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Molecular responses of Jurkat T-cells to 1763 MHz radiofrequency radiation

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Abstract

Purpose: The biological effects of exposure to mobile phone emitted radiofrequency (RF) radiation are the subject of intense study, yet the hypothesis that RF exposure is a potential health hazard remains controversial. In this paper, we monitored cellular and molecular changes in Jurkat human T lymphoma cells after irradiating with 1763 MHz RF radiation to understand the effect on RF radiation in immune cells.

Materials and methods: Jurkat T-cells were exposed to RF radiation to assess the effects on cell proliferation, cell cycle progression, DNA damage and gene expression. Jurkat cells were exposed to 1763 MHz RF radiation at 10 W/kg specific absorption rate (SAR) and compared to sham exposed cells.

Results: RF exposure did not produce significant changes in cell numbers, cell cycle distributions, or levels of DNA damage. In genome-wide analysis of gene expressions, there were no genes changed more than two-fold upon RF-radiation while ten genes change to 1.3 $\sim$ 1.8-fold. Among ten genes, two cytokine receptor genes such as chemokine (C-X-C motif) receptor 3 (CXCR3) and interleukin 1 receptor, type II (IL1R2) were down-regulated upon RF radiation, but they were not directly related to cell proliferation or DNA damage responses.

Conclusion: These results indicate that the alterations in cell proliferation, cell cycle progression, DNA integrity or global gene expression was not detected upon 1763 MHz RF radiation under 10 W/kg SAR for 24 h to Jurkat T cells.

Keywords: *Radiofrequency radiation, cell proliferation, DNA damage, cell cycle, microarray, gene expression*

Introduction

The number of mobile phone users continues to expand throughout the world, raising concerns that growing exposure to radiofrequency (RF) radiation from mobile phones poses a serious risk to public health. In order to determine if RF exposure is a health hazard, studies have analyzed whether it increases tumor incidence, or interferes with immune system or brain functions (Smialowicz et al. 1981, Imaida et al. 1998, Chagnaud et al. 1999, Chagnaud and Veyret 1999, Higashikubo et al. 1999, Preece et al. 1999, Adey et al. 2000, Sienkiewicz et al. 2000, Zook and Simmens 2006). While these studies failed to firmly link RF exposure to tumorigenesis, or impairment of immune or brain functions, additional studies suggested that RF radiation could produce detrimental biological changes at the cellular level.

RF exposure has been found to affect cell cycle progression, cell proliferation and to produce DNA damage in certain cell types. For example, 2 W/kg of 900 MHz RF radiation increased the basal cell proliferation rate of rat skin cells (Ozguner et al. 2004). In direct contrast, similar levels of RF exposure did not increase the proliferation rate of primary rat glial cells, C6 rat glioma cells, C3H 10T1/2 mouse embryonic fibroblasts, or U87MG

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human glioma cells (Higashikubo et al. 1999). The ability of RF radiation to produce DNA damage is also controversial and variations may depend upon experimental conditions and model systems. A comprehensive review of studies from 1990–2003 reporting on RF exposure and DNA damage found that 23% reported increases in DNA damage, including DNA strand breaks, incidence of chromosomal aberrations, micronuclei, and sister chromatid exchanges (Vijayalaxmi and Obe 2004). However, the majority of publications reported that RF exposure did not produce DNA damage, and the remaining 19% of studies were inconclusive (Vijayalaxmi and Obe 2004). The variable ability of RF exposure to produce DNA damage in these studies may depend on cell type, exposure time, or radiation dose.

In order to resolve the conflicting results, the appropriate cellular model system and exposure conditions must be chosen. In addition, new techniques like Microarray gene expression profiling, can provide valuable molecular information about changes produced by RF exposure that may underlie pathological conditions. For example, microarray analysis was successfully used to detect alterations in the expression pattern of genes related to RF radiation in HL-60 cells exposed to 2.45 GHz of RF radiation (Lee et al. 2005). In an effort to find the molecular responses to 1763 MHz RF radiation, we irradiated Jurkat T cells at 10 W/kg specific absorption rate (SAR) with the maximum power of our exposure chamber. In particular, we wanted to check the effect of RF radiation to immune cells by monitoring gene expression using microarray.

