Increase	CCCCATCGTC	3		67
	CCCATCGTTC	4		29
	GCCATCGTCC	1		18
Decrease	AAGCGGCCGC	268		54
	ACTAACACCC	220		40
	CTAAGACTTC	203		45
Common in 2- and 6-h radiated cells				
Turn-on	CCCACCGTCC	0	5	8
Turn-off	ATCTGAGTTC	8	0	0
	CCCCCGTGAT	5	0	0
Increase	GCTGCGTTAG	1	6	5
Decrease	TCGGTTGCAT	13	2	1
	AGTAGGTGGC	6	1	1
	AGTTGCGAAC	6	1	0

their cell division activities. The delayed cell division may provide opportunities for repairing on one hand and apoptosis on the other.

In addition to the SAGE tags that represent RF-response known genes, there are 36 and 131 novel SAGE tags identified in the 2- and 6-h exposed cells, respectively. Since these novel SAGE tags do not match currently known genes in the human genome, they are novel transcripts such as alternatively spliced isoforms from either known genes, or potential novel genes [32]. Determination of the gene origins of these novel SAGE tags are of special interest, as they might represent specific RF-response genes in the genome.

It has been questioned that the biological effect of RF, if any, may be due to its thermal effect [33,34]. Heat shock genes are the biosensors to the thermal environment. They increase rapidly their level of expression in response to thermal increase [35]. We compared all transcripts, including not only the dominant forms but also the alternative forms, expressed from classical heat-shock genes. The results show no significant increase at the expression levels for these heat shock genes upon RF-irradiation, confirming that the RF conditions used in the experiment were thermal stable. Therefore, the altered gene expression in the RF exposed cells was due to non-thermal mechanism(s).

Our main goal of this study is to use genome approach to address if RF has biological effects. Data from our study indicate that RF indeed has biological effects, or in other words, the living cells can sense the RF insulation. We should point out that this is an in vitro study and the RF system used for the study is not exactly identical to those used in telecommunications. Our study supports further study for RF effects on biological system.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2005.07.063.

Table 7
Expression levels of heat shock genes in RF-radiated cells

Gene	UniGene ID	SAGE tag	SAGE tag copy			*P* value	
			Control	2 h	6 h	Control to 2 h	Control to 6 h
Heat shock 10 kDa protein 1 (chaperonin 10)	Hs.1197	AGCCACCTTG	3	0	1	0.06	0.13
Heat shock 22 kDa protein 8	Hs.111676	CCTGGCCTAA	1	0	0	0.25	0.25
Heat shock protein, alpha-crystallin-related, B6	Hs.351558	GAGACCTTCT	0	0	1		0.25
Heat shock protein, alpha-crystallin-related, B9	Hs.238094	ACCTGCTGCC	0	1	0	0.25	
Heat shock 27 kDa protein 1	Hs.76067	ATTGCAGCAC	2	0	2	0.13	0.19
Heat shock 60 kDa protein 1 (chaperonin)	Hs.79037	TACCAGTGTA	23	20	18	0.06	0.05
Heat shock 70 kDa protein 1A	Hs.75452	AAGAGCGCCG	0	1	0	0.25	
Heat shock 70 kDa protein 1B	Hs.274402	AAGAGCCCCG	1	0	0	0.25	0.25
Heat shock 70 kDa protein 4	Hs.90093	GATCCAGTTG	4	6	1	0.10	0.08
Heat shock 70 kDa protein 5	Hs.310769	TGCATCTGGT	68	84	87	0.01	0.01
Heat shock 70 kDa protein 8	Hs.180414	CCAGGAGGAA	16	19	2	0.06	0.00
Heat shock 70 kDa protein 9B (mortalin-2)	Hs.184233	AGTGAAACCC	1	1	5	0.25	0.05
Heat shock 70 kDa protein 12A	Hs.372597	AGACAAGCTG	4	2	1	0.12	0.08
Heat shock 70 kDa protein 14	Hs.430666	CACAGATCAA	1	1	1	0.25	0.25
Heat shock 90 kDa protein 1, alpha	Hs.446579	TACTAGTCCT	35	39	15	0.04	0.00
Heat shock 90 kDa protein 1, beta	Hs.74335	TGATTTCACT	64	88	8	0.00	0.00
Heat shock transcription factor 1	Hs.132625	AGCCTGCCTG	0	1	0	0.25	
Heat shock transcription factor 2	Hs.158195	CACACTCACT	2	1	0	0.19	0.13

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