2-GHz Band CW and W-CDMA Modulated Radiofrequency Fields Have No Significant Effect on Cell Proliferation and Gene Expression Profile in Human Cells

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Mobile phone/Whole-body average/DNA microarray.

We investigated the mechanisms by which radiofrequency (RF) fields exert their activity, and the changes in both cell proliferation and the gene expression profile in the human cell lines, A172 (glioblastoma), H4 (neuroglioma), and IMR-90 (fibroblasts from normal fetal lung) following exposure to 2.1425 GHz continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) RF fields at three field levels. During the incubation phase, cells were exposed at the specific absorption rates (SARs) of 80, 250, or 800 mW/kg with both CW and W-CDMA RF fields for up to 96 h. Heat shock treatment was used as the positive control. No significant differences in cell growth or viability were observed between any test group exposed to W-CDMA or CW radiation and the sham-exposed negative controls. Using the Affymetrix Human Genome Array, only a very small (< 1%) number of available genes (ca. 16,000 to 19,000) exhibited altered expression in each experiment. The results confirm that low-level exposure to 2.1425 GHz CW and W-CDMA RF fields for up to 96 h did not act as an acute cytotoxicant in either cell proliferation or the gene expression profile. These results suggest that RF exposure up to the limit of whole-body average SAR levels as specified in the ICNIRP guidelines is unlikely to elicit a general stress response in the tested cell lines under these conditions.

INTRODUCTION

The arrival of the new millennium has seen rapid growth in mobile phone usage. A number of studies have investigated the effects of mobile communication radiation on biological systems, most of which are still ongoing. One way in which cells adjust to changes in their environment is by altering gene expression patterns, and measurement of the changes in expression when exposed to radiofrequency (RF) signals can thus help to determine how RFs act on cells and organisms. This technology has been used to discover gene function, understand biochemical pathways and regulatory mechanisms, classify disease specimens, and discover new drug targets.1) Given general acceptance that the specific absorption rate (SAR) of RF energy, particularly in the human head, is insufficient to cause significant heating of tissues, any theory linking mobile phone RF radiation and cancer must be based on the ‘non-thermal’ effects of RF on cells and tissues, notwithstanding that a mechanism has yet to be determined. The existence of biological effects has fuelled concerns of potential health effects. French et al.2) and Leszczynski et al.3,4) proposed a hypothetical mechanism by which RF exposure could be associated with cancer via the induction of the heat shock response. Activation of heat shock proteins (hsp) is a normal defense response to cellular stress.5) Hsp27 expression has been shown to inhibit significantly JNK-induced apoptosis as well as Akt activation in PC12 cells and superior cervical ganglion neurons.6,7) On chronic expression, however, hsp is known to induce or promote oncogenesis, metastasis, and/or resistance to anticancer drugs.8,9) These previous studies provide an important focus for research in this controversial area.

The aim of the present study is to assess the biological effects of low-level irradiation from a 2-GHz mobile radio base station by investigating changes in the gene expression...
profile in three human cell lines following exposure to 2.1425 GHz continuous wave (CW) and Wideband-Code Division Multiple Access (W-CDMA) RF fields from mobile radio base station at three SAR levels. The first objective of this study is to confirm the gene expression profile in human cell lines exposed to microwaves at a SAR of 80 mW/kg, which corresponds to the limit of the whole-body average SAR for general public exposure as defined by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines. The second objective is to investigate whether CW and W-CDMA modulated signal RF fields at 2.1425 GHz, which corresponds to the center frequency of the IMT-2000, induces different levels of changes in the gene expression profile.

MATERIALS AND METHODS

Cells and culture

Three human cell lines, A172 (glioblastoma, CRL-1620), H4 (neuroglioma, HTB-148), and IMR-90 (fibroblasts from normal fetal lung, CCL-186) were obtained from the American Type Culture Collection (ATCC, Rockville, MD). Stock cultures were maintained in 100-mm diameter culture dishes at 37°C in a 5% CO2 incubator. A172 and H4 cells were maintained with Dulbecco’s modified Eagle medium (Invitrogen), Carlsbad, CA supplemented with 10% fetal calf serum (FCS; Invitrogen), containing streptomycin and penicillin. IMR-90 cells were grown in Minimum Essential Medium-Earle’s (Invitrogen) with 10% FCS, containing 0.1 mM non-essential amino acids (Invitrogen), 1.0 mM sodium pyruvate (Invitrogen), streptomycin, and penicillin. We used Ca2+ and Mg2+-free phosphate buffered saline [PBS(−)] with 0.25% trypsin and 0.02% EDTA to harvest the cells. All cell lines were immediately expanded to obtain sufficient cells to freeze 100 vials each.