Materials and methods

Cell culture and RF exposure

Jurkat cells were grown in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) (GIBCO-BRL, Invitrogen Corp., Carlsbad, CA, USA), 100 mg/ml streptomycin, and 100 U/ml penicillin (Invitrogen, Carlsbad, CA, USA) at 37°C in a 5% CO_2 incubator (Forma Scientific, Marietta, OH, USA).

The conditions for RF exposure were as described previously (Lee et al. 2006a). For the RF exposure system, a real Code Domain Multiple Access (CDMA) signal at 1762.5 MHz was applied. Cells in 100 mm Petri dishes containing 18 ml of growth medium were exposed to 1763 MHz RF radiation. The exposure system was first equilibrated to 37°C for 30 min without cells. RF radiation exposure was then conducted at SAR values of 2 W/kg or 10 W/kg. During exposure, the temperature in the chamber was maintained at 37 ± 0.2°C by circulating water within the cavity. After exposure for up to 24 h, the cells were immediately harvested or transferred to a cell culture incubator for the indicated time prior to harvesting. For the sham exposure, the cells were incubated in the RF radiation device, but were not exposed to RF radiation.

Cell proliferation and cell cycle analysis

Jurkat cells were seeded at a density of 1×10^5 cells/ml and exposed to 2 W/kg or 10 W/kg SAR of 1763 MHz RF radiation. Cells were exposed for 1 hour per day for 1, 2, or 3 days. After the last exposure, cells were incubated for 24 h before harvesting.

The cells were counted with a Coulter Counter®, and the numbers of harvested cells were compared to seeded cells as a measure of cell proliferation (Beckman Coulter, Inc., Fullerton, CA, USA).

For cell cycle analysis, cells were washed with chilled Phosphate Buffered Saline (PBS, 150 mM NaCl, 10 mM Phosphate, 2.5 mM KCl, pH 7.4) and fixed overnight with 70% ethanol at 4°C Fixed cells were washed with chilled PBS and resuspended in a final volume of 1 ml of PBS. Fifty μl of 100 μg/ml DNase-free RNase (Gentra, Minneapolis, MN, USA) was added and incubated at 37°C for 30 min. DNA was then stained by adding 200 μl of 50 μg/ml propidium iodide (Sigma, St Louis, MO, USA) for 5–10 min. The stained cells were analyzed for DNA content by flow cytometry using a FACSCalibur™ flow cytometer (Becton Dickinson, Hunt Valley, MD, USA).

Comet assay

Comet assays were performed as previously described with the following modifications (Kim et al. 2006). Solutions of 1% normal melting point agarose (NMA) (Ameresco, Solon, OH, USA) and 0.5% or 1% low melting point agarose (LMA) (Ameresco, Solon, OH, USA) were dissolved in PBS. Dissolved NMA was kept 65°C and dissolved LMA was kept at 37°C. Slides were coated with 50 μl of 1% NMA, followed by 100 μl of 1% NMA. Cover slips were added and the slides incubated at 4°C for 5 min. Cover slips were then removed and 50 μl Jurkat cells, followed by 50 μl of 1% LMA, were layered on the slides. New coverslips were added and the slides were incubated at 4°C for 5 min. The coverslips were removed and final layer 100 μl of 0.5% LMA was added before a final incubation at 4°C for 5 min. These slides were submersed in a lysis solution (2.5 M NaCl, 100 mM ethylenedinitrilotetraacetic acid (EDTA), 10 mM Tris–HCl, pH 10; 1% Triton X-100 and 10% dimethyl sulfoxide (DMSO), pH 10) overnight at 4°C. After lysis, the slides were

