Radiofrequency Radiation and Gene/Protein Expression: A Review

Authors: McNamee, J. P., and Chauhan, V.

Source: Radiation Research, 172(3) : 265-287

Published By: Radiation Research Society

URL: https://doi.org/10.1667/RR1726.1

RADIATION RESEARCH **172**, 265–287 (2009)
0033-7587/09 $15.00

DOI: 10.1667/RR1726.1

REVIEW

Radiofrequency Radiation and Gene/Protein Expression: A Review

J. P. McNamee[1] and V. Chauhan

Consumer and Clinical Radiation Protection Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario, Canada, K1A 1C1

McNamee, J. P. and Chauhan, V. Radiofrequency Radiation and Gene/Protein Expression: A Review. *Radiat. Res.* 172, 265–287 (2009).

Mobile telecommunications have developed considerably in recent years. With the proliferation of wireless technologies, there is much public anxiety about the potential health impact associated with exposure to radiofrequency (RF) radiation from these novel products. Contradictory scientific evidence, often reported in the popular media, has further fueled public concern. Some epidemiological studies have reported that ipsilateral use of a mobile phone is associated with an increased risk for brain tumors, while other studies have reported an association between brain tumor risk and mobile phone use for longer than 10 years. However, other large epidemiological studies have failed to find similar associations. Despite the existence of national and international RF-radiation exposure guidelines, there are increasing public demands for precaution with respect to human exposure to RF radiation. Since current epidemiological evidence is insufficient to make a definitive judgment on the health risks of low-level RF radiation exposure, laboratory evidence assessing biological plausibility and theoretical mechanisms of interaction are important. A number of studies have reported that RF radiation may induce alterations in gene/protein expression in a variety of cells/tissues that may be associated with potentially harmful health outcomes, while other studies have shown no clear effects related to RF radiation. This review focuses on the current scientific evidence related to changes in protein and gene expression induced by low-level RF radiation.

INTRODUCTION

Electromagnetic radiation is emitted by many natural and man-made sources. We are warmed by electromagnetic radiation emitted from the sun and our eyes can detect the visible light portion of the electromagnetic spectrum. Radiofrequency (RF) radiation is a portion of the electromagnetic spectrum with frequencies ranging from 3 kHz to 300 GHz, below that of visible light and above that of extremely low-frequency (ELF) fields. RF radiation is produced by many man-made sources, including mobile phones and base stations, television and radio broadcasting facilities, radar, medical equipment, microwave ovens and radiofrequency heaters as well as a diverse assortment of other electronic devices within our living and working environments.[2]

Sufficiently intense RF radiation can cause heating of materials with finite conductivity, including biological tissues. A number of well-established biological effects and adverse health effects from acute exposure to intense RF radiation have been documented (*1, 2*). For the most part, these effects relate to localized heating or stimulation of excitable tissue from intense RF-radiation exposure. The specific biological responses to RF radiation are generally related to the rate of energy absorption. The rate and distribution of RF-radiation energy absorbed depend strongly on the frequency, intensity and orientation of the incident fields as well as the body size and the constitutive properties of the tissues (dielectric constant and conductivity). At frequencies above 1 MHz, absorption of RF radiation is commonly described in terms of the specific absorption rate (SAR), which is a measure of the rate of energy deposition per unit mass of body tissue and is usually expressed in units of watts per kilogram (W/kg). Based on a large amount of historical knowledge, national and international exposure limits have been established to protect the general public against adverse effects associated with acute RF-radiation exposures (*3, 4*). However, the safety of exposure to long-term, low-level RF radiation remains controversial and the risk of development of cancer remains a primary public health concern.

[1] Address for correspondence: Consumer and Clinical Radiation Protection Bureau, Health Canada, 775 Brookfield Road, PL 6303B, Ottawa, Ontario, Canada, K1A 1C1, e-mail: James_McNamee@hc-sc.gc.ca.

[2] Portions of the Introduction have been reproduced from "Chromosomal Alterations: Methods, Results and Importance in Human Health" (2007), pp. 427–431, with kind permission from Springer Science + Business Media.

The scientific literature on the possible health implications of RF radiation is full of conflicting results, and the question of whether exposure can contribute to cancer risk remains unresolved (*5, 6*). Since the energy per photon produced by RF radiation is not considered strong enough to induce direct chemical changes in the cells, biological effects produced by RF radiation are most likely subtle and indirect. Proposed changes in cellular functions by RF radiation include inhibition of DNA synthesis, transcription, RNA processing and translation, inhibition of cell cycle progression, denaturation of proteins, alterations in cellular metabolism, and changes in membrane permeability. One proposed mechanism suggests that RF radiation may act as a stressor, inducing chronic overexpression of heat-shock proteins (HSPs) that may influence the risk of cancer (*7*). Research into these effects has recently led to some controversial findings. This article reviews the current state of the literature with respect to possible effects on gene and/or protein expression after exposure to RF radiation. Since the thermal effects of RF radiation are well understood, this review will focus on the evidence for such changes from low-level, non-thermalizing RF radiation.

Challenges when Conducting RF-Radiation Research

Researchers must overcome several challenges when attempting to conduct high-quality RF-radiation bioeffects research that are related to the physical properties of this form of radiation. Of primary importance is the interaction between RF radiation and matter. Rotational friction is caused when small polar molecules (such as those in water and biological tissues) reorient themselves in the presence of the oscillating RF field, thereby producing heat. The rate at which heat energy is applied to the sample must not exceed the rate at which it is removed; otherwise the temperature within the sample will rise and thermal confounding may occur. If RF-radiation energy is applied to biological samples at a low rate (e.g. <1 W/kg) or for a short duration, then passive cooling and/or sweating may permit the tissue/body/sample temperature to remain within a normal physiological range. However, if higher SARs (>1 W/kg) are employed and/or prolonged, then some form of active cooling mechanism may be required to ensure that excessive sample heating does not occur. When performing RF-radiation experiments, it is crucial to include appropriate temperature controls within the experimental setup to avoid thermal confounding in the study results. To this end, the use of non-perturbing temperature probes and active cooling with either forced air or water is particularly beneficial.

Another significant challenge that must be addressed when studying the biological effects of RF radiation relates to the homogeneity of energy absorption within the sample (SAR distribution) and the maintenance of a relatively homogeneous, non-perturbing temperature throughout the biological sample. These are two separate yet related phenomena. While controlling volume-averaged temperature within the biological sample is important, it is also important to ensure that RF-radiation energy is deposited in a homogeneous manner, or "hot spots" can occur within the biological sample. This phenomenon is akin to focusing light energy on an object, whereby the localized temperature at the focal point is considerably elevated while the surrounding area is not. In such a situation with RF radiation, the local temperature for some part of the sample may be greatly elevated while the volume-averaged temperature remains relatively unaffected due to heat convection and diffusion. With some *in vitro* exposure systems, heterogeneous RF-radiation absorption can also cause convective flow within aqueous biological samples due to the presence of temperature gradients within the sample. Such convection currents in an RF-radiation-exposed sample can be a source of artifacts.

Since RF radiation behaves in a similar fashion as light with respect to its ability to reflect off of metallic surfaces, care must be taken to ensure that samples are exposed in a reverberant-free environment or at least that all sources of reflection are taken into account in the dosimetry analysis. This is particularly problematic for *in vitro* studies, where cells must be maintained under appropriate conditions (37°C, 50–60% humidity, 5% CO_2 in air) in a tissue culture incubator. Most incubators are built with a stainless-steel coated interior that acts as an ideal reflector for RF radiation. As a result, when conducting *in vitro* RF-radiation research, great care must be taken either to contain RF radiation within a closed exposure system inside a standard tissue culture incubator or to design alternative non-RF radiation perturbing tissue culture environments. In either case, maintenance of sample temperature, humidity, osmolarity and pH is a challenge and must be carefully controlled.

Due to the above considerations, it is critical that RF-radiation bioeffects studies encompass certain experimental controls to ensure that confounding variables do not result in artifactual biological observations. For *in vitro* studies, monitoring the temperature, pH and cell viability within the sample is paramount. For both *in vivo* and *in vitro* studies, empirical and/or numerical analysis of SAR distribution patterns within the sample is fundamental. The inclusion of unexposed (negative) controls, sham (non-RF-radiation-exposed) controls, and positive controls are imperative to ensure that the assay methodology is responding appropriately and that experimental conditions are identical. Inclusion of these controls is particularly important when evaluating studies where RF-radiation-induced effects were ob-

served. On the other hand, analysis of statistical power in studies where no evidence of RF-radiation-induced effects were observed is important to determine whether a sufficient number of samples (independent experiments) were run to allow the detection of significant differences between groups. For all studies, the investigators should be blinded with respect to the exposure status of the samples until all laboratory experiments, data acquisition and statistical analyses are complete.

In summary, when evaluating the literature related to RF-radiation-induced effects, all of the above considerations must be taken into account. This paper is not only a review but also a critique of the literature, highlighting certain deficiencies in the design of some of the studies that should be taken into account when evaluating the significance of certain RF-radiation research findings.

CURRENT STATE OF KNOWLEDGE

Studies Assessing the Effects of RF Radiation on Heat-Shock Proteins (HSPs)

To date, the majority of RF-radiation research has focused on identifying sensitive stress markers of RF-radiation-induced effects. The ''stress proteins'', also known as the heat-shock proteins (HSPs), are one group of proteins that have been reported to be affected by RF-radiation emissions. HSPs are a family of chaperone proteins that are found in all cell types and are highly conserved and abundantly expressed with diverse functions. They are expressed in response to cold, heat and other environmental stresses, although some are constitutively expressed. HSPs increase heat tolerance and perform functions essential to cell survival under these conditions. Some HSPs serve to stabilize proteins in abnormal configurations, while others play a role in the folding and unfolding of proteins, acting as molecular chaperones. HSPs are also believed to act as major immunogens in many infections and disease states (*8*). Stress-induced transcription of HSPs requires activation of heat-shock factors (*9, 10*) that bind to the heat-shock promoter element, thereby activating its transcription activity. Because HSPs and their associated factors are induced by a variety of stressors, they have been proposed as possible biomarkers of RF-field exposure. French *et al.* (*7*) hypothesized that repeated exposure to RF radiation may act as a repetitive stressor, leading to continuous overexpression of HSPs in exposed cells and tissues.

Table 1 provides a list of studies that have assessed the effect of RF radiation on HSP gene and protein expression. One of the earliest studies in this area of research was conducted by Parker *et al.* (*11*). In this study, L5178Y and Chinese hamster ovary (CHO) cells were exposed for 3–20 min to 2.45 GHz continuous-wave (CW) RF radiation at extremely high SARs of 51.75 and 103.5 W/kg. The investigators measured temperature within the sham- and RF-field-exposed samples and conducted experiments at culture temperatures ranging from 37–45°C. Despite the high SARs, the investigators did not observe any detectable RF-field-induced changes in mRNA expression for HSP70 and a variety of proto-oncogenes (v-fos, v-myc, v-H-ras) using Northern blot analysis when compared to the corresponding sham temperature controls. However, it was unclear whether any temperature changes occurred in the exposed flasks relative to the sham-exposed flasks, and there was no indication that any statistical analysis was performed on the data. After this work, Fritze *et al.* (*12*) examined HSP70 response in the central nervous system of rats exposed to 900 MHz global system for mobile communication (GSM)-modulated RF radiation for 4 h at SARs of 0.3, 1.5 and 7.5 W/kg. Rectal temperature monitoring indicated no detectable temperature change at the highest dose tested (7.5 W/kg). Immediately after exposure, a slight induction of HSP70 mRNA (but not protein) was observed in the cerebellum and hippocampus at 7.5 W/kg exposures but not at lower levels. Twenty-four hours after exposure, immunohistochemical analysis of HSP70 did not reveal any alterations. The authors concluded that acute high-intensity radiofrequency-field exposure of rats may induce some minor stress response in the thermal range but does not result in lasting adaptive changes in the brain. Cleary *et al.* (*13*) assessed protein expression levels as a possible ''biomarker'' of RF-field-induced stress effects. HeLa cells were exposed for 2 h at mean SARs of 25 W/kg to 27 or 2450 MHz CW RF radiation while CHO cells were exposed for 2 h to 27 MHz CW RF radiation at an SAR of 100 W/kg. Temperature measurements indicated that RF-field-exposed samples were maintained at 37.0 ± 0.1°C. The authors evaluated protein expression at 24 h after RF-field exposure and observed no RF-field-induced effects on HSP70 or on lower-molecular-weight stress protein induction.