RF-EMF exposure system and exposure protocol

The W-CDMA cellular system is a component system of the IMT-2000 cellular system. W-CDMA adopts Direct Sequence CDMA (DS-CDMA) and Frequency Division Duplex (FDD) as a multiple access and duplex scheme, respectively. The chip rate of the spread code of this system is 3.84 Mcps. A beam-formed RF exposure incubator employing a horn antenna, a dielectric lens, and a culture case in an anechoic chamber was developed for large-scale in vitro studies. A detailed description of the exposure system was published by Iyama et al. Briefly, two identical RF field exposure incubators, one for RF field exposure and the other for sham exposure, were established in separate anechoic chambers, and a mechanical switch in a dummy box allows the selection of RF field exposure or sham exposure. This system allows simultaneous exposure of 49 (7 × 7 array) 35-mm culture dishes to a 2.1425 GHz RF electromagnetic field, which corresponds to the center frequency of the IMT-2000 downlink band, with a uniform SAR distribution in the medium of all 49 culture dishes. The main unit for the cell exposure provides identical air to the two culture units through sealed ducts at the appropriate temperature (37°C), CO2 (5%), and humidity (> 90%). The SAR dosimetry was also shown by Iyama et al. The combination of an electric field measurement technique and the Finite Difference Time Domain (FDTD) calculation method was used to assess the SAR distribution in the culture fluid. At 2.1425 GHz, the measurement results using the coaxial probe (85070C, Agilent Technologies) and the vector network analyzer (8722ES, Agilent Technologies) indicated that the culture fluid had a relative permittivity of 76.4 and a conductivity of 2.5 S/m. The mean SAR of the culture fluid at the bottom of the 49 culture dishes used in the in vitro experiments was 175 mW/kg for an antenna input power of 1 W, and the standard deviation of the SAR distribution was 59%. When only the inner 25 culture dishes (5 × 5 array) were evaluated, the mean SAR was 139 mW/kg for the same antenna input power and the standard deviation of the SAR distribution was 47%. The dishes in the inner dish positions were used in this study.

For each experiment, a new vial of frozen cells was thawed, expanded for 14 days, and used at the same passage number (third or fourth). Four hours prior to exposure, cells were centrifuged (1000 rpm, 5 min) and re-suspended in fresh medium at a concentration calculated to give the desired cell number (third or fourth). Four hours prior to exposure, cells were centrifuged (1000 rpm, 5 min) and re-suspended in fresh medium at a concentration calculated to give the desired cell number (third or fourth). Four hours prior to exposure, cells were centrifuged (1000 rpm, 5 min) and re-suspended in fresh medium at a concentration calculated to give the desired cell number (third or fourth). Four hours prior to exposure, cells were centrifuged (1000 rpm, 5 min) and re-suspended in fresh medium at a concentration calculated to give the desired cell number (third or fourth). Four hours prior to exposure, cells were centrifuged (1000 rpm, 5 min) and re-suspended in fresh medium at a concentration calculated to give the desired cell number (third or fourth). Four hours prior to exposure, cells were centrifuged (1000 rpm, 5 min) and re-suspended in fresh medium at a concentration calculated to give the desired cell number (third or fourth). Four hours prior to exposure, cells were centrifuged (1000 rpm, 5 min) and re-suspended in fresh medium at a concentration calculated to give the desired cell number (third or fourth). Four hours prior to exposure, cells were centrifuged (1000 rpm, 5 min) and re-suspended in fresh medium at a concentration calculated to give the desired cell number (third or fourth). Four hours prior to exposure, cells were centrifuged (1000 rpm, 5 min) and re-suspended in fresh medium at a concentration calculated to give the desired cell number (third or fourth). Four hours prior to exposure, cells were centrifuged (1000 rpm, 5 min) and re-suspended in fresh medium at a concentration calculated to give the desired cell number (third or fourth). Four hours prior to exposure, cells were centrifuged (1000 rpm, 5 min) and re-suspended in fresh medium at a concentration calculated to give the desired cell number (third or fourth). Four hours prior to exposure, cells were centrifuged (1000 rpm, 5 min) and re-suspended in fresh medium at a concentration calculated to give the desired cell number (third or fourth).
**Heat shock treatment**