placed into Unwinding Buffer (UB) (1 mM EDTA, 300 mM NaOH, pH 13) for 20 min, and electrophoresed in UB for 20 min at 25 V and 300 mA (0.8 V/cm). After electrophoresis, the slides were neutralized with neutralization buffer (400 mM Tris–HCl, pH 7.4) and stained with 50 μl of 10 μg/ml ethidium bromide (Sigma, St Louis, MO, USA). Stained slides were examined using a Komet 4.0 image analysis system (Kinetic Imaging, Liverpool, UK), fitted with an Olympus BX50 fluorescence microscope, equipped with an excitation filter of 515–560 nm and a barrier filter of 590 nm. For each treatment group, two slides were prepared and 50 randomly chosen cells (total 100 cells) were scored manually. Three parameters were calculated automatically using the Komet 4.0 image analysis system. Tail length (μm) is the distance of DNA migration from the center of the body of the nuclear core and is used to evaluate the extent of DNA damage. Tail DNA (%) is the percentage of the genomic DNA that migrates during electrophoresis from the nuclear core to the tail. Both tail length and tail intensity are measured automatically by image analysis software. Olive tail moment was calculated according to the following formula:

$$\text{Tail moment} = (\text{tail mean} - \text{head mean}) \times \text{tail DNA}/100.$$

These were used for a global comet description.

Statistical analysis

Each sample were characterized for the extent of DNA damage by considering the mean $\pm$ standard deviation (SD) for the comet parameters measured in three independent experiments. In order to normalize distribution and to equalize the variance, a logarithmic transformation of data was applied. The Wilcoxon signed rank test was used to analyze the effect of RF radiation on DNA damage. For cell cycle and cell proliferation, we used Friedman test, a non-parametric statistical test to detect any difference between sham and exposure groups.

Microarray analysis

Five biologically replicated samples in each group were harvested in triplicates. RNA was extracted with TriZol (Invitrogen, Carlsbad, CA, USA) and purified using Qiagen RNAeasy columns (Qiagen, Valencia, CA, USA) (Park et al. 2002). We used the Applied Biosystems 1700 full genome expression human microarray (Applied Biosystems, Foster City, CA, USA) which includes 30,000 human genes from the public and Celera databases (http://www.pantherdb.org/). Fluorescence intensity was processed and measured using the Applied Biosystems 1700 Chemiluminescent Microarray Analyzer. Intensity data were imported to an in-house microarray database as described previously (Lee et al. 2006b). To perform reliable microarray data analysis, some probes were filtered out based on unreliable intensities. A final set of 16, 215 probes were defined as reliable based on flag information and high signal to noise intensity ratios (S/N).

Quantile normalization was applied to remove systematic variance between arrays in a set of arrays. Quantile normalization was chosen because it reduces the variance slightly better than Lowess normalization (Bolstad et al. 2003). To identify genes whose expression levels are associated with an exposure to RF radiation, the normalized expression values were analyzed using statistical tests based on *t*-statistics. A Multiple testing procedure for controlling the expected proportion of falsely rejected hypotheses, defined as the false discovery rate (FDR) (Hochberg and Benjamini 1990), was applied using Multi-test R package. The multiple testing procedure is based on the proportion of false positive rates, and is therefore more appropriate for large scale statistical analysis of genomic data than reporting error rates based on the number of false discoveries (Dudoit et al. 2004).

Results

RF radiation does not alter cell proliferation rates or cell cycle distributions

To determine if RF radiation affects cell proliferation rates or cell cycle progression, log-phase Jurkat cells were exposed to RF radiation and then analyzed for changes in number and DNA content. Cells were exposed to 1763 MHz RF radiation at 2 W/kg or 10 W/kg SAR for 1 h per day for three days. After RF exposure, cells were incubated for 24 h before harvesting and counting. In the absence of RF exposure, the cell numbers of sham group increased with population doubling in every 24 h (Figure 1). When we compared the cell numbers of 2 W/kg and 10 W/kg RF-exposure group to sham group, either one did not show significant alterations in cell numbers in all pairs of exposure groups of any time period according to Friedman tests ($p = 0.202$) (Figure 1). Furthermore, the RF exposure did not significantly affect the distribution of cells in sub-G0/1, G0/G1, S, and G2/M phases of the cell cycle as detected by measuring DNA content by flow cytometry (Figure 2). Comparing RF-exposed and sham-exposed groups by Friedman tests, no statistically significant changes were observed in all pairs of exposure groups of any time period ($p = 0.497$) (Figure 2).