A limited number of studies have shown that RF radiation may cause a strong induction of the stress proteins using unique *in vivo* model systems. In a study that was later withdrawn, De Pomerai *et al.* (*14*) reported that when transgenic *Caenorhabditis elegans* (containing β-galatoctosidase or GFP reporter genes) were exposed overnight to 750 MHz RF radiation at an SAR of ~0.001 W/kg, HSP16 reporter gene activity was elevated. The authors suggested that this might have been a non-thermal bioeffect. However, in a later study, the authors retracted their original interpretation of a non-thermal effect, because they observed that their exposure conditions actually caused a 0.2°C change in culture temperature and that in the absence of RF radiation such changes in temperature were capable of causing a similar induction of HSP16-reporter gene activity (*15*). Weisbrot *et al.* (*16*) exposed *Drosophila*

TABLE 1
Summary of Studies Assessing the Effect of RF Radiation on Heat-Shock Proteins (HSPs)

Reference	Tissue/cell line(s)	Signal(s)	SAR(s)
Parker *et al.* (*11*)	L5178Y, RAW 264.7 P388-D1, CHO cells	2.45 GHz, CW	51.75 or 103.5 W/kg
Fritze *et al.* (*12*)	male Wistar rats	900 MHz, CW and GSM	0.3 W/kg (GSM), 1.5 W/kg (GSM), 7.5 W/kg (CW)
Cleary *et al.* (*13*)	HeLa cells CHO cells	27 MHz, CW 2450 MHz, CW	25 or 100 W/kg
De Pomerai *et al.* (*14*)	*Caenorhabditis elegans*	750 MHz, CW	~0.001 W/kg
Leszczynski *et al.* (*21*)	EA.hy926 cells	900 MHz, GSM	~2 W/kg
Tian *et al.* (*18*)	MO54 cells	2.45 GHz, CW	5, 20, 50, 100 W/kg
Weisbrot *et al.* (*16*)	*Drosophila melanogaster*	900 MHz, GSM 1900 MHz, GSM	SAR unknown
Czyz *et al.* (*17*)	mouse embryonic carcinoma cells, embryonic stem cells ($p53^{-/-}$, p53wt)	1.71 GHz, GSM	0.1–2.0 W/kg
Laszlo *et al.* (*34*)	HeLa S3, HA-1, C3H 10T½	835 MHz, FDMA 847 MHz, CDMA	0.6 W/kg (low dose) 5 W/kg (high dose)
Lee *et al.* (*25*)	HSP70.1-deficient and wild type mice	849 MHz, CDMA 1763 MHz, CDMA	0.4 W/kg
Lim *et al.* (*26*)	human blood	900 MHz, CW or GSM	0.4, 2.0 and 3.6 W/kg
Miyakoshi *et al.* (*27*)	MO54 cells	1950 MHz, CW	1, 2, and 10 W/kg
Chauhan *et al.* (*36*)	TK6 cells	1.9 GHz, pulse-modulated	1 and 10 W/kg
Chauhan *et al.* (*37*)	HL60 and MM6 cells	1.9 GHz, pulse-modulated	1 and 10 W/kg
Dawe *et al.* (*15*)	*Caenorhabditis elegans*	1.0 GHz, CW	4–40 mW/kg
Lantow *et al.* (*29*)	MM6 and K562 cells	1800 MHz; CW and GSM	0.5, 1.0, 1.5, and 2.0 W/kg
Lee *et al.* (*35*)	Jurkat cells, rat primary astrocytes	1763 MHz, CDMA	2 or 20 W/kg
Nylund and Leszczynski (*22*)	EA.hy926, EA.hy926v1 cells	900 MHz, GSM	2.8 W/kg
Sanchez *et al.* (*31*)	human keratinocytes, fibroblasts and reconstructed epidermis	900 MHz, GSM	2 W/kg
Simko *et al.* (*28*)	MM6 cells	1800 MHz, CW and GSM	2 W/kg
Wang *et al.* (*19*)	A172 cells	2450 MHz, CW	5–200 W/kg
Chauhan *et al.* (*38*)	MM6 and U87MG cells	1.9 GHz, pulse-modulated	0.1, 1 and 10 W/kg
Vanderwaal *et al.* (*23*)	HeLa, S3 and EA.hy296 cell lines	837 MHz, TDMA 900 MHz, GSM	5.0 W/kg (TDMA) 3.7 W/kg (GSM)
Hirose *et al.* (*24*)	A172 cells and IMR-90 fibroblasts	2.1425 GHz, CW and W-CDMA	80 and 800 mW/kg
Sanchez *et al.* (*32*)	human primary keratinocytes and fibroblasts	1800 MHz, GSM	2 W/kg
Zhadobov *et al.* (*30*)	U-251MG cells	60 GHz, CW	5.4 $\mu W/cm^2$ or 0.54 mW/cm^2
Sanchez *et al.* (*33*)	hairless female rats	900 MHZ, GSM 1800 MHz, GSM	2.5 or 5 W/kg

Note. Abbreviations: CDMA: code domain multiple access; CHO: Chinese hamster ovary; CW, continuous wave; FDMA: frequency domain multiple access; GSM, global system for mobile communication; HSC, heat-shock cognate; HSF, heat-shock factor; HSP: heat-shock protein; p-HSP27, phosphorylated HSP27; SAR, specific absorption rate; SRE: serum-response element; W-CDMA: wideband-code division multiple access.

TABLE 1
Extended

Duration	End point	Target(s)	Results and comments
3–20 min	mRNA	HSP70	-no effect -SAR values very high (thermal confounding possible)
4 h	mRNA, protein	HSP70	-induction of HSP70 immediately after 7.5 W/kg RF radiation -no effect after 24 h
2 h	protein	stress protein induction	-no effect -SAR values very high
24 h	HSP16 reporter gene activity	HSP16.1	-increased expression of HSP16 reporter gene activity -a later study (Dawe *et al.*, 2006) indicated this result may be due to athermal effects
1 h	protein	protein, p-HSP27	-transient change in p-HSP27 and phosphorylation of other unidentified proteins -transient change in protein level of HSP27
2–16 h	protein	HSP70	-increased expression of HSP70 at SARs above 20 W/kg -SAR values very high (thermal confounding possible)
2 × 1 h/day for 10 days	protein	HSP70	-increased expression of HSP70 -poorly defined exposure conditions -no information on SAR distribution or possible temperature within tissue
6–72 h	mRNA	HSP70	-HSP70 increased in $p53^{-/-}$ embryonic stem cells
5–60 min, 24 h	HSF protein-DNA binding	HSF DNA binding	-no effect
2 × 45 min/day, 5 days/week for 4, 8 or 10 weeks	protein	HSP25, HSP70, HSP90	-no effect
20 min, 1 h and 4 h	protein	HSP27, HSP70	-no effect
1 or 2 h	protein	HSP27, HSP70, p-HSP27	-decrease in p-HSP27 at SAR of 10 W/kg
6 h, (5 min ON, 10 min OFF)	mRNA	HSP27, HSP70	-no effect
6 h, (5 min ON, 10 min OFF)	mRNA	HSP27, HSP70	-no effect
2.5 and 20 h	HSP16 reporter gene activity	HSP16-1 reporter gene activity	-no effect
45 min	protein	HSP70	-no effect
30 min or 1 h	protein	HSP27, HSP70, HSP90	-no effect
1 h	mRNA, protein	HSP27, HSP40, HSP60, HSC71, HSP90	-no effect
48 h	protein	HSP27, HSC70, and HSP70	-no effect on keratinocytes -slight increase in HSP70 in epidermis -significant decrease in HSC70 in fibroblasts
1 h	protein	HSP70	-no effect
1–3 h	protein	HSP27, HSP70, p-HSP27	-no effect at SARs less than 50 W/kg -SAR values very high (thermal confounding possible)
6–24 h, (5 min ON, 10 min OFF)	mRNA	HSP27, HSP40, HSP70, HSP90, HSP105	-no effect
1, 2, or 24 h for TDMA; 1, 2, or 5 h for GSM	protein	p-HSP27	-no effect
2–48 h	mRNA, protein	HSP27, HSP40, HSP70, HSP105, p-HSP27	-no effect
48 h	protein	HSP27, HSP70 and HSC70	-no effect
1–33 h	mRNA, protein	HSP70	-no effect
2 h or 2 h/day, 5 days/week for 12 weeks	protein	HSP25, HSP70, HSC70	-no effect -animals restrained during exposure

melanogaster to RF-field emissions created by placing the flies in proximity to the antennae of an active 900/1900 MHz GSM phone. The flies were exposed in culture tubes for 2 × 1 h/day for 10 days during their developmental period. While the authors measured the electric and magnetic fields around the phone, they did not provide an accurate assessment of the SARs to which the flies were exposed. The authors reported that non-thermal radiation from the GSM mobile phone increased the numbers of offspring, elevated the protein levels of HSP70, enhanced serum response element (SRE) DNA binding, and increased the phosphorylation of the nuclear transcription factor, ELK-1. However, since the exposure system was relatively crude (a cell phone placed next to incubation tubes) and the exposure characteristics in the study were not established, the possibility of SAR hot spots and/or thermal confounding cannot be excluded. Czyz *et al.* (*17*) reported that exposure of pluripotent embryonic stem cells to 1.71 GHz GSM-modulated RF radiation at SARs of 1.5 W/kg for 48 h and 2.0 W/kg for 72 h could induce a significant and stable up-regulation in transcript levels of HSP70 in p53-deficient cells but not in wild-type cells. Interestingly, no effects were observed when the 1.71 GHz signal was modulated using a "GSM-Talk" paradigm at the same slot-averaged SARs. The authors speculated that certain signal characteristics (e.g. 217 Hz modulation of the carrier signal) and biological/genetic conditions (e.g. p53 function) may be important for the detection of RF-field-related cellular responses. Tian *et al.* (*18*) exposed MO54 cells to 2.45 GHz RF radiation at SARs of 5 to 100 W/kg for 2 to 16 h. Protein was extracted and changes in HSP70 protein levels and heat-shock factor (HSF) activity were assessed by Western blotting. A slight increase in HSP70 protein levels was observed after a 2-h exposure at SARs of 25 and 78 W/kg, but this effect was likely due to thermal confounding due to the relatively high SARs employed in this study. Similar results were observed by Wang *et al.* (*19*), who did not detect any alterations in HSP27, HSP70 or phosphorylated-HSP27 protein expression in human glioblastoma-derived A172 cells after 0–3 h exposure to 2.45 GHz RF radiation at SARs less than 50 W/kg. At SARs exceeding 50 W/kg, the authors observed elevated HSP70 and phosphorylated-HSP27 protein expression, but these results have been attributed to thermal confounding (e.g. hot spots) (*20*).

In a highly publicized study, Leszczynski *et al.* (*21*) exposed cells of a human endothelial cell line (EA.hy926) to GSM-modulated 900 MHz RF radiation for 1 h at an average SAR of 2.8 W/kg. The authors performed both numerical dosimetry (finite difference time domain, FDTD) and empirical temperature measurements to characterize the heterogeneity of SAR distribution within their samples and to that ensure thermal confounding was not an issue. Temperatures within the samples were reported to remain within 37.0 ± 0.3°C over the exposure period. Immediately after exposure, samples were collected and analyzed for protein expression and phosphorylation. The authors reported altered phosphorylation status for a number of proteins. Specifically, HSP27 was found to undergo a transient increase in phosphorylation immediately after exposure but not 1 or 4 h after exposure. Additionally, the protein levels of both HSP27 and p38 mitogen-activated protein kinase (p38MAPK) were reported to be transiently increased. The authors suggested that RF-field exposure may induce a cellular stress response in these cells. In a follow-up study, this group exposed two variants of the human endothelial cell line (EA.hy926) to similar RF radiation as in their original study and assessed the effect of 1 h exposure on gene and protein expression (*22*). The authors identified 14 differentially expressed genes and 83 differentially expressed proteins in response to RF-field exposure but did not observe any measurable effects on HSP gene/protein expression.