Log-phase A172, H4, and IMR-90 cells were incubated for 24, 48, or 72 h at 39°C or 41°C in 5% CO₂ incubators, and collected immediately after incubation. Several studies suggested that RF fields exposure caused activation of a cellular stress response as heat shock response.² ³

**Cell proliferation assay**

To determine cell numbers, enzymatically dissociated log-phase cells were plated (0.5 to 1 × 10⁵ cells in 3 ml) in 35-mm culture dishes, incubated for 2 h, and then continuously exposed to RF fields. Plates were then harvested at 24, 48, 72, and 96 h after the start of exposure. Cultures were harvested by enzymatic dissociation, washed in medium without FCS, and counted with a hemocytometer to determine total cells. Viable cells were measured by trypan blue dye exclusion. For heat treatment, the relative cell number was determined by the quantitation of ATP, as measured by CellTiter-Glo™ Luminescent Cell Viability Assay (Promega, Madison, WI). Mean cell count and standard deviation were calculated for each group. Statistical analysis was carried out using Student’s t- or Welch’s t-test after application of the preliminary F-test for the homogeneity of variance.

**Microarray hybridization and analysis**

Immediately after sham or RF field exposure, three culture dishes from each exposure were placed on ice, and the cell culture medium was aspirated. The cells were dissolled in 0.5 ml of lysis buffer provided in a Qiagen RNeasy Mini-Kit (Qiagen, Tokyo, Japan), with 1% 2-mercaptoethanol. The cell lystate was transferred to a centrifuge tube and stored at -80°C. Total RNA was isolated using the kit according to the manufacturer’s instructions. RNA concentration and purity were determined spectrophotometrically by measuring the absorbance at 230, 260, 280, and 320 nm. Human genome HG-U133A and B (Affymetrix, Santa Clara, CA) are ribonucleotide probe-based gene arrays containing > 100,000 unique oligos representing about 45,000 transcripts, which in turn represent > 33,000 well-substantiated human genes.

Double-stranded cDNA was synthesized with 10 μg of total RNA using the T7oligo (dT)₂₄ primer (Affymetrix) and SuperScript double-stranded cDNA synthesis kit (Invitrogen) following the manufacturer’s protocol. Precipitated double-stranded cDNA was used as a template for cRNA synthesis with a BioArray High Yield RNA Transcipt Labeling Kit (Enzo, Farmingdale, NY). Biotin-labeled cRNA was then purified (RNeasy Mini Kit, Qiagen) and fragmented to a mean size of 200 bp before hybridization. Total RNA and biotin-labeled cRNA were tested for integrity and size by resolution on an Agilent RNA 6000 Nano LabChip (Agilent, Palo Alto, CA). Hybridization was performed at 45°C for 16 h [0.1 M 4-morpholinepropanesulfonic acid (MES, pH 6.6), 1 M NaCl, 0.02 M EDTA, and 0.01% Tween 20] with washing under both non-stringent (6x SSPE and 0.01% Tween 20, 25°C) and stringent (0.1 M MES, 0.1 M [Na⁺], and 0.01% Tween 20, 50°C) conditions. Chips were stained with phycoerythrin-streptavidin (10 μg/ml), scanned, and analyzed with Microarray Suite 5.0 software (MAS 5.0; Affymetrix). Each exposure was repeated at least twice, and each sample was hybridized to two chips (HG-U133A & B). The primary data captured using MAS 5.0 resulted in a signal raw value for each probe set based on the mean of the difference between the hybridization intensity for the perfect match features and the mismatch for features for a particular transcript.

Statistical analysis was carried out using one-way analysis of variance (ANOVA) with GeneSpring™ ver.6.1 (Silicon Genetics, CA). Genes were considered to be differentially expressed if (i) expression changed by at least two-fold in two independent experiments performed with duplicate RNA samples, (ii) the mRNAs were assigned at least one “present” detection call by the Affymetrix software in both experiments and (iii) the change in gene expression was in the same direction (“increased” or “decreased”) in both experiments. The multiplicity problem was controlled by the Benjamini and Hochberg false discovery rate.¹⁷ For comparison between RF exposure and sham exposure for each condition, the unpaired t test was applied to identify the subsets of genes that had a significant (P < 0.05) increase or decrease in frequency values.

**RESULTS**

**Cell proliferation assay**

During the incubation phase, each cell line was exposed at a SAR of 80, 250, or 800 mW/kg with either W-CDMA or CW electromagnetic fields at 2.1425 GHz for up to 96 h. The temperature was maintained at 37.0°C ± 0.5 for the W-CDMA and CW experiments. Typical growth profiles of cell lines exposed to W-CDMA at 80 mW/kg are shown in Fig. 1. No differences were observed in either cell morphological character or cell growth profile between cells exposed during the incubation period versus sham-exposed cells. Under some exposure conditions, an increase or decrease (or both) in the number of cells was observed, but these changes were not consistently repeated under the same RF field exposure conditions. In contrast, the change in proliferation profile of the human cell lines exposed to heat shock at 41°C was significantly different from that of cells maintained at 37°C. A decrease was observed in the number of cells treated with heat shock (Fig. 2).

**Microarray hybridization and analysis**

The effect of RFs on gene expression in the tested human cell lines was determined at 96 h after exposure to several RF fields. For each treatment (cell lines, RF, and SAR), genes with statistically significant changes in expression...
were identified using the approach of Bushel et al. (2001). Of the 44760 (100%) gene probe sets analyzed, 17759 (39.7%), 16503 (36.9%), and 16582 (37.0%) genes were “present” in A172, H4, and IMR-90 cells, respectively, according to the stringent criteria detailed in Materials and Methods. Under RF exposure conditions, the number of genes present was as follows: A172, 17679 (39.5%); H4, 16301 (36.4%); and IMR-90, 16592 (37.1%). A total of 11125 genes were commonly expressed in the three cell lines. We identified a total of eight genes in H4 cells that changed two-fold or more on RF exposure at both 80 and 250 mW/kg. The known genes were ribosomal protein S2, growth arrest specific transcript 5, and integrin, beta 5. The number of genes exhibiting two-fold changes in A172 and IMR-90 cells was five and one, respectively. These genes were expressed sequence tags (ESTs), which are not well annotated. In contrast, the change in the gene expression profile in H4 cells exposed to heat shock at 39 or 41°C was different from that in RF radiation- and sham-exposed cells (data not shown). To identify genes modulated by heat treatment at 41°C for 24 h in another cell line, we compared the gene expression profiles of A172 cell RNA isolated from thermal treatment and under normal conditions. A total of 108 genes had statistically significant up- and down-regulation in the heat treatment condition. These profiles were compared to the respective gene expression profiles of A172, H4, and IMR-90 cells exposed to RF with a SAR of 80 or 800 mW/kg in 2.1425-GHz W-CDMA modulated signals. Sixty genes showed a significant increase in expression, of which 28 were categorized by information from several

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**Fig. 1.** Cell lines were seeded into 35-mm diameter culture dishes, pre-cultured for 2 h in a conventional incubator, and then exposed to several RFs for up to 96 h. The cell growth and proliferation ratio of human cell lines that were exposed to 80 mW/kg W-CDMA RF fields were plotted against those of sham exposure. Exposure began 2 h after the plating on day 1. Data are expressed as the mean (S.E.M. of triplicate plates) a: A172 (glioblastoma), b: H4 (neuroglioma), and c: IMR-90 (fibroblasts from fetal lung). The number of cells is shown as solid (RF exposed) or open (sham) bars. The open circle is the ratio of the number of viable cells in the RF-exposed dishes to that in the sham-exposed dishes.

**Fig. 2.** Log-phase A172, H4, and IMR-90 cells were incubated for 24, 48, or 72 h at 37°C, 39°C or 41°C in 5% CO₂ incubators. Three different cultures used in each. The relative cell number was determined by the quantitation of ATP. Treatment of the cells to heat shock at 41°C resulted in a remarkable decrease compared to the cells cultured at 37°C or 39°C.
| Accession No. | Gene Ontology / Biological Process | Description | Thermal Stress | W-CDMA | A172 | A172 | H4 | IM-R90 |
|--------------|-----------------------------------|-------------|---------------|--------|------|------|----|--------|
| AF003934     | Signal transduction               | Prostate differentiation factor | 6.21 < 0.001 | 1.11 – | 1.15 – | 1.04 – |      |        |
| *N71923      | Signal transduction               | ADP, ATP carrier protein, fibroblast isofrom | 5.23 < 0.001 | 1.08 < 0.05 | 1.03 – | 1.11 – |      |        |
| AB007830     | UV protection                     | Scavenger receptor class A, member 3 | 3.99 < 0.001 | 1.02 – | 0.86 – | 1.02 – |      |        |
| NM_003287    | Oncogenesis                       | Tumor protein D52-like 1 | 3.36 < 0.001 | 0.98 – | 0.79 – | 0.87 – |      |        |
| *NM_001673   | Metabolism                        | Asparagine synthetase | 3.20 < 0.001 | 1.11 – | 1.14 < 0.05 | 1.00 – |      |        |
| M61715       | Tryptophanyl-tRNA aminoacylation   | Tryptophanyl-tRNA synthetase | 2.96 < 0.001 | 0.93 – | 1.14 – | 0.93 – |      |        |
| *NM_006905   | Pregnancy                         | Pregnancy specific beta-1-glycoprotein 1 | 2.85 < 0.001 | 0.89 < 0.05 | 1.50 – | 0.95 – |      |        |
| NM_004184    | Tryptophanyl-tRNA aminoacylation   | Tryptophanyl-tRNA synthetase | 2.75 < 0.001 | 1.05 – | 1.16 – | 0.88 – |      |        |
| AD13324      | Chromosome organization and biogenesis | Histone 2 | 2.74 < 0.001 | 1.20 – | 1.11 – | 1.15 – |      |        |
| NM_004585    | Negative regulation of cell proliferation | Retinoic acid receptor responder (tiazotrate induced) 3 | 2.71 < 0.001 | 1.12 – | 1.15 – | 0.78 – |      |        |
| NM_014905    | Glutamine catabolism              | Glutaminase | 2.59 < 0.001 | 1.10 – | 1.19 – | 1.04 – |      |        |
| BG434174     | Intracellular protein transport    | TFHA-alpha/beta-like factor | 2.59 < 0.001 | 0.96 – | 1.07 – | 0.90 – |      |        |
| NM_004199    | Protein metabolism                | Procollagen-proline, 2-oxoglutarate 4-dioxygenase | 2.58 < 0.005 | 1.04 – | 1.13 – | 0.97 – |      |        |
| U77914       | Cell communication                | Jagged 1 (Alagille syndrome) | 2.57 < 0.001 | 0.99 – | 1.13 – | 1.03 – |      |        |
| M25915       | Lipid metabolism                  | Clusterin (complement lysis inhibitor, SP-40,40, sulfated glycoprotein 2, testosterone-repressed prostate message 2, apolipoprotein J) | 2.53 < 0.001 | 1.09 – | 1.10 – | 0.99 – |      |        |
| BG231932     | Proteolysis and peptidolysis      | Tripeptidyl peptide I | 2.47 < 0.001 | 1.05 – | 1.17 – | 1.12 – |      |        |
| NM_014210    | Cell proliferation                | Ecotropic viral integration site 2A | 2.44 < 0.001 | 1.17 – | 1.04 – | 1.02 – |      |        |
| NM_001235    | Heat shock response               | Heat shock protein 47 | 2.40 < 0.001 | 1.04 – | 1.15 – | 0.98 – |      |        |
| NM_014454    | Response to DNA damage            | P53 regulated PA26 nuclear protein | 2.39 < 0.001 | 1.11 – | 1.12 – | 0.88 – |      |        |
| AC004770     | Electron transport                | Fatty acid desaturase 3 | 2.36 < 0.001 | 1.04 – | 1.34 – | 1.04 – |      |        |
| AB014573     | Protein folding                   | Peptidylprolyl isomerase D | 2.35 < 0.001 | 1.04 – | 1.09 – | 0.97 – |      |        |
| BC002842     | Nucleosome assembly               | Histone 1, H2bd | 2.31 < 0.001 | 1.14 – | 1.16 – | 1.03 – |      |        |
| NM_030918    | Intracellular signaling cascade    | Sorting nexin family member 27 | 2.26 < 0.001 | 0.87 – | 1.18 – | 0.91 – |      |        |
| AF217990     | Response to unfolded protein       | Homocysteine-inducible ubiquitin-like domain | 2.23 < 0.001 | 1.02 – | 1.05 – | 0.90 – |      |        |
| NM_006644    | Heat shock response               | Heat shock 105 kDa/110 kDa protein 1 | 2.20 < 0.001 | 1.04 – | 1.12 – | 1.05 – |      |        |
| *NM_000391   | Lipid metabolism                  | Cereoid-lipofuscinosis, neuronal 2, late infantile (Jansky-Bielschowsky disease) | 2.18 < 0.001 | 1.04 < 0.05 | 1.08 – | 1.04 – |      |        |
| BC002439     | Induction of apoptosis             | HIV-1 Tat interactive protein 2, 30 kDa | 2.16 < 0.005 | 1.15 – | 1.01 – | 0.93 – |      |        |
| NM_014362    | Metabolism                        | 3-hydroxyisobutyryl-Coenzyme A hydrolase | 2.14 < 0.005 | 1.04 – | 0.98 – | 1.00 – |      |        |

Each cell line was exposed to several RFs for 96 h. Three different cultures were used in two to four independent RF exposure experiments. *The value is the ratio of gene expression in the RF field-exposure (or thermal stress) to that in the sham-exposure. Some genes (*) were observed to be statistically significantly different at P < 0.05 in the RF field-exposure. A172 cells were treated with thermal stress for 24 hr. Significantly different at P < 0.005 or 0.001 under thermal stress at 41°C.
databases including NetAffx from Affymetrix, SwissProt, and Pubmed. Representative genes for these thermal inducible categories are listed in Table 1. Comparison of the gene expression results of thermal stress-regulated genes in the A172 cells with the respective gene expression profiles of the three human cell lines after exposure to RF showed that RF exposure had only a small effect on both the A172 and H4 cells at a SAR of 800 mW/kg W-CDMA, but no effect in IMR-90 cells (Table 1).

**DISCUSSION**

We evaluated if RF signals induce changes in the gene expression profile, which is not only induced by hyperthermia, but can be triggered by a wide variety of stresses. It is crucial to clearly define the RF exposure period and sampling time point for evaluating the biological responses to RF radiation. The RF exposure period was defined as the time required for one cell to divide (doubling time), so that all cells could divide third time from once during exposure to the RF signals.

*In-vitro* study of the proliferation and transformation of cells is an important way of assessing the influence of RF on cancer promotion and progression. If RF fields are capable of influencing tumor promotion, there is a strong possibility that this will correspond to an increase in the cell division rate. A number of investigations on proliferation have appeared, but no general conclusion can be drawn as the few positive results obtained at low levels were bidirectional.\(^{19-22}\)

Here, we tested the ability of CW and W-CDMA RF fields at 2.1425 GHz, which is the whole-body average SAR for general public exposure, to alter the proliferative response of three cultured human cell lines. Cultures were exposed for periods from 24 to 96 h, which was equivalent to three or four population-doubling levels (PDLs) in each human cell line, and then assayed for proliferation using the number of cells and cell cycle profile. The hypothesis that the tested modulated RF fields might act as a cytotoxicant was tested using two experimental parameters, cellular morphology and cell proliferation profile. No difference in cell morphological character was detected when the exposure took place during the period of incubation. In contrast, we did observe a significant change in the cell proliferation ratio in some tested cell lines, but where two to four independent exposures were performed, statistical significance was not repeated. Furthermore, no substantial effects were identified between sham and exposed cells in terms of the growth ratio or cell cycle profile after exposure to either CW or W-CDMA at SAR levels up to 800 mW/kg. The results confirm that neither CW nor W-CDMA RF exposure at 2.1425 GHz has a critical effect on cell growth at the low levels corresponding to the field around the base station of a cellular system. On the other hand, heat shock treatment induced a marked decrease in cell proliferation ratio at 41°C for 48 and 72 h, but no effect at 39°C for the same time periods (Fig. 2). Our experimental procedures are sensitive enough and properly performed to detect the changes of cell proliferation.

Several papers have reported that the expression of hsps, which play a role in the response to stress stimuli such as heat, UV, or oxidative stress, may be induced in response to RF exposure at non-thermal levels in different models (drosophila and human cells).\(^{3,4,23}\) On the other hand, these studies were unable to verify the hypothesis that GMS RF at 1800 MHz modified expression levels of hsps at low level SARs from 76 mW/kg to 1.4 and 2.0 W/kg.\(^{24}\) In contrast, other groups reported that RF field exposure did not induce expression of hsps.\(^{13,17,25}\) In addition, several studies on gene expression in PC12 rat pheochromocytoma cells,\(^{26}\) C3H 10T 1/2 cells,\(^{27}\) and tumor suppressor p53-deficient embryonic stem cells\(^{28}\) reported a slight increase or decrease (or both) in c-jun, c-fos, c-myc, and hsp70 as early response genes in tested cells exposed to RF fields. Nylund and Leszczynski\(^{29}\) reported that exposure to 900 MHz GSM radiation at 2 W/kg for 1 h did not change gene expression of hsp27 in human endothelial cell lines. Based on these findings, we further investigated whether or not non-thermal exposure of cells to 2.1425 GHz mobile phone radiation activates signal transduction pathways and induces cellular stress response in a human model. To determine unknown mechanisms of action in RF exposure effects, we performed microarray analysis of gene expression in RF-exposed human cells in two tumor (A172 and H4) and a cell line (IMR-90) derived from normal tissue. We used the Affymetrix GeneChip® system, since it covers a wide range of human genes encoding proteins related to the cell cycle and cell proliferation, cell death and apoptosis, response to stress and signal transduction, etc.

Several studies suggested that RF fields exposure caused activation of a cellular stress response as heat shock response.\(^{2,3}\) In the present study, statistically significant increased gene expression was detected in A172 cells stimulated with thermal stress at 41°C, indicating that our system is able to detect gene expression difference in the cells activated by some RF field exposure, if present.

The number of 2-fold change genes was 20 in all of our trials. There likely to be false positives due to statistics, because the raw signal values were low. In general, the values of the gene expression changes were high in cases where their raw signal values were low. With the Affymetrix GeneChip® system however, the expression of only a very small (< 1%) number of available genes (~20,000) was altered in each experiment, and almost all genes exhibiting fold-changes were ESTs or unknown functional genes. However, the same results were not repeated in the two independent trials for exposure to the same RF exposure. Thus, no common effect was observed in any tested cell or under any RF exposure condition.

We found that changes in the gene expression profile in
two types of human cells, which have very different characteristic; A172 and H4 cells are a transformed cell line from adult brain tumor and IMR-90 fibroblasts are an untransformed cell line from fetal lung, was not induced by exposure to 2.1425 GHz W-CDMA signals up to 800 mW/kg. Our experimental results suggested that the exposure to RF signals up to 800 mW/kg could not induce changes in the gene expression profile in many kinds of human cells.

In this study, we evaluated the hypothesis that CW and W-CDMA RF fields at 2.1425 GHz, which corresponds to the center frequency of the IMT-2000 downlink band, alter the cell proliferation-related gene expression profile using three human cell lines; two tumor cell lines and a cell line derived from normal tissue. The variables are known to be influenced by environmental stress factors such as UV, ionizing radiation, chemical oxidants, and high temperatures. Here, we present new findings at the genome-wide gene expression level of encoding proteins related to DNA damage and repair, cell death and apoptosis, response to stress and signal transductions, etc. To the best knowledge of the authors, these have not been previously investigated. The control treatment with heat shock treated cells produced cell damage and showed changes in the related gene expression consistent with that in literature. In contrast, no significant effect on cell proliferation or the gene expression profile was observed for any RF signal tested (2.1425 GHz W-CDMA and CW RF fields at 80 to 800 mW/kg). The findings should contribute to the understanding of the overall evaluation of risks related to the limits of whole-body average SAR level for RF exposure in humans. The results did not indicate any mechanisms that would explain the association between RF exposure and carcinogenesis.

In conclusion, the findings under our experimental conditions used microarray suggest that 2.1425 GHz RF field exposure from mobile radio base station does not appear to effect cancer promotion, progression, or carcinogenesis at the limit of the average whole-body SAR level according to ICNIRP guidelines.

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