RF radiation exposure does not produce gross DNA damage

Comet assay were carried out to evaluate DNA damage in Jurkat T cells exposed to 10 W/kg SAR of 1763 MHz RF radiation once for 24 h. Three comet assay parameters (olive tail moment, tail DNA, and tail length) were measured, as shown in Table I. Although the mean value of the olive tail moment after 1 h of sham exposure was higher than that of control, the mean value of the olive tail moment of RF-exposed group was not increased in comparison to that of control group. Cells harvested after 4 and 24 h of RF exposure had no observable alterations in the three measured parameters. Using Wilcoxon signed rank test analysis between sham- and RF-exposed groups we could not find any statistically significant alteration in the parameters of comet assay.

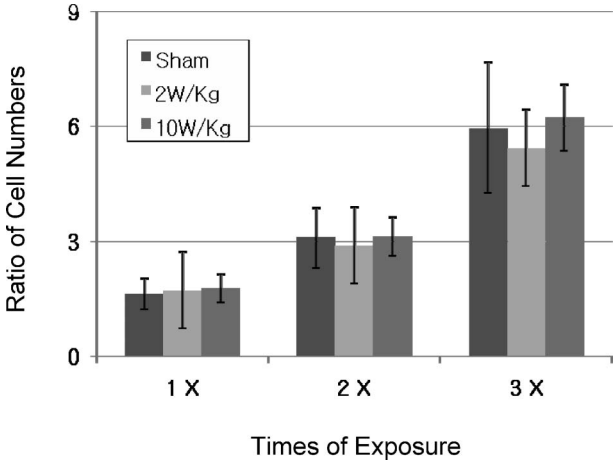

Figure 1. The biological effects of 1763 MHz RF radiation on cell proliferation. Jurkat cells were exposed to RF radiation for one hour per day and the number of cells counted 24 hours after the last exposure. Graphs represent the average values and standard deviations for three independent experiments. The cell numbers of each group (Sham, 2 W/kg and 10 W/kg) were not significantly different by Friedman tests ($p = 0.202$).

RF radiation alters the gene expression profile of Jurkat cells

In an effort to characterize the cellular responses to RF radiation in Jurkat cells, we used the Applied Biosystems 1700 full genome expression human microarray system to examine the gene expression profiles of exposed cells. We made five biological replicates of sham-treated cells and cells exposed to 10 W/kg SAR of 1763 MHz RF radiations once for 24 h. From 10 microarray experiments, 16,215 probes were selected as reliable, but no genes were

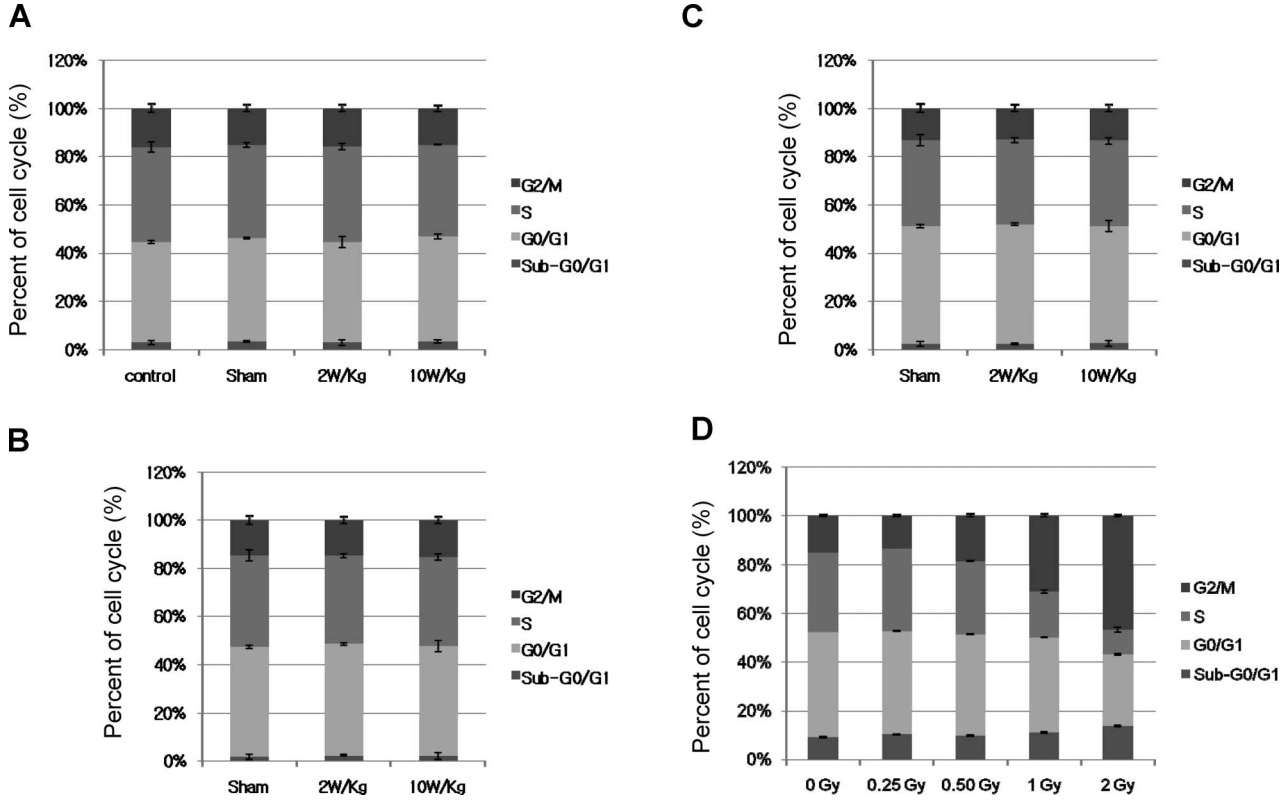

Figure 2. The biological effects of 1763 MHz RF radiation on cell cycle distribution. Jurkat cells were exposed to 2 or 10 W/kg SAR for one hour per day, for 1 day (A), 2 days (B), or 3 days (C). Flow cytometric profiles are presented in average values of each cell cycle with standard errors for six independent experiments ($n = 6$). The percentages of cells in each stage of the cell cycle were not statistically different between groups by Friedman tests ($p = 0.497$).

Table I. Gross anaylsis of DNA damage by comet assay of Jurkat T cells exposed to 10W/kg SAR of 1763 MHz RF radiations.

Exposure group	Hour after exposure	Tail DNA	Olive tail moment	Tail Length
Control	1	9.65 ± 0.24	0.58 ± 0.03	17.32 ± 0.27
	4	9.75 ± 0.28	0.59 ± 0.02	17.11 ± 0.28
	24	9.12 ± 0.33	0.56 ± 0.01	17.67 ± 0.29
Sham	1	14.11 ± 0.41	1.02 ± 0.02	17.91 ± 0.14
	4	9.14 ± 0.28	0.46 ± 0.01	16.26 ± 0.05
	24	7.77 ± 0.38	0.35 ± 0.02	12.99 ± 0.24
RF (10 W/kg)	1	9.53 ± 0.32	0.57 ± 0.02	16.27 ± 0.04
	4	9.52 ± 0.43	0.56 ± 0.02	16.11 ± 0.27
	24	9.31 ± 0.20	0.74 ± 0.02	15.56 ± 0.14
H_2O_2 (200 μM)	4	16.96 ± 1.18	2.62 ± 0.25	40.63 ± 3.18

Each data point represents the mean ± standard error of means (SEM) of three independent experiments.

Table II. List of genes up- or down-regulated > 1.3-fold after RF radiation ($p < 0.1$).

Symbol	Gene name	Log2 [Activation Fold Ratio]	p value
Down-regulated genes (5 genes)			
CXCR3	chemokine (C-X-C motif) receptor 3	−0.850	0.035
ATAD3C	ATPase family, AAA domain containing 3C	−0.758	0.045
hCG2019717	ABI clone	−0.555	0.046
IL1R2	interleukin 1 receptor, type II	−0.533	0.072
CHD7	chromodomain helicase DNA binding protein 7	−0.449	0.001
Up-regulated genes (5 genes)			
hCG2042600	ABI clone	0.414	0.071
TRBV5-4	T cell receptor beta variable 5–4	0.419	<0.001
COX17	COX17 homolog, cytochrome c oxidase assembly protein (yeast)	0.457	<0.001
LSM4	LSM4 homolog, U6 small nuclear RNA associated (*S. cerevisiae*)	0.470	0.046
KRTAP10-12	keratin associated protein 10–12	0.583	0.054

changed more than two-fold in their expressions upon RF radiation. In further analysis we lowered the cutoff of activation fold ratio to 1.3-fold to find 10 genes in RF-exposed cells ($p < 0.1$) (Table II). Ten differentially expressed genes (DEG) represent only 0.06% of the 16,215 tested genes. Five genes such as chemokine (C-X-C motif) receptor 3 (CXCR3), ATPase family, AAA domain containing 3C (ATAD3C), hCG2019717 (ABI clone number), interleukin 1 receptor, type II (IL1R2), chromodomain helicase DNA binding protein 7 (CHD7) were down-regulated. On the other hand, five genes like hCG2042600 (ABI clone number), T cell receptor beta variable 5–4 (TRBV5-4), COX17 homolog cytochrome c oxidase assembly protein (yeast) (COX17), LSM4 homolog, U6 small nuclear RNA associated (S. cerevisiae) (LSM4), keratin associated protein 10–12 (KRTAP10-12) were up-regulated in Jurkat T cells exposed to RF radiation (Table II). Notably, two of the DEG were cytokine receptors, CXCR3 (1.8-fold decrease, $p = 0.035$) and IL1R2 (1.4-fold decrease, $p = 0.072$).

Discussion and conclusion

Although most direct studies have not established RF exposure as a risk factor for cancer development (La Regina et al. 2003, Huang et al. 2005, Zook and Simmens 2006), the reported cellular effects of RF exposure have established that the health risks of RF radiation are still controversial. In addition, our understanding of the health risks associated with RF radiation are further complicated by conflicting results of studies on the ability of RF radiation to affect cell proliferation, cell cycle progression, and DNA damage. For example, in neuroblastoma cells exposed to 900 MHz RF radiation at 1 W/kg of SAR for up to 72 h, WST-I assays showed that RF exposure does not induce significant alterations in cell proliferation (Merola et al. 2006). Similarly, cell cycle analysis or BrdU incorporation also showed no differences between RF- or sham-exposed Mono Mac 6 cells exposed to 1800 MHz RF radiation at 2 W/kg of SAR for 72 h (Lantow et al. 2006). Exposure of human peripheral blood leukocytes to 900 MHz RF SAR levels of 0.3 and 1 W/kg used as exposure conditions did not induce DNA damage (Zeni et al. 2005). DNA damage was also undetectable in Molt-4 cells exposed to CDMA, Frequency Division Multiple Access (FDMA), Integrated Digital Enhanced Network (iDEN), or Time Division Multiple Access (TDMA) modulated RF radiation (Hook et al. 2004). In contrast to these negative findings, 1800 MHz RF radiation from mobile phones significantly increased DNA single-strand and double-strand breaks in human diploid fibroblasts and in rat granulosa cells in culture (Diem et al. 2005). We exposed cells at 10 W/kg for 24 h, which condition is much harder than other condition. Even at 10 W/kg SAR, we could hardly detect cellular and molecular changes upon RF radiation.

In the present study, cell proliferation and cell cycle progression were analyzed for Jurkat cells

exposed to 2 or 10 W/kg SAR of 1763 MHz RF radiation for 1 h per day for up to 3 days. No significant differences between RF exposed cells and sham-treated cells were detectable under these conditions. This result appears contrary to the finding that the cell cycle regulatory growth arrest and DNA-damage-inducible (GADD45) gene was up-regulated in embryonic stem cell-derived neural progenitor cells after RF radiation exposure (Nikolova et al. 2005). However, the GADD45 gene up-regulation was not associated with a detectable change in cell physiology (Nikolova et al. 2005). Cell cycle distribution was also measured in monocytic U937 and neuroblastoma SK-N-SH cells after Global System of Mobile communications (GSM) exposure, which resulted in no significant difference between sham-exposed and RF-exposed cells (Gurisik et al. 2006). These negative results were confirmed in our study using Jurkat cells.

Alkaline comet assay was developed to detect DNA strand breakage of single cells by electrophoresis in alkaline condition (pH $>$ 13) (Olive et al. 1991, Collins 2002). This method can provide quite sensitive tools to detect DNA damages because it can measure the wide variety forms of DNA damages including double strand breaks (DSB), single strand breaks (SSB), alkali labile sites, oxidative DNA base damage, DNA-DNA/DNA-protein/DNA-Drug cross-linking and DNA repair (Abt et al. 1997). Moreover, comet assay is quite sensitive to detect 50–100 breaks per cell (Olive and Banáth 2006). The alkaline comet assay has been used to determine whether DNA damage was induced in C3H 10T1/2 mouse fibroblasts and U87MG human glioblastoma cells exposed to RF (Malyapa et al. 1997). RF exposure did not induce DNA damage immediately after exposure or 4 h post exposure in these cell lines (Malyapa et al. 1997). These results were confirmed in the present study. To detect DNA damage in Jurkat cells exposed to 1763 MHz RF radiation, comet assays were performed after 24 h of RF exposure at a SAR level of 10 W/kg. Our results also showed no detectable DNA damage in RF-exposed Jurkat cells. This appears to contradict a report by Lai and Singh that 2450 MHz RF radiation increased DNA single-strand breaks in rat brain cells (Lai and Singh 1995). However, this study was later independently repeated, and the results did not confirm that DNA damage was induced in cells of the cerebral cortex or the hippocampus after a two hour exposure to 2450 MHz RF radiation (Malyapa et al. 1998). In our comet assay, 1763 MHz RF radiation at a SAR level of 10 W/kg did not induce any detectable DNA damage in Jurkat T cells in comparison to sham and control groups. Although the possibility of microscopic lesions in DNA cannot be ruled out, we could not find any evidence supporting DNA damage upon 1763 MHz RF radiation.

Microarray analysis of RF- exposed cells consistently shows that there are no statistically significant changes in global gene expression after exposure to RF radiation. In the experiments with C3H 10T 1/2 mouse fibroblasts exposed to 5 W/kg SAR to CDMA RF radiation for 24 h, the number of genes with altered expression was less than that of false positives (Whitehead et al. 2006). Another study using SK-N-SH human neuroblastoma and U937 human monocytic leukemia cells showed that exposure to 900 MHz RF signal at 0.2 W/kg for 2 h down-regulated only six of 8,400 genes tested (Gurisik et al. 2006), which was not statistically significant. Our studies produced similar results. Treatment of Jurkat cells with 1763 MHz RF radiation at 10 W/kg for 24 h did not produce statistically significant changes in global gene expression.

While RF exposure does not alter the global level of gene expression, alterations in ten individual genes were detectable in RF-exposed Jurkat cells including CXCR3, ATAD3C, hCG2019717, IL1R2, CHD7, hCG2042600, TRBV5-4, COX17, LSM4, and KRTAP10-12. CXC motif chemokine receptor 3 (CXCR3) is the receptor for interferon (IFN)-inducible protein-10 (IP10, or CXCL10) and IFN-γ-induced monokine-2 (MIG, or CXCL9). Binding of CXCR3 to these ligands induces the chemotaxis of activated lymphocytes (Loetscher et al. 1996). Mice lacking CXCLl10 exhibit enhanced susceptibility to experimental autoimmune encephalomyelitis (EAE) (Liu et al. 2006). The level of CXCR3 mRNA was decreased to 1.8-fold in RF-exposed Jurkat T cells. In addition to CXCR3, a second cytokine receptor called interleukin 1 receptor type II (IL1R2) was also down-regulated in RF-exposed Jurkat cells. IL1R2 can inhibit IL1 activity by acting as a decoy target for IL1 (Colotta et al. 1993). Soluble IL1R2 found in the serum of endometriosis patients can block the secretion of monocyte chemotactic protein-1 secretion of monocytes (Kharfi and Akoum 2002). In summary, the expression of two cytokine receptor genes was decreased in RF-exposed Jurkat cells, and this finding suggests that RF exposure can influence the chemotaxis of various immune cells. Further study is needed to quantify the level of CXCR3 and IL1R2 transcripts in RF-exposed Jurkat cells and RF-exposed animal models, and to determine if these changes in cytokine receptor gene expression produce immunological disturbances in RF-exposed animals.

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