In contrast to the above studies, Vanderwaal *et al.* (*23*) found no evidence of altered HSP27 phosphorylation in a series of cell lines (HeLa, S3 and EA.hy296) after exposure to either 837 MHz TDMA-modulated RF radiation for 1, 2 or 24 h at an SAR of 5.0 W/kg or 900 MHz GSM-modulated RF radiation for 1, 2 or 5 h at an SAR of 3.7 W/kg. Interestingly, the authors also evaluated the effect of mild (42°C for 2 h) to moderate (45°C for 30 min) hyperthermia on HSP27 phosphorylation. It was observed that multiple isoforms of phosphorylated HSP27 were formed after hyperthermia and that the specific distribution depended on the severity of the heat-shock conditions. More recently, Hirose *et al.* (*24*) examined the effect of RF radiation on HSP27 phosphorylation and on gene and protein expression in two cell lines. Human glioblastoma-derived A172 cells and human fetal lung-derived IMR-90 fibroblasts were exposed for 2–48 h to 2.1425 GHz RF radiation at SARs up to 800 mW/kg. No evidence of altered HSP27 phosphorylation or increased expression of mRNA for a variety of HSPs was observed in either cell line. Other recent studies have also found no evidence of RF-field-induced effects using well-characterized exposure systems. Lee *et al.* (*25*) exposed HSP70.1-deficient mice twice daily (45 min/session) for up to 10 weeks to 849 or 1763 MHz RF radiation at a whole-body average SAR of 0.4 W/kg. No effect was observed on HSP25, HSP70 or HSP90 protein expression in a variety of organs. Lim *et al.* (*26*) exposed human peripheral blood to 900 MHz CW or GSM-modulated RF radiation at three average SARs (0.4, 2.0 and 3.6 W/kg) for 20 min to 4 h. No statistically significant differences were detected in the frequency of lymphocytes or monocytes expressing elevated HSP27 or HSP70 after RF-radiation exposure. Miyakoshi *et al.* (*27*) exposed human malignant glioma cells to

1950 MHz CW RF radiation for up to 2 h at SARs of 1, 2 and 10 W/kg. Cells exposed to RF radiation did not show increased HSP27 and HSP70 protein expression. However, cells exposed to RF radiation at an SAR of 10 W/kg for 1 and 2 h exhibited a significant decrease in the level of phosphorylated HSP27. The authors concluded that the reduction in phosphorylated HSP27 was likely to contribute to adverse effects in cultured cells. Simko *et al.* (*28*) exposed a human monocyte-derived cell line (Mono-Mac-6) to 1800 MHz CW or GSM-modulated RF radiation for 1 h an SAR of 2 W/kg either alone or in conjunction with ultrafine particles. The authors observed no effects on HSP70 protein expression. In a follow-up study by the same group, Lantow *et al.* (*29*) investigated whether 1800 MHz RF radiation could cause altered expression of HSP70 in Mono-Mac-6 and K562 cells. The cells were exposed to CW and a variety of GSM-modulated signals for 45 min at SARs of 0.5–2.0 W/kg either alone or in combination with chemicals. No significant effects were detected in HSP70 protein expression in either cell line after RF-radiation exposure under any of the conditions tested.

Zhabodov *et al.* (*30*) investigated whether low-power 60 GHz RF radiation (as used in wireless local area networks) could alter stress-sensitive gene and protein expression in cells of a human glial cell line. Glial cells were exposed for 1–33 h to 60 GHz RF radiation at power densities of 5.4 $\mu W/cm^2$ or 0.54 mW/cm^2. The authors found no evidence of altered stress-gene expression, as determined by reporter assays and RT-PCR. Similarly, Western blot analysis indicated no effects from RF radiation on the protein levels of clusterin or HSP70. Sanchez *et al.* (*31*) evaluated possible stress effects in isolated human skin cells and in reconstructed human epidermis after a 48-h exposure to 900 MHz GSM-modulated RF radiation at an SAR of 2 W/kg. Immunohistochemical analysis demonstrated no detectable changes in the protein expression of HSP27 or inducible HSP70 in keratinocytes after RF-radiation exposure. However, heat-shock cognate (HSC) 70 protein levels were decreased significantly in dermal cells isolated from human skin after RF-radiation exposure. The authors did not observe a similar result in reconstructed human epidermis, and they concluded that human cutaneous cells may react to RF radiation by modulating the expression of some HSPs, but this response may depend on the cell model. In a follow-up study, the same investigators found that primary human skin cells (keratinocytes and fibroblasts) did not display any alterations in inducible or constitutive HSP70 protein levels or in the levels of HSP27 after a 48-h exposure to 1800 MHz GSM-modulated RF radiation (*32*). The authors did not speculate on the differences in the responses observed in these two studies. Recently, Sanchez *et al.* (*33*) exposed the skin on the back of hairless female rats to 900 and 1800 MHz GSM-modulated RF radiation using a loop antenna for either a single 2-h exposure at SARs of 0 or 5 W/kg or a repeated exposure regimen (2 h/day, 5 days/week for 12 weeks) at SARs of 0, 2.5 or 5 W/kg. The animals were killed immediately after the single RF-radiation exposure or at 72 h after the repeated RF-radiation exposures. The exposed portion of skin was then preserved for immunohistochemical analysis. The authors included cage (unhandled) control, sham (handled) control, and positive (400 mJ/cm^2 UVB radiation) control groups. Immunohistochemical analysis of the protein levels of HSC70, HSP25 and HSP70 in the epidermis yielded no significant differences in expression between the RF-radiation-exposed animals and the controls, while the positive (UVB radiation) control was markedly increased for each of these proteins. The authors concluded that they found no evidence that either 900 or 1800 MHz GSM-modulated RF radiation could alter HSP expression in rat skin.

The transcription of HSPs are regulated by the DNA-binding activity of the heat-shock transcription factors (HSFs). These factors bind to specific regulatory elements in the promoter regions of heat-shock protein genes. In a study conducted by Laszlo *et al.* (*34*), the activity of HSF protein–DNA binding was examined in hamster, mouse and human cells after exposure to 835.62 MHz frequency domain multiple access (FDMA)- or 847.74 MHz code domain multiple access (CDMA)-modulated RF radiation at SARs of ~0.6 W/kg and ~5 W/kg. The authors did not detect any increase in the DNA-binding ability of HSF in cultured mammalian cells as a consequence of any RF-radiation exposure paradigm tested. Similar results were observed by Lee *et al.* (*35*), who found no detectable alterations in HSP27, HSP70 or HSP90 transcript expression after exposure of human T-lymphocyte-derived Jurkat cells and rat primary astrocytes to 1763 MHz RF radiation at SARs of 2 or 20 W/kg for up to 1 h. These findings are supported by a series of studies conducted at Health Canada. Chauhan *et al.* (*36–38*) investigated whether non-thermalizing 1.9 GHz pulse-modulated (50 Hz, 1/3 duty cycle) RF-radiation exposure could alter the transcript expression of HSP27, HSP40, HSP70, HSP90 and HSP105 in a variety of human-derived cell lines. None of these studies found any evidence of altered HSP transcriptional activation after non-thermal RF-radiation exposure.

Despite a large number of studies, there have been conflicting results with respect to RF-radiation exposure and HSP expression (*39*). As demonstrated by the studies described above, there are a vast number of research studies suggesting that non-thermal RF-radiation exposures do not elicit a cellular stress response characterized by altered HSP gene or protein expression. A small number of studies exist where exposure conditions seem well controlled, yet an RF-radiation-

TABLE 2
Summary of Studies that have Investigated the Effect of RF Fields on the Expression of Proto-oncogenes

Reference	Tissue/cell line(s)	Signal(s)	SAR(s)
Ivaschuk *et al.* (*40*)	Rat PC12 cells	836.55 MHz, TDMA	0.09, 0.9, 9.0 mW/cm^2
Fritze *et al.* (*12*)	Rat brain (male Wistar)	900 MHz, CW and GSM	0.3 W/kg (GSM), 1.5 W/kg (GSM), 7.5 W/kg (CW)
Morrissey *et al.* (*43*)	Mouse brain (male Balb/c)	1.6 GHz; CW or IRIDIUM	from ~ 0.2 to 7.8 W/kg (local brain SAR)
Goswami *et al.* (*41*)	C3H 10T½ cells (serum-deprived and exponentially growing)	835.6 MHz, FMCW 847.7 MHz, CDMA	0.6 W/kg
Stagg *et al.* (*44*)	Rat brain (male Fisher 344)	1.6 GHz IRIDIUM	0.16, 1.6, 5 W/kg (brain averaged)
Czyz *et al.* (*17*)	Mouse embryonic carcinoma cells, embryonic stem cells (p53$^{-/-}$, p53 wt)	1.71 GHz, GSM	0.1–2.0 W/kg
Whitehead *et al.* (*42*)	C3H 10T½ cells (serum stimulated)	847.7 MHz, CDMA 835.6 MHz, FDMA 836.6 MHz, TDMA	5 and 10 W/kg
Finnie (*45*)	Mouse brain (female C57BL/6NTac)	900 MHz, GSM	4 W/kg (whole body)
Chauhan *et al.* (*36*)	TK6 cells	1.9 GHz, pulse-modulated	1 and 10 W/kg
Chauhan *et al.* (*37*)	HL-60, MM6 cells	1.9 GHz, pulse-modulated	1 and 10 W/kg
Finnie *et al.* (*46*)	Fetal mouse brain (Balb/c)	900 MHz, GSM	4 W/kg, (whole body, pregnant dams)
Finnie *et al.* (*48*)	Mouse brain (female C57BL/6NTac)	900 MHz, GSM	4 W/kg

Note. Abbreviations: CDMA, code division multiple access; CW, continuous-wave; FDMA, frequency division multiple access; FMCW, frequency-modulated continuous wave;.GSM, global system for mobile communication; SAR, specific absorption rate; TDMA, time division multiple access.

induced response on HSP expression was observed. Such studies leave sufficient doubt to require further investigation to determine whether these observations are ''false-positive events'' that occurred due to some unknown uncontrolled variable or whether they represent a cell/tissue-, frequency-, modulation- or model-specific response. Additional well-characterized replication studies are required to further evaluate these observations further. However, the majority of the research to date does not suggest a role for non-thermal RF radiation in inducing a generalized cellular stress response.

Studies Assessing the Effects of RF Radiation on Proto-oncogenes

Another group of proteins that has gained considerable attention in bioelectromagnetics research are the proto-oncogenes and the related transcription factors. Proto-oncogenes are genes whose protein products have the capacity to induce cellular transformation. The transcription of these genes is often low and is easily stimulated by various agents. Some proto-oncogenes, often considered as immediate early response genes (IEG), include c-fos, c-myc and c-jun. These genes are constitutively expressed at low levels but are rapidly and transiently induced in response to stressful external stimuli. Each of these genes encodes for a transcription factor that regulates expression of genes through binding on Enhancer Box sequences (E-boxes) and recruiting histone acetyltransferases. Given their ability to regulate cellular growth, proliferation and differentiation, they have become an important topic in RF-radiation bioeffects research.

Several studies have reported that RF radiation may affect the expression of a number of proto-oncogenes in cell cultures (Table 2). In an early study conducted by Ivaschuk *et al.* (*40*), rat PC12 pheochromocytoma cells were exposed to intermittent (20 min ON/20 min OFF) 836.55 MHz TDMA-modulated RF radiation at 0.09, 0.9 and 9 mW/cm^2 for exposure times of 20, 40 and 60 min. Northern blot analysis revealed a decrease in the expression of c-jun after a 20-min 9 mW/cm^2 RF radiation exposure, but no changes were observed after 40- or 60-min exposures at the same intensity. No changes in c-jun expression were observed at lower

TABLE 2
Extended

Duration	End point	Target(s)	Results and comments
20–60 min, (20 min ON/ 20 min OFF)	mRNA	c-fos, c-jun	-no effect on c-fos -decrease in c-jun expression after 20 min exposure to 9 mW/cm^2
4 h	mRNA, protein	c-fos, c-jun, fos-B, jun-B, jun-D	- no effect
1 h	mRNA	c-fos	-increased expression of c-fos in forebrain at local brain SARs > 2.75 W/kg (IRIDIUM and CW) -response consistent with restraint stress and possible thermal perception by animals
24 h (serum-deprived) or 4 days (exponential growth phase)	mRNA	c-fos, c-myc, c-jun	-increase in c-fos expression in cells transitioning from exponential to plateau phase and in the plateau phase, but no effect on AP1-DNA binding activity
2 h	mRNA	c-fos, c-jun	-no effect on c-fos expression
6–72 h		c-jun, c-myc, c-fos	-transient upregulation in c-jun and c-myc in p53$^{-/-}$ embryonic stem cells
4 days	mRNA	c-fos	-no effect on c-fos expression -authors suggest that small changes in background levels could produce biologically insignificant, statistically elevated fold-changes (Goswami *et al.*, 1999)
1 h	protein	c-fos	-no effect from RF radiation -immobilization of mice in exposure chamber resulted in stress response leading to elevated c-fos in sham and exposed groups when compared to cage control group
6 h, (5 min ON/ 10 min OFF)	mRNA	c-fos, c-myc, c-jun	-no effect
6 h, (5 min ON/ 10 min OFF)	mRNA	c-fos, c-myc, c-jun	-no effect
1 h/day from gestational days 1–19	protein	c-fos	-no effect
1 h/day, 5 days/week, for 104 weeks	protein	c-fos	-no effect from RF radiation -immobilization of mice in exposure chamber resulted in stress response leading to elevated c-fos in sham and exposed groups when compared to cage control group

power densities or longer times, and no changes were observed in c-fos transcript expression. Interestingly, Czyz *et al.* (*17*) reported that exposure of p53-deficient embryonic stem cells to 1.71 GHz GSM-modulated RF radiation caused a transient increase in c-jun expression immediately after a 48-h RF-radiation exposure and an increase in c-myc expression at 5 days after RF-radiation exposure. However, no changes in the expression of these proto-oncogenes were detected in the exposed wild-type embryonic stem cells. These results suggest that loss of p53 function increases the sensitivity of embryonic stem cells to RF radiation. Goswami *et al.* (*41*) investigated the effects of 835.62 MHz FDMA and 847.74 MHz CDMA RF radiation at an SAR of 0.6 W/kg on proto-oncogene expression in serum-deprived and in exponentially growing C3H 10T½ cells. The authors found no effects on c-myc, c-jun, c-fos, NF-κB, AP-1 or AP-2 in serum-deprived cells; however, RF radiation induced a statistically significant increase in c-fos expression in exponentially growing cells. In an attempt to confirm these findings, Whitehead *et al.* (*42*) exposed C3H 10T½ cells under similar conditions, albeit at higher SARs of 5 and 10 W/kg, and also to 836.55 MHz time difference multiple-access (TDMA)-modulated RF radiation. In contrast to their original study, no effects on c-fos expression were observed in C3H 10T½ cells after any RF-radiation exposure. The authors speculated that small changes in the background levels of some transcripts could cause falsely elevated changes that are statistically significant yet are biologically insignificant. Recently, Chauhan *et al.* (*36, 37*) exposed cells of three human-derived cell lines to intermittent (5 min ON/10 min OFF) 1.9 GHz pulse-modulated RF radiation at SARs of 1 and 10 W/kg for 6 h; total RNA was then harvested immediately or 18 h after exposure. Concurrent sham, negative (incubator) and positive (heat-shock) controls were conducted for each experiment. No significant differences were observed in the relative expression levels of the proto-oncogenes c-jun, c-fos and c-myc in any of the cell lines examined. However, the heat-shock positive control demonstrated a significantly elevated expression of both c-fos and c-jun in all three cell lines.

The effects of non-thermal RF radiation on proto-oncogene expression have been investigated in a number of animal-based studies. Fritze *et al.* (*12*) reported no evidence of altered c-fos or c-jun protein expression in the rat brain at 24 h or 7 days after an acute 4-h exposure to 900 MHz RF radiation at SARs of 0.3–7.5 W/kg. Interestingly, a transient increase in HSP70 mRNA was observed after exposure to 7.5 W/kg RF

radiation, but no corresponding change in the level of HSP70 protein was observed. This study deserves particular distinction for the use of various novel positive controls to ensure the quality and sensitivity of their end points. Morrissey *et al.* (*43*) exposed mice for 1 h to a 1.6 GHz Iridium signal (CW or pulse-modulated at 11 Hz) at brain SARs ranging from ~0.2 to 7.8 W/kg and examined c-fos mRNA expression in two brain regions. The authors reported that c-fos expression was elevated in several regions of the forebrain but only when local brain SARs (>2.75 W/kg) exceeded current international RF-radiation exposure limits (*3, 4*). The authors speculated that this response was consistent with a generalized stress response, possibly due to thermal perception coupled with restraint stress. In a related study, Stagg *et al.* (*44*) exposed loosely restrained Fischer 344 rats for 2 h to 1.6 Hz (11 Hz, pulse-modulated) RF radiation (Iridium signal) at brain-averaged SARs ranging from 0.16 to 5 W/kg and then killed the animals immediately after exposure. The authors failed to observe any alterations in either ornithine decarboxylase, c-jun or c-fos transcript levels in the cerebrum or cerebellum of RF-radiation-exposed rats. Interestingly, the authors did observe that a significant stress response (as determined by core body temperature changes, corticosterone and ACTH levels) was evident even in loosely restrained animals. This suggested that preconditioning animals to the exposure environment was necessary to minimize RF-radiation exposure system/handling-related stress responses and thereby avoid false-positive responses and/or allow detection of true stress-related effects.

In a series of studies by the same group, the expression of c-fos protein was assessed in the brains of mice exposed to 900 MHz RF radiation. Finnie (*45*) exposed partially restrained mice to 900 MHz GSM-modulated RF radiation for 1 h at a whole-body SAR of 4 W/kg. After exposure the mice were immediately killed, coronal brain sections were prepared, and possible alterations in c-fos expression in various mouse brain regions were assessed immunohistochemically. The authors observed no differences in c-fos expression in five cortical areas between sham- and RF-radiation-exposed animals; however, both groups demonstrated significantly more c-fos expression than in the cage (unhandled) control group, implying a stress response due to partial immobilization rather than to RF radiation. These results are consistent with the findings of Stagg *et al.* (*44*). In a follow-up study, Finnie *et al.* (*46*) evaluated c-fos immunoreactivity in archived brains from the study of Utteridge *et al.* (*47*) of partially restrained mice exposed for 104 weeks (1 h/day, 5 days/week; 4 W/kg) to 900 MHz GSM-modulated RF radiation. In concordance with their previous study, there was no evidence of altered c-fos expression between sham- and RF-radiation-exposed mice for any of the brain regions examined; however, both groups displayed restraint-induced elevated c-fos expression compared to the cage (unhandled control). Interestingly, despite 104 weeks of daily partial-restraint stress, there was no apparent habituation of c-fos expression in the cerebral cortical or hippocampal regions. This finding challenges the notion that restraint stress in RF-radiation studies can ever be eliminated effectively through conditioning of animals to the RF-radiation exposure environment. Finally, the effect of gestational exposure to RF radiation on the fetal brain was recently evaluated by Finnie *et al.* (*48*). Pregnant dams were exposed to 900 MHz GSM-modulated RF radiation at 4 W/kg for 1 h/day from gestational days 1 through 19. After exposure on gestational day 19, the fetuses were surgically removed and the brains were immediately preserved. c-fos expression was assessed in three coronal slices, allowing seven brain regions to be examined. The authors found no evidence of altered c-fos protein expression between corresponding brain regions of either the sham-exposed, RF-radiation-exposed or negative (cage) control mice.

Based upon current results, there is no convincing evidence of non-thermal RF-radiation-induced alterations of proto-oncogene mRNA or protein expression in cell culture or animal-based studies. Most cases of altered proto-oncogene expression appear to have occurred under unique exposure conditions with no clear dose–response effects, and many of these findings remain unconfirmed and/or internally inconsistent (e.g. dose response, time response). It is clear that restraint stress can be a major confounding variable in animal RF-radiation studies. Future studies should take special precautions to ensure that animal restraint is either avoided or properly accounted for. At present, the weight of evidence does not support the hypothesis that non-thermal RF radiation can alter proto-oncogene activation.

Studies Assessing the Effects of RF Radiation on other Signal Transduction Pathways

Another major mechanism used by cells to regulate transcriptional activity in response to stressors is through the mitogen-activated protein kinase (MAPK) pathways that include the extracellular-signal regulated kinase (ERK), p38 and the 1/2 c-Jun N-terminal kinase (JNK) cascades. These pathways are complex, and they regulate a variety of cellular processes including proliferation, differentiation, metabolism and the stress-or response. Upon phosphorylation of these kinases, a large number of regulatory proteins and transcription factors (Egr-1, Elk-1) can become activated, thereby altering cellular processes and allowing further gene transcription.

One of the earliest studies to assess the possible effect of RF radiation on cellular signal transduction pathways was conducted by Leszczynski *et al.* (*21*). In this study, cells of a human endothelial cell line were exposed to 900 MHz GSM-modulated RF radiation at 2 W/kg for 1 h, and then changes in the levels of p38MAPK protein were assessed. The authors reported a transient increase in hsp27-phosphorylation and observed that this effect could be inhibited by SB203580 (a specific inhibitor of p38MAPK). Since temperature measurements indicated no alterations in cell culture temperature during the exposure period, the authors speculated that activation of the p38MAPK stress response pathway might be a potential mode of non-thermal molecular interaction of RF radiation with biological tissue. Similarly, Friedman *et al.* (*49*) reported that low-level exposure of serum-deprived Rat1 and HeLa cells to 875–950 MHz RF radiation at power densities ranging from 0.07–0.31 mW/cm^2 for 5–30 min significantly activated the ERK1/2 signal transduction pathway. Interestingly, neither the p38MAPK nor the JNK1/2 stress-responsive pathways were activated by RF radiation. It is clear that this study suffers from some methodological shortcomings and that these results require confirmation under more tightly controlled exposure conditions where the SAR level, SAR heterogeneity and actual sample temperature are precisely controlled.

Recently, the expression levels of transcription factors (Egr-1, Bcl-2, Elk-1) downstream of the MAPK pathways have been investigated. Buttiglione *et al.* (*50*) assessed Egr-1 transcript expression and the phosphorylation of ERK1/2 and JNK in a human neuroblastoma cell-line (SH-SY5Y) after exposure (5 min–24 h) to 900 MHz GSM-modulated RF radiation at an SAR of 1 W/kg. The authors observed a transient increase in Egr-1 levels after 5–30 min of RF-radiation exposure, but the effect was no longer evident after 6–24 h of RF-radiation exposure. Phosphorylation of ERK1/2, JNK 1/2 and Elk-1 were also transiently increased after various RF-radiation exposure times (5 min–6 h) while significant decreases in the transcript levels of Bcl-2 and Survivin were observed after 24 h RF-radiation exposure. However, the authors also observed a significant decrease in cell viability (as determined by the MTT assay) and the appearance of sub-G_1 nuclei and a G_2/M block (as determined by flow cytometry) after 24 h RF-radiation exposure. This may indicate either that the RF radiation was inducing apoptosis or that the environmental conditions of the exposure (temperature, pH, osmolarity) were not ideal and may have confounded the results of this study. Further investigation is required.

Signal transduction pathways have also been studied in *Drosophila melanogaster* after exposure to RF radiation. Weisbrot *et al.* (*16*) exposed flies to 900/1900 MHz GSM-modulated RF radiation emitted from a commercial mobile phone for 10 days during their developmental period. The authors observed an increased level of phosphorylated Elk-1 protein in RF-radiation-exposed flies. However, the exposure conditions in this study were poorly defined, and the authors provided no information on SAR distribution or possible temperature within the biological tissue. Recently, Lee *et al.* (*51*) exposed two strains of *Drosophila melanogaster* to 835 MHz RF radiation at SARs of 1.6 or 4.0 W/kg for 12–36 h. The authors reported activation of ERK and JNK but not p38MAPK after RF-radiation exposure. Interestingly, flies exposed to RF radiation at 1.6 W/kg predominately showed activation of ERK while flies exposed at 4.0 W/kg showed activation of JNK. However, these results should be interpreted with caution. It was observed that flies exposed at 4.0 W/kg had only a 10% survival rate after 36 h of RF-radiation exposure relative to the sham controls. Furthermore, the authors reported up-regulation of HSP27 and HSP70 protein expression after RF-radiation exposure at both 1.6 and 4.0 W/kg, indicating that thermal confounding may be an issue. While the flies were exposed at room temperature, the presence of a wet paper filter (to maintain humidity) within the exposure apparatus may have caused absorption of more RF-radiation energy than anticipated and therefore elevated the ambient temperature within the exposure apparatus. Since the authors did not actually record the temperature within the exposure apparatus where the flies were housed, the possibility of thermal confounding cannot be discounted.

In contrast to the above studies, other studies have failed to observe activation of stress-responsive signal transduction pathways in various cell and animal models after RF-radiation exposure. Lee *et al.* (*35*) exposed Jurkat cells and rat primary astrocytes to 1763 MHz CDMA-modulated RF radiation at SARs of 2 or 20 W/kg for 30 min–1 h in the presence or absence of the phorbol-ester, 12-O-tetradecanoylphorbol 13-acatate (TPA). The authors observed no evidence of phosphorylation of ERK1/2, JNK1/2 or p38MAPK after RF-radiation exposure in either the presence or absence of TPA. Similar results were obtained when 8-week-old hsp70.1-deficient mice were exposed twice daily (for 45 min), 5 days/week for up to 10 weeks to 849 or 1763 MHz RF radiation at an SAR of 0.4 W/kg (*25*). Histological analysis of the lungs and brains of RF-radiation-exposed mice showed no differences in the phosphorylation status of the stress-activated kinases ERK1/2 and JNK1/2 relative to the sham-exposed group. The authors also reported no evidence of increased proliferation, apoptosis or HSP expression in tissues in the RF-radiation-exposed group relative to the sham controls.

TABLE 3
Summary of Studies that have Investigated the Effect of RF Radiation on Proteins/Genes Associated with Signal Transduction Pathways

Reference	Tissue/cell line(s)	Signal(s)	SAR(s)
Li *et al.* (*54*)	WS1neo fibroblasts	837 MHz, CW	0.9 or 9.0 W/kg
Leszczynski *et al.* (*21*)	EA.hy926 cells	900 MHz, GSM	~2 W/kg
Weisbrot *et al.* (*16*)	*Drosophila melanogaster*	900/1900 MHz, GSM	SAR unknown
Czyz *et al.* (*17*)	mouse embryonic carcinoma cells, embryonic stem cells ($p53^{-/-}$, p53 wt)	1.71 GHz, GSM	0.1–2.0 W/kg
Lee *et al.* (*25*)	HSP70.1-deficient and wild type mice	849 MHz, CDMA 1763MHz, CDMA	0.4 W/kg
Nikolova *et al.* (*52*)	Mouse embryonic stem cells	1.71 GHz, GSM	1.5 W/kg
Lee *et al.* (*35*)	Jurkat cells and rat primary astrocytes	1763 MHz, CDMA	2 or 20 W/kg
Hirose *et al.* (*53*)	A172 and IMR-90 cells	2.1425 MHz, CW and W-CDMA	80, 250 and 800 mW/kg
Buttiglione *et al.* (*50*)	Human neuroblastoma (SH-SY5Y) cells	900 MHz, GSM	1.0 W/kg
Friedman *et al.* (*49*)	Rat1 and HeLa cells	875, 900 and 950 MHz	0.07–0.31 mW/cm^2
Lee *et al.* (*51*)	*Drosophila melanogaster*	835 MHz	1.6 and 4 W/kg

Note. Abbreviations: CDMA, code division multiple access; CW, continuous-wave; GSM, global system for mobile communication; SAR, specific absorption rate; TDMA, time division multiple access; W-CDMA, wideband-code division multiple access.

A limited number of studies have examined the effects of RF radiation on the expression of other eukaryotic transcriptional regulators, tumor suppressor genes, cell cycle proteins, signaling molecules and growth factors. Nikolova *et al.* (*52*) exposed pluripotent embryonic mouse stem cells to 1.71 GHz GSM-modulated RF radiation at an average SAR of 1.5 W/kg for up to 48 h. The authors observed transient changes in the expression of bax, GADD45 and Nurr1 at specific (isolated) times after exposure, but no consistent alterations in the expression of these genes were observed at other times. The authors observed no corresponding changes on cellular proliferation or apoptosis, leading them to speculate that the effect of RF radiation on these genes may be compensated for at the translational and post-translational levels. Similarly, Czyz *et al.* (*17*) exposed wild-type and p53-deficient mouse embryonic stem cells to 1.71 GHz GSM-modulated RF radiation at SARs ranging from 0.11–2.0 W/kg for up to 72 h. No statistically significant changes were observed in the expression of egr-1 or bcl-2; however, a transient induction of p21 was observed in $p53^{-/-}$ but not wild-type mice. To examine the effects of RF radiation on regulators of apoptosis, Hirose *et al.* (*53*) examined gene transcript levels in A172 (a cell line derived from an adult brain tumor) and IMR-90 (a cell line derived from fetal lung) cells after RF-radiation exposure. A series of genes known to be components of p53-mediated apoptosis (including APAF1, TP53, TP53BP2 and CASP9) were assessed after cells were exposed to 2.1425 GHz CW and W-CDMA-modulated RF radiation at SARs of 80–800 mW/kg for up to 48 h. The authors observed no significant differences in the expression of these p53-related apoptosis genes relative to the sham control groups under any conditions tested. Similarly, Li *et al.* (*54*) exposed human fibroblasts to 837 MHz CW RF radiation at SARs of 0.9 to 9.0 W/kg for 2 h and did not observe any evidence of altered TP53 protein expression.

In conclusion, as with the HSPs and the proto-oncogenes, investigations that have examined proteins and genes associated with signal transduction pathways involved in the stress response after RF-radiation

TABLE 3
Extended

Duration	End point	Target(s)	Results and comments
2 h	protein	p53	-no effect
1 h	protein	p38MAPK	-transient change in p38MAPK
2 × 1 h/day for 10 days	protein phosphorylation status	ELK1	-increase in ELK1 phosphorylation -poorly defined exposure conditions -no information on SAR distribution or possible temperature within tissue
6–72 h	mRNA	Egr-1, Bcl-2, p21	-transient increase in p21 in $p53^{-/-}$ embryonic stem cells -no effect on egr-1 or bcl-2
2 × 45 min/day, 5 days/week for 4, 8 or 10 weeks	protein phosphorylation status	MAPK, ERK1/2, JNK1/2 p38MAPK	-no effect
48 h, (5 min ON/30 min OFF)	mRNA	Nurr1, *bax* and GADD45	-transient increase in GADD45 and *bax*, decrease in Nurr1 -no changes in cell proliferation, chromosomal stability or apoptosis
30 min or 1 h	protein phosphorylation status	p38MAPK, ERK1/2, JNK1/2	-no effect in the presence or absence of TPA -exposure conditions/temperature properly controlled
24, 28 and 48 h	protein levels and phosphorylation status	APAF1, TP53, TP53BP2 and CASP9	-no effect
5 min–24 h	mRNA, protein phosphorylation	Egr-1, ERK1/2, JNK 1/2, Bcl-2, Survivin	-transient increase in Egr-1 transcript levels -transient increase in ERK1/2, JNK phosphorylation -evidence of apoptosis after 24 h RFR exposure -possible confounding due to environmental factors
5–30 min	protein, phosphorylation	p38MAPK, ERK1/2, JNK1/2	-increase in ERK1/2 phosphorylation/activation, no effect on p38MAPK or JNK1/2 -insufficient details on RFR exposure system, temperature control and negative control conditions -no inferential statistics
12–36 h	mRNA, protein, protein phosphorylation	ERK1/2, JNK1/2, p38MAPK,	-increased protein expression of ERK and JNK -no significant effects on p38MAPK -possible thermal confounding -insufficient number of independent experiments -no details on statistical methods applied

exposure have yielded mixed results. At present, there is inconclusive experimental evidence to support the hypothesis that RF radiation can affect signal transduction pathways associated with the stress response. Clearly, further well-controlled investigations are required to elucidate whether RF radiation can influence these pathways and result in sustained biological effects.

Microarray Studies Assessing the Effects of RF Radiation on Gene Expression

The use of DNA microarrays is becoming increasingly popular for high-throughput analysis of differential gene expression in response to a variety of chemical, pharmaceutical and environmental exposures/conditions. This technology, while extremely useful for screening large numbers of genes for potential interaction with a test substance, is often employed under less than ideal conditions. Common deficiencies in microarray studies include poor experimental design and/or an inadequate number of biological replicates (presumably due to the high cost of microarrays and associated reagents), improper data acquisition/normalization procedures, and a lack of validation of the data using other techniques (e.g. semi-quantitative RT-PCR). It is particularly important that a study encompasses enough biological replicates to carry out a proper statistical analysis of the data. Data preprocessing steps (including applying the appropriate normalization, filtering and quality control checks) are required before proceeding to data analysis. Appropriate statistical approaches must be applied to correctly model nuisance parameters and interpret real biological variability. Finally, biological replication of important genes or of a subset of differentially expressed genes is important to identify possible false-positive responses. It is imperative to take all of these factors into account before undertaking and carrying out an experiment using DNA microarrays (*55*). Vanderstraeten and Verschaeve (*56*) have recently reviewed the literature on the evidence for RF-radiation-induced effects on gene and protein expression using high-throughput screening technologies. To date, many of the microarray studies assessing RF-radiation-induced transcriptional changes have been plagued by one or more critical methodological deficiencies. A summary of microarray studies evaluating the effect of RF radiation on gene expression is presented in Table 4.

The first RF-radiation-related microarray study was conducted by Harvey and French (*57*). In this study, human mast cells (HMC-1) were exposed to 864.3 MHz CW RF radiation in three 20-min exposures a day (at 4-h intervals) for 7 days at an average SAR of 7 W/kg. A total of 588 genes were screened for differential expression using the Human Atlas cDNA array, but only three genes were observed to be affected after RF-radiation exposure. The affected genes included the proto-oncogene c-kit, the transcription factor nucleoside diphosphate kinase B, and the apoptosis-associated gene DAD-1. However, the conclusions that can be drawn from this study are limited because the authors used relative changes instead of inferential statistics to identify differentially expressed genes and the conclusions were based on the results from only two experiments. Furthermore, there was no confirmation of differential expression of the responding genes by the use of an alternative technology. However, it must be noted that this study was conducted during the infancy of high-throughput genomics analysis and that the methodological approaches employed were deemed appropriate at the time of publication.

Pacini *et al.* (*58*) exposed cells of a human fibroblast cell line for 1 h to 902.4 MHz GSM-modulated RF radiation by placing the culture dishes atop an energized cell phone engaged in a simulated voice conversation. The authors assessed differential gene expression after hybridizing their samples to the Atlas Human Array trial kit (containing 82 human cDNAs). Several mitogenic signal transduction genes, cell growth inhibitor genes, and genes controlling apoptosis were found to be altered after RF-radiation exposure. However, like the study by Harvey and French (*57*), this study suffered from several critical deficiencies. To begin, the authors estimated an SAR of 0.6 W/kg for the cells, based upon the cell phone manufacturer's technical information and the European maximal human SAR limits. No empirical or numerical dosimetry was performed to assess the actual SARs within the six-well culture plates. It is known that SAR distributions in petri dishes and culture plates exposed from uniform fields are significantly inhomogeneous. The SAR inhomogeneity would be further exacerbated by the highly non-uniform fields produced close to a cell phone. Thus the actual SARs may have ranged from well above 0.6 W/kg in some places within the cell culture to much lower than 0.6 W/kg in other areas; the average SAR is unknown. Furthermore, the conclusions of this study were apparently based on crude differences between sham- and RF-radiation-exposed samples from a single experiment. Finally, there was no confirmation of the results by other techniques such as RT-PCR, thereby limiting the conclusions that can be derived from this study.

More recently, Lee *et al.* (*59*) used Serial Analysis of Gene Expression (SAGE) to evaluate differential gene expression in HL-60 cells immediately and 6 h after a 2-h exposure to 2.45 GHz RF radiation at an SAR of 10 W/kg. Immediately after exposure, the authors observed 221 differentially expressed genes in the sample exposed to RF radiation relative to the 2-h sham-exposed sample. At 6 h after exposure, 759 genes were differentially expressed in the radiation-exposed sample relative to the 2-h sham-exposed control. Interestingly, there was no evidence of differential HSP gene expression after exposure. Unfortunately, this study consisted of SAGE analysis of RNA collected from a single experiment, with a single sham control used as a reference for two postirradiation exposure times. Therefore, it is not surprising that this study reported a relatively large number of genes as being differentially expressed between groups, the possibility that many of the differentially expressed genes are false-positive events cannot be discounted. This study also failed to validate the SAGE results using RT-PCR or other verification techniques.

In a study funded by the REFLEX project (European Union Fifth Framework Programme), Remondini *et al.* (*60*) isolated RNA from six human-derived cell lines/types (NB69, EA.hy926, T-lymphocytes, U937, CHME5, and HL-60) after exposure to 900 or 1800 MHz RF radiation at various SARs, signal modulations and times, then analyzed gene expression changes using human Unigene whole genome cDNA arrays (containing 75000 cDNA clones). For each of the 11 conditions tested, total RNAs from two to five independent experiments were pooled for each of the sham- and RF-radiation-exposed samples. Thereafter, each of the 11 sham- and 11 RF-radiation-exposed RNA pools were processed simultaneously and hybridized in triplicate, for a total of 66 hybridizations. The authors reported no evidence of differential gene expression in three of the cell lines tested, but alterations in gene expression (up to 34 differentially expressed genes) were observed after RF-radiation exposure in the other three cell lines under various exposure conditions. The authors commented that these data may indicate a differential sensitivity of some cell lines/tissues to RF radiation. Unfortunately, since data analysis was carried out using cDNA from a single RNA pool for each experimental group, it is impossible to estimate the true biological variance for statistical inference testing. Ideally, either truly independent experiments or multiple independent pools of RNA are required. Although each gene was spotted four times on the same nylon membrane, the authors did not present any meaningful technical replicates. Duplicate spots on a single microarray slide are not independent, because the same cDNA sample is hybridized onto the same membrane at the same time, and therefore replicate spots on a microarray do not constitute valid technical replicates. Multiple hybridizations from the same pooled cDNA sample

onto separate arrays would be required. The statistical inference testing employed by Remondini *et al.* (*60*) is therefore based upon unsubstantiated assumptions of biological and technical variance in their experimental model. As a result, gene specific variance components cannot be estimated well and outliers cannot be appropriately identified. In the absence of such information, external validation of the experimental findings becomes more critical, yet no such information was provided in this study. For these reasons, the conclusions that can be drawn from this study are limited.

In a related study, Nylund and Leszczynski (*22*) examined gene and protein expression in two variants of the human endothelial cell line EA.hy926 and EA.hy926v1 after a 1-h exposure to 900 MHz GSM-modulated RF radiation at an SAR of 2.8 W/kg. The authors performed three independent experiments, and the samples from each experiment (non-pooled RNA) were independently hybridized to Atlas Human v1.2 cDNA expression arrays (containing 1167 genes). After data filtering (for signal intensity), a total of 136 genes across the two cell lines were compared for RF-radiation-induced changes in gene expression. The authors observed that one gene was down-regulated in the EA.hy926 cell line, while 13 genes were up-regulated in the EA.hy926v1 cell line after RF-radiation exposure. The authors did not observe similar changes at the protein level for the microarray-identified responsive genes and did not verify the microarray results using RT-PCR. It is interesting to note that the endothelial cell line EA.hy926 continues to demonstrate an apparent responsiveness to RF radiation. Further investigation of these results are required to elucidate whether some aspect of these cells make them more responsive to RF radiation or whether these cells are sensitive to other factors related to the RF-radiation exposure conditions (minor fluctuations in temperature, vibration, osmolarity, etc). More recently, this research group evaluated changes in protein expression in skin punch biopsies obtained from 10 women whose forearms were exposed to 900 MHz GSM-modulated RF radiation at an estimated average SAR of 1.3 W/kg for 1 h (*61*). Using 2D gel electrophoresis, the authors compared the expression of proteins in RF-radiation-exposed skin relative to that in unexposed skin (punch biopsies acquired from the other forearm). The authors observed eight differentially expressed protein spots in the RF-radiation-exposed skin biopsies relative to the biopsies from unexposed skin. Interestingly, two of the protein spots were present in all 10 volunteers. At present, the identity of these proteins is unknown, and further investigation is required to confirm these findings and determine whether this response is related to a thermoregulatory response or represents a non-thermal effect.

Zhao *et al.* (*62*) investigated the expression of genes related to cell death pathways in primary cultured neurons and astrocytes isolated from 15-day-old ICR mouse embryos. The cultured cells were exposed for 2 h in a 35-mm petri dish to a 1900 MHz GSM-modulated RF radiation from a cell phone placed atop the culture dish. After the 2-h exposure, RNA was harvested and the resulting cDNA was hybridized to GEArray Q series Mouse Apoptosis Arrays (containing probes to 96 apoptosis-related genes). The authors reported an up-regulation of several genes involved in the apoptosis pathway, including caspase 2, caspase 6 and Asc, in the two cell lines. These results were confirmed by RT-PCR analysis. It is important to note, however, that this study suffered from many methodological deficiencies. First, the cell cultures were not exposed to RF radiation under controlled experimental conditions. The authors acknowledged that they did not measure or attempt to control electromagnetic-field components (such as SAR levels and/or distribution) within the cell cultures during exposure. Furthermore, since the cultures were apparently exposed to RF radiation outside of a standard tissue culture incubator with the lids removed, while the reference control remained inside the incubator, the pH/osmolarity/temperature of the exposed cultures were likely quite different than those of the reference controls. Thus, while the authors confirmed the responding genes by RT-PCR, it cannot be determined whether the responding genes arose from exposure to RF radiation or were due to environmental factors related to the exposure conditions. Significant gene expression changes were also observed in a study conducted by Zhao *et al.* (*63*). In this study, primary rat neurons were exposed to intermittent (5 min ON/10 min OFF) 1800 MHz GSM-modulated RF radiation at 2 W/kg for 24 h, and RNA was isolated immediately after exposure. After conversion to cDNA and transcription to cRNA the samples were hybridized to Affymetrix Rat Neurobiology U34 arrays (containing 1200 probes). The authors identified 10 down-regulated genes and 24 up-regulated genes among the 1200 genes that were screened, using fold change (±15%) as the analysis criterion. While the microarray results are based on a single independent experiment, the authors confirmed many of the observed gene changes using RT-PCR. However, there was no evidence of any form of active cooling or temperature monitoring within the RF-radiation-exposed samples; therefore, the possibility of thermal confounding cannot be excluded. The results of this study need to be confirmed using high-quality exposure conditions.

Other recent microarray studies have found no evidence that non-thermal exposure can affect gene expression in cultured cells. Gurisik *et al.* (*64*) studied the effect of a 2-h exposure to 900 MHz GSM-modulated RF radiation at an SAR of 0.2 W/kg on gene expression in a human neuroblastoma cell line (SK-N-SH). At 2 h after sham or RF-radiation exposure, RNA was harvested and the samples were hybridized

TABLE 4
Summary of Microarray Studies Assessing the Effect of RF Radiation on Gene Expression

Reference(s)	Tissue/cell line(s)	Signal(s)	SAR(s)	Duration
Harvey and French (*57*)	HMC-1 cells	864.3 MHz, CW	7.3 W/kg	3 × 20 min/day for 7 days
Pacini *et al.* (*58*)	Detroit 550 fibroblasts	902.4 MHz, GSM	0.6 W/kg	1 h
Lee *et al.* (*59*)	HL-60 cells	2.45 GHz, pulse-modulated	10 W/kg	2 or 6 h
Belyaev *et al.* (*69*)	Rat cerebellum (*in vivo*)	915 MHz, GSM	0.4 W/kg (whole-body)	2 h
Gurisik *et al.* (*64*)	SK-N-SH cells	900 MHz, GSM	0.2 W/kg	2 h
Hirose *et al.* (*53*)	A172 and IMR-90 cells	2.1425 GHz, CW and W-CDMA	80, 250 and 800 mW/kg	24, 28 and 48 h exposure times
Nylund and Leszczynski (*22*)	EA.hy926, EA.hy926v1	900 MHz, GSM	2.4 W/kg	1 h
Qutob *et al.* (*68*)	U87MG cells	1.9 GHz, pulse-modulated	0.1, 1.0 and 10.0 W/kg	4 h
Remondini *et al.* (*60*)	NB69, U937 EA.hy926, CHME5, HL60, lymphocytes	900 MHz, GSM 1800 MHz, GSM	0.77 W/kg and 1.8–2.5 W/kg	1, 24 and 44 h
Whitehead *et al.* (*66, 67*)	C3H 10T½ cells	847.74 MHz, CDMA 835.2 MHz, FDMA	5 W/kg	24 h
Zeng *et al.* (*65*)	MCF-7 cells	1800 MHz, GSM	2.0 and 3.5 W/kg	24 h, (5 min ON, 10 min OFF)
Chauhan *et al.* (*38*)	U87MG and MM6 cells	1.9 GHz, pulse-modulated	0.1, 1 and 10 W/kg	6 and 24 h (5 min ON, 10 min OFF)
Hirose *et al.* (*24*)	A172 cells and IMR-90 fibroblasts	2.1425 GHz, CW and W-CDMA	80 and 800 mW/kg	2–48 h

TABLE 4
Extended

Experiments	Platform	Results and comments
$N = 2$	Atlas Human cDNA array (588 genes screened)	-3 genes differentially expressed (c-kit, nucleoside diphosphate kinase B, DAD-1) -no inferential statistics -no confirmation of results using RT-PCR -insufficient number of biological experiments -exposure conditions/temperature properly controlled
$N = 1$	Atlas Human cDNA array trial kit (82 genes screened)	-14 genes differentially expressed (mitogenic signal transduction genes, cell cycle inhibitors, apoptosis) -no inferential statistics -no confirmation of results using RT-PCR -insufficient number of biological experiments -uncontrolled exposure conditions
$N = 1$	SAGE	-221 genes differential expressed after 2 h exposure, 759 genes after 6 h -no inferential statistics -no confirmation of results using RT-PCR -insufficient number of biological experiments -exposure conditions/temperature properly controlled
$N = 3$	Affymetrix U34 GeneChips (8800 genes screened)	-11 genes up-regulated, 1 gene down-regulated (diverse functions) -changes in gene expression were relatively small, may have resulted from heterogeneous tissue -no confirmation of results using RT-PCR -minimum number of biological replicates
$N = 1$	Affymetix Human Focus Gene arrays (8400 genes screened)	-6 genes down-regulated in response to RF exposure -RT-PCR analysis of 2 genes did not confirm the microarray results -insufficient number of biological experiments
$N = 2$	Affymetrix HG-U133 Plus2.0 Genechip (38000 probes screened)	-no consistent changes in gene expression across 2 experiments -lack of response on p53-related gene expression (TP53, TP53BP2, APAF1 and CASP9) confirmed by RT-PCR -insufficient number of biological experiments
$N = 3$	Atlas Human v1.2 cDNA arrays (1167 genes screened)	-1 gene down-regulated in EA.hy926 cells, 13 genes up-regulated in EA.hy926v1 cells -no evidence of protein changes for these genes -no confirmation of results using RT-PCR -minimum number of biological replicates
$N = 5$	Agilent Human 1A arrays (~22,000 probes screened)	-no effect -lack of effect on several HSP confirmed by RT-PCR -assessed multiple RF-radiation doses -included concurrent positive, negative and sham controls -exposure conditions/temperature properly controlled
used pooled RNA, $N = 1$ for hybridizations	Human Unigene RZPD-2 cDNA array (~75000 probes screened)	-differential gene expression in 3 cell lines (EA.hy926, U937, HL60) -no confirmation of results using RT-PCR -insufficient number of biological replicates -exposure conditions/temperature properly controlled
$N = 3$	Affymetrix U74AV2 GeneChips (~9200 genes screened)	~400 genes differentially expressed in sham-sham comparison, a total of ~200 genes altered between RFR-sham comparison -no confirmation of results using RT-PCR -minimum number of biological replicates -included positive controls -exposure conditions/temperature properly controlled
$N = 2$	Affymetrix GeneChip Test3 arrays (~22000 probes screened)	-no effect at 2.0 W/kg, 5 genes up-regulated at 3.5 W/kg -RT-PCR analysis did not confirm differential expression of the 5 candidate genes identified by microarray analysis -insufficient number of biological replicates
$N = 5$	Agilent Human 1Av2 arrays (~22,000 probes screened)	-no effect -lack of effect on several HSP confirmed by RT-PCR -assessed multiple RFR doses -included concurrent positive, negative and sham controls -exposure conditions/temperature properly controlled
$N = 2$	Affymetrix HG-U133 Plus2.0 Genechip (38000 probes screened)	-no effect -no confirmation of results using RT-PCR -insufficient number of biological replicates

TABLE 4
Continued

Reference(s)	Tissue/cell line(s)	Signal(s)	SAR(s)	Duration
Zhao *et al.* (*62*)	Mouse primary neurons and astrocytes	1900 MHz, GSM	unknown	2 h
Zhao *et al.* (*63*)	Rat neurons	1800 MHz, GSM	2.0 W/kg	24 h (5 min ON, 10 min OFF)
Paparini *et al.* (*70*)	Mouse brain (*in vivo*)	1800 MHz, GSM	1.1 W/kg (whole-body)	1 h

Note. Abbreviations: CDMA: code domain multiple access; CHO: Chinese hamster ovary; CW, continuous wave; FDMA: frequency domain multiple access; GSM, global system for mobile communication; HSC, heat shock cognate; HSF, heat-shock factor; HSP: heat-shock protein; p-HSP27, phosphorylated HSP27; RT-PCR, reverse transcription polymerase chain reaction; SAR, specific absorption rate; SRE: serum response element; W-CDMA: wideband-code division multiple access.

against Affymetrix Human Focus Gene Arrays (containing probes to 8400 genes). Six genes were found to be slightly down-regulated as a result of RF-field exposure relative to the sham control. However, these results were based on a single independent experiment. Primers were prepared for two of the six genes and RT-PCR analysis failed to verify the microarray results. The remaining four genes (LIM, Nap1L1, ACADM, Rbbp4) were not examined by RT-PCR and require further investigation. Zeng *et al.* (*65*) exposed cells of a human breast cancer cell line (MCF-7) to intermittent (5 min ON/10 min OFF) 1800 MHz GSM-modulated RF radiation for 24 h at 2.0 and 3.5 W/kg. RNA was harvested immediately in two independent experiments, and each sample was hybridized in duplicate onto Affymetrix GeneChip Test3 arrays (~22,000 probes screened). To reduce the number of false positives detected, the authors performed four pairwise comparisons among the duplicate arrays for each of the RF-radiation- and sham-exposed groups in each experiment. Using this approach, no statistically significant differences were observed at an SAR of 2.0 W/kg, but five differentially expressed genes were observed in cells exposed at an SAR of 3.5 W/kg. However, none of these genes could be confirmed by RT-PCR analysis. The authors concluded that 1800 MHz RF radiation at intensities up to an SAR of 3.5 W/kg for 24 h did not result in reproducible changes in gene expression in MCF-7 cells. Hirose *et al.* (*24*) exposed human glioblastoma-derived cells (A172) and human fetal lung fibroblast-derived (IMR-90) cells to 2.1425 GHz CW and wideband-CDMA RF radiation for 2–28 h at SARs ranging from 0.08 to 0.8 W/kg. The authors conducted two independent experiments (each consisting of three cultures exposed to sham and RF radiation), isolated RNA immediately after exposure, and hybridized samples to Affymetrix HG-U133 Plus2.0 microarrays (containing probes to over 38,000 genes). Despite assessing a variety of exposure conditions, including different exposure durations, signal modulation and SARs, the authors reported no differential gene expression among all conditions tested in either cell line. However, heat treatment at 42°C for 2 h was observed to elicit a profound change in a number of HSP genes (as expected). In a previous study, Hirose *et al.* (*53*) also observed no noticeable changes in p53-related gene expression in A172 or IMR-90 cells under similar RF-radiation exposure conditions. In this study the authors confirmed the absence of a response by microarray analysis on four genes (APAF1, TP53, TP53BP2 and CASP9) involved in p53-mediated apoptosis using RT-PCR. While these studies assessed the entire human genome for responsiveness, the results are based on an inadequate number of independent experiments. In summary, each of these studies found no convincing evidence that RF radiation caused any alterations in gene expression; however, an insufficient number of independent biological experiments were performed for proper microarray analysis.

In contrast to the above investigations, several recent microarray studies have incorporated a larger number of independent biological experiments ((*3–5*)), applied the appropriate normalization and filtering techniques of image data, and performed appropriate statistical inference testing. Whitehead *et al.* (*66, 67*) exposed C3H 10T½ mouse cells to 847.74 MHz CDMA and 835.2 MHz FDMA RF radiation at 5 W/kg for 24 h. The authors conducted three independent experiments for each of the signal modulations tested and used

TABLE 4
Extended Continued

Experiments	Platform	Results and comments
$N = 2$ for microarray, $N = 3$ for RT-PCR	GEArray Q series mouse apoptosis array (96 apoptosis-related genes were screened)	-up-regulation of 3 genes (caspase-2, caspase-6 and Asc) in both cell lines, Bax also up-regulated in astrocytes -no inferential statistics (based on 35% fold change) -RT-PCR confirmed microarray results -RFR environmental conditions did not match control sample conditions -insufficient number of biological experiments for microarray analysis -imprecise exposure conditions (exposure system-related confounding possible)
$N = 1$ for microarray, $N = 3$ for RT-PCR	Affymetrix Rat Neurobiology U34 arrays (~1200 probes screened)	-10 down-regulated and 24 up-regulated genes -no inferential statistics (based upon 15% fold change) -insufficient number of biological replicates for microarray analysis -RT-PCR analysis confirmed microarray results -no evidence of active cooling (temperature confounding possible)
used pooled RNA, $N = 3$ for hybridizations	Affymetrix MOE 430A arrays (22000 probes screened)	-no effect on gene expression using high-stringency microarray data analysis, 75 differentially expressed genes identified using low-stringency (1.5 fold change) analysis. -RT-PCR analysis of 30 genes did not confirm the low-stringency microarray data analysis -exposure conditions/temperature properly controlled

matching samples exposed to X radiation (0.68 Gy) as positive controls. RNA was harvested immediately after sham/RF-radiation exposure or at 4 h after X irradiation, and the samples were then hybridized to Affymetrix U74AV2 GeneChips (containing 12,422 probes). In an interesting approach, the authors made sham-sham comparisons to empirically estimate the false discovery rate (FDR) in their experimental model. Using this approach, they observed that fewer genes (~200) were found to be differentially expressed after exposure to CDMA or FDMA RF radiation than would have occurred by chance alone (~400). These genes were disregarded based upon the estimated FDR and the fact that only two genes were similarly altered for the exposures to CDMA and FDMA RF radiation. However, none of the RF-radiation-induced differentially expressed genes were actually tested by RT-PCR. Qutob *et al.* (*68*) exposed human glioblastoma-derived (U87MG) cells to 1.9 GHz pulse-modulated RF radiation for 4 h at SARs of 0.1, 1.0 and 10.0 W/kg. At 6 h after sham or RF-radiation exposure, RNA was harvested and samples were subsequently hybridized to Agilent Human 1A arrays (containing probes to over 22,000 genes). Similar to the approach of Whitehead *et al.* (*66, 67*), this study included concurrent negative (incubator) and positive (heat-shock) controls to evaluate the responsiveness of the experimental protocol and assess the influence of possible RF-radiation exposure system environmental factors. The authors observed no evidence of differential gene expression in any of the RF-radiation-exposed samples relative to the sham controls. However, the positive (heat-shock) control showed an up-regulation in 99 genes, including a number of typical HSPs and stress-responsive genes. Semi-quantitative RT-PCR was conducted on a variety of HSP and verified that RF radiation had no effect on HSP gene transcription. In an extension of this study, this group exposed U87MG cells to intermittent (5 min ON, 10 min OFF) 1.9 GHz pulse-modulated RF radiation for 24 h at SARs of 0.1, 1.0 and 10.0 W/kg and RNA was harvested 6 h after exposure (*38*). Human-derived monocyte cells (Mono-Mac-6) were also exposed under similar RF-radiation conditions for 6 h and RNA was harvested either immediately or 18 h after exposure. No evidence of differential gene expression was observed in either cell line at any SAR or time tested; however, heat shock (42°C for 1 h) was observed to elicit a profound change in gene expression relative to the sham-treated controls. These studies are unique in that multiple SARs, incubator, sham and positive-controls were performed concurrently in five independent experiments. The inclusion of a larger number of independent experiments reduces the likelihood of observing false-positive events that occur when performing statistical inference testing on thousands of end points. Furthermore, RT-PCR was used to verify HSP responses in the positive controls and lack of response in the RF-radiation-exposed samples. The authors concluded that they observed no evidence of differential gene expression under any RF-radiation conditions tested in their experimental system at SARs up to 10 W/kg and exposures up to 24 h.

To date, only two studies have evaluated the effect of RF radiation on global gene expression changes *in vivo*. Belyaev *et al.* (*69*) examined gene expression changes in rat cerebellum after a 2-h *in vivo* exposure to 915 MHz GSM-modulated RF radiation at a whole-body SAR of 0.4 W/kg. In this study, three sham-exposed and three RF-radiation-exposed rats were killed immediately after exposure. RNA was isolated from the cerebellum and subsequently hybridized to Affymetrix U34A GeneChips (containing probes to ~8800 genes). The authors identified 11 genes that were up-regulated and one gene that was down-regulated in the cerebellum of RF-

radiation-exposed rats relative to sham-exposed rats. These genes encoded proteins with a variety of functions, including metabolic enzymes, blood-brain barrier function, glia function and miscellaneous proteins. The changes in expression were relatively small and were not confirmed by the use of other methods. The authors concluded that the relatively small changes in gene expression may have resulted from the use of highly heterogeneous tissue and that future studies should examine specific regions of the brain. Finally, Paparini *et al.* (*70*) exposed immobilized mice to 1800 MHz GSM-modulated RF radiation for 1 h at a whole-body SAR of 1.1 W/kg (~0.2 W/kg in brain). A total of 15 animals were exposed to sham or RF radiation, RNA was harvested from the whole brains of animals immediately after exposure, and then three pools of RNA (prepared from five animals each) were prepared for each of the sham- and RF-radiation-exposed mice. The pooled RNA samples were hybridized to Affymetrix Mouse Expression Array 430A GeneChip arrays (containing over 22,600 probe sets). The microarray results were filtered on flags (present calls), fold change (>1.5- or >twofold) and *P* value. A total of 301 genes were observed to display changes greater than 1.5-fold, while only 30 genes were altered by more than twofold; however, none of these genes was considered significantly different by inferential statistics. When less stringent conditions were applied for data analysis by removing the Benjamini-Hochberg correction for multiple comparisons, 75 probes were identified as differentially expressed. To confirm these results, the authors performed RT-PCR on RNA from each animal for 20 up-regulated and 10 down-regulated genes identified using low-stringency data analysis. However, the authors found no evidence of differential gene expression for any of these genes using RT-PCR, supporting the accuracy of the high-stringency analysis. While the authors conducted this study with an appropriate experimental design and a commendable statistical analysis (using both high- and low-stringency approaches), the use of crude whole-brain homogenates as the RNA source may have masked possible specific brain region/nuclei-specific responses.

In conclusion, there is no convincing evidence of differential gene expression in either cell culture experiments or rodent brain tissue after non-thermal RF-radiation exposures. The studies that have reported differential gene expression are typically based on observations from a single experiment, where high rates of false positives are expected and where no verification of the microarray results by alternate methodologies have been performed. Additional high-quality microarray studies are required, but they should be conducted according to best practices for the proper conduct of microarray analysis (*55*). In particular, the examination of gene responses in specific brain nuclei/regions related to neurophysiological dysfunction purportedly attributed to electromagnetic hypersensitivity (e.g. memory, attention, motor function) or observed in human volunteer studies (e.g. cognitive reaction time) would be highly informative from a public health perspective.

CONCLUDING REMARKS

It is always a difficult task to translate contradictory science into the context of human health risk. The basic question to be considered by risk assessors is: Which results are correct? In the case of RF radiation, is it the investigations that find no evidence of radiation-induced effects or is it the studies that report radiation-induced alterations in genes/proteins? It has become clear through a number of studies that replication of purported RF-radiation-induced gene/protein effects by other laboratories does not always produce the same findings. However, one must consider whether all the essential variables in the experimental matrix of the replication study have been properly accounted for or whether they are being compromised by some unknown masked factors that have been missed in the experimental design. Were the duration of exposure, tissue penetration and/or SAR homogeneity, heat generation, type of the field (static or oscillatory), waveform (sinusoidal, square, etc.), biological status and type of the cells similar in the replication studies? Questions also remain relating to the actual exposure conditions of the original studies. Was the SAR level and distribution modeled accurately and/or measured correctly? Could thermal confounding (e.g. local hot spots) be responsible for the observed results? Could other environmental conditions (e.g. cooling systems) affect the outcome of the experiments? Important questions also need to be considered with the use of modern high-throughput technologies. Are they reliable? Are the data being interpreted properly? Are all the correct controls employed, and are they behaving appropriately? Is the vast amount of data output being appropriately corrected for false positives as well as for false negatives? All these questions need to be considered when evaluating the scientific literature before making weight-of-evidence decisions with respect to the potential effect of non-thermal RF radiation on human health.

In the last decade, numerous studies have investigated the potential ability of RF radiation to modify gene transcription and protein levels in a variety of cellular and animal models. A selected few of these investigations have reported RF-radiation-induced effects, but under conditions where the possibility of thermal confounding cannot be excluded. Other studies report RF-radiation-induced alterations in gene/protein expression under non-thermal RF-radiation exposure conditions, but typically in unique (unreplicated) conditions/

models or under experimental conditions with methodological shortcomings. Furthermore, there are no clear trends in the list of responsive genes/proteins across studies of various experimental designs, thereby diminishing the biological plausibility that responses observed in individual studies are genuine. When taken collectively, the weight of evidence does not support the notion of specific, non-thermal responses to RF radiation at the gene or protein level. Nevertheless, a few well-conducted studies have observed sufficient evidence of possible RF-radiation-induced gene/protein interaction to warrant further investigation. The increasing application of high-throughput technologies to study possible RF-radiation-induced bioeffects may help to resolve these discrepancies, but only if such studies are conducted with a sound experimental design and rigorous statistical analysis.

ACKNOWLEDGMENT

This review was funded entirely by Health Canada.

Received: January 27, 2009; accepted: April 24, 2009

REFERENCES

1. E. R. Adair and D. R. Black, Thermoregulatory responses to RF energy absorption. *Bioelectromagnetics Suppl.* **6**, S17–S38 (2003).
2. K. R. Foster and R. Glaser, Thermal mechanisms of interaction of radiofrequency energy with biological systems with relevance to exposure guidelines. *Health Phys.* **92**, 609–620 (2007).
3. International Commission for Non-Ionizing Radiation Protection (ICNIRP), Guidelines for limiting exposure to time-varying electric, magnetic, and electromagnetic fields (up to 300 GHz). *Health Phys.* **74**, 494–522 (1998).
4. *IEEE Standard for Safety Levels with Respect to Human Exposure to Radiofrequency Electromagnetic Fields, 3 kHz to 300 GHz*. Institute of Electrical and Electronic Engineers, New York, 2005.
5. J. E. Moulder, K. R. Foster, L. S. Erdreich and J. P. McNamee, Mobile phones, mobile phone base stations and cancer: a review. *Int. J. Radiat. Biol.* **81**, 189–203 (2003).
6. P. A. Valberg, T. E. van Deventer and M. H. Repacholi, Workgroup report: base stations and wireless networks-radiofrequency (RF) exposures and health consequences. *Environ. Health Perspect.* **115**, 416–424 (2007).
7. P. W. French, R. Penny, J. A. Laurence and D. R. McKenzie, Mobile phones, heat shock protein and cancer. *Differentiation* **67**, 93–97 (2001).
8. R. I. Morimoto, Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. *Genes Dev.* **12**, 3788–3796 (1998).
9. J. Lis and C. Wu, Protein traffic on the heat shock promoter: parking, stalling, and trucking along. *Cell* **74**, 1–4 (1993).
10. R. I. Morimoto, Cells in stress: transcriptional activation of heat shock genes. *Science* **259**, 1409–1410 (1993).
11. J. E. Parker, J. L. Kiel and W. D. Winters, Effect of radiofrequency radiation of mRNA expression in cultured rodent cells. *Physiol. Chem. Phys. Med. NMR* **20**, 129–134 (1988).
12. K. Fritze, C. Wiessner, N. Kuster, C. Sommer, P. Gass, D. M. Hermann, M. Kiessling and K. A. Hossmann, Effect of global system for mobile communication microwave exposure on the genomic response of the rat brain. *Neuroscience* **81**, 627–639 (1997).
13. S. F. Cleary, G. Cao, L-M. Liu, P. M. Egle and K. R. Shelton, Stress proteins are not induced in mammalian cells exposed to radiofrequency or microwave radiation. *Bioelectromagnetics* **18**, 499–505 (1997).
14. D. De Pomerai, C. Daniells, H. David, J. Allan, I. Duce, M. Mutwakil, D. Thomas, P. Sewell, J. Tattersall, D. Jones and P. Candido, Non-thermal heat-shock response to microwaves. *Nature* **405**, 417–418 (2000).
15. A. S. Dawe, B. Smith, D. W. Thomas, S. Greedy, N. Vasic, A. Gregory, B. Loader and D. I. De Pomerai, A small temperature rise may contribute towards the apparent induction by microwaves of heat-shock gene expression in the nematode *Caenorhabditis elegans*. *Bioelectromagnetics* **27**, 88–97 (2006).
16. D. Weisbrot, H. Lin, L. Ye, M. Blank and R. Goodman, Effects of mobile phone radiation on reproduction and development in *Drosophila melanogaster*. *J. Cell Biochem.* **89**, 48–55 (2003).
17. J. Czyz, K. Guan, Q. Zeng, T. Nikolova, A. Meister, F. Schonborn, J. Schuderer, N. Kuster and A. M. Wobus, High frequency electromagnetic fields (GSM signals) affect gene expression levels in tumor suppressor p53-deficient embryonic stem cells. *Bioelectromagnetics* **25**, 296–307 (2004).
18. F. Tian, T. Nakahara, K. Wake, M. Taki and J. Miyakoshi, Exposure to 2.45 GHz electromagnetic fields induces hsp70 at a high SAR of more than 20 W/kg but not at 5 W/kg in human glioma MO54 cells. *Int. J. Radiat. Biol.* **78**, 433–440 (2002).
19. J. Wang, S. Koyama, Y. Komatsubara, Y. Suzuki, M. Taki and J. Miyakoshi, Effects of a 2450 MHz high-frequency electromagnetic field with a wide range of SARs on the induction of the induction of heat-shock proteins in A172 cells. *Bioelectromagnetics* **27**, 479–486 (2006).
20. A. Lerchl, Comments on ''Effects of a 2450 MHz high-frequency electromagnetic field with a wide range of SARs on the induction of heat-shock proteins in A172 cells'' by Wang *et al.* (*Bioelectromagnetics* 27: 479–486, 2006). *Bioelectromagnetics* **29**, 583–584 (2008)
21. D. Leszczynski, S. Joenvaara, J. Reivinen and R. Kuokka, 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 (2002).
22. R. Nylund and D. Leszczynski, 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 (2006).
23. R. P. Vanderwaal, B. Cha, E. G. Moros and J. L. Roti Roti, HSP27 phosphorylation increases after 45°C or 41°C heat shocks but not after non-thermal TDMA or GSM exposures. *Int. J. Hyperthermia* **22**, 507–519 (2006).
24. H. Hirose, N. Sakuma, N. Kaji, K. Nakayama, K. Inoue, M. Sekijima, T. Nojima and J. Miyakoshi, Mobile phone base station-emitted radiation does not induce phosphorylation of hsp27. *Bioelectromagnetics* **28**, 99–108 (2007).
25. J. S. Lee, T. Q. Huang, J. J. Lee, J. K. Pack, J. J. Jang and J. S. Seo, Subchronic exposure of hsp70.1-deficient mice to radiofrequency radiation. *Int. J. Radiat. Biol.* **81**, 781–792 (2005).
26. H. B. Lim, G. G. Cook, A. T. Barker and L. A. Coulton, Effect of 900 MHz electromagnetic fields on nonthermal induction of heat-shock proteins in human leukocytes. *Radiat. Res.* **163**, 45–52 (2005).
27. J. Miyakoshi, K. Takemasa, Y. Takashima, G. R. Ding, H. Hirose and S. Koyama, Effects of exposure to a 1950 MHz radio frequency field on expression of Hsp70 and Hsp27 in human glioma cells. *Bioelectromagnetics* **26**, 251–257 (2005).
28. M. Simko, C. Hartwig, M. Lantow, M. Lupke, M. O. Mattsson, Q. Rahman and J. Rollwitz, Hsp70 expression and free radical release after exposure to non-thermal radio-frequency electro-

magnetic fields and ultrafine particles in human Mono Mac 6 cells. *Toxicol. Lett.* **161**, 73–82 (2006).

29. M. Lantow, J. Schuderer, C. Hartwig and M. Simko, Free radical release and HSP70 expression in two human immune-relevant cell lines after exposure to 1800 MHz radiofrequency radiation. *Radiat. Res.* **165**, 88–94 (2006).
30. M. Zhadobov, R. Sauleau, L. Le Coq, L. Debure, D. Thouroude, D. Michel and Y. Le Dréan, Low-power millimeter wave radiations do not alter stress-sensitive gene expression of chaperone proteins. *Bioelectromagnetics* **28**, 188–196 (2007).
31. S. Sanchez, A. Milochau, G. Ruffie, F. Poulletier de Gannes, I. Lagroye, E. Haro, J-E. Surleve-Bazeille, B. Billaudel, M. Lassegues and B. Veyret, Human skin cell stress response to GSM-900 mobile phone signals: *In vitro* study on isolated primary cells and reconstructed epidermis. *FEBS J.* **273**, 5491–5507 (2006).
32. S. Sanchez, E. Haro, G. Ruffie, B. Veyret and I. Lagroye, *In vitro* study of the stress response of human skin cells to GSM-1800 mobile phone signals compared to UVB radiation and heat shock. *Radiat. Res.* **167**, 572–580 (2007).
33. S. Sanchez, H. Masuda, G. Ruffié, F. P. De Gannes, B. Billaudel, E. Haro, P. Lévêque, I. Lagroye and B. Veyret, Effect of GSM-900 and -1800 signals on the skin of hairless rats. III: Expression of heat shock proteins. *Int. J. Radiat. Biol.* **84**, 61–68 (2008).
34. A. Laszlo, E. G. Moros, T. Davidson, M. Bradbury, W. Straube and J. L. Roti Roti, The heat-shock factor is not activated in mammalian cells exposed to cellular phone frequency microwaves. *Radiat. Res.* **164**, 163–172 (2005).
35. J-S. Lee, T-Q. Huang, T-H. Kim, J. Y. Kim, H. J. Kim, J-K. Pack and J-S. Seo, Radiofrequency radiation does not induce stress response in human T-lymphocytes and rat primary astrocytes. *Bioelectromagnetics* **27**, 578–588 (2006).
36. V. Chauhan, A. Mariampillai, P. V. Bellier, S. S. Qutob, G. Gajda, E. Lemay, A. Thansandote and J. P. McNamee, Gene expression analysis of a human lymphoblastoma cell line exposed *in vitro* to an intermittent 1.9 GHz pulse-modulated radiofrequency field. *Radiat. Res.* **165**, 424–429 (2006).
37. V. Chauhan, A. Mariampillai, G. B. Gajda, A. Thansandote and J. P. McNamee, 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. J. Radiat. Biol.* **82**, 347–354 (2006).
38. V. Chauhan, S. S. Qutob, S. Lui, A. Mariampillai, P. V. Bellier, C. L. Yauk, G. R. Douglas, A. Williams and J. P. McNamee, 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 (2007).
39. I. A. Cotgreave, Biological stress responses to radiofrequency electromagnetic radiation: are mobile phones really so (heat) shocking? *Arch. Biochem. Biophys.* **435**, 227–240 (2005).
40. O. I. Ivaschuk, R. A. Jones, T. Ishida-Jones, W. Haggren, W. R. Adey and J. L. Phillips, Exposure of nerve growth factor-treated PC12 rat pheochromocytoma cells to a modulated radiofrequency field at 836.55 MHz: effects on c-jun and c-fos expression. *Bioelectromagnetics* **18**, 223–229 (1997).
41. P. C. Goswami, L. D. Albee, A. J. Parsian, J. D. Baty, E. J. Moros, W. F. Pickard, J. L. Roti Roti and C. R. Hunt, Proto-oncogene mRNA levels and activities of multiple transcription factors in C3H 10T½ murine embryonic fibroblasts exposed to 835.62 and 847.74 MHz cellular phone communication frequency radiation. *Radiat. Res.* **151**, 300–309 (1999).
42. T. D. Whitehead, B. H. Brownstein, J. J. Parry, D. Thompson, B. A. Cha, E. G. Moros, B. E. Rogers and J. L. Roti Roti, Expression of the proto-oncogene Fos after exposure to radiofrequency radiation relevant to wireless communications. *Radiat. Res.* **164**, 420–430 (2005).
43. J. J. Morrissey, S. Raney, E. Heasley, P. Rathinavelu, M. Dauphinee and J. H. Fallon, IRIDIUM exposure increases c-fos expression in the mouse brain only at levels which likely result in tissue heating. *Neuroscience* **92**, 1539–1546 (1999).
44. R. B. Stagg, L. H. Hawel, K. Pastorian, C. Cain, W. R. Adey and C. V. Byus, Effect of immobilization and concurrent exposure to a pulse-modulated microwave field on core body temperature, plasma ACTH and corticosteroid, and brain ornithine decarboxylase, Fos and Jun mRNA. *Radiat. Res.* **155**, 584–592 (2001).
45. J. W. Finnie, Expression of the immediate early gene, c-fos, in mouse brain after acute global system for mobile communication microwave exposure. *Pathology* **37**, 231–233 (2005).
46. J. W. Finnie, Z. Cai, P. C. Blumbergs, J. Manavis and T. R. Kutchel, Expression of the immediate early gene, c-fos, in fetal brain after whole of gestation exposure of pregnant mice to Global System for Mobile Communication microwaves. *Pathology* **38**, 333–335 (2006).
47. T. D. Utteridge, V. Gebski, J. W. Finnie, B. Vernon-Roberts and T. R. Kuchel, Long-term exposure of Eμ-*Pim1* transgenic mice to 898.4 MHz microwaves does not increase lymphoma incidence. *Radiat. Res.* **158**, 357–364 (2002).
48. J. W. Finnie, Z. Cai, P. C. Blumbergs, J. Manavis and T. R. Kuchel, Stress response in mouse brain after long-term (2 year) exposure to mobile telephone radiofrequency fields using the immediate early gene, c-fos. *Pathology* **39**, 271–273 (2007).
49. J. Friedman, S. Kraus, Y. Hauptman, Y. Schiff and R. Seger, Mechanism of short-term ERK activation by electromagnetic fields at mobile phone frequencies. *Biochem. J.* **405**, 559–568 (2007).
50. M. Buttiglione, L. Roca, E. Montemurno, F. Vitiello, V. Capozzi and G. Cibelli, Radiofrequency radiation (900 MHz) induces Egr-1 gene expression and affects cell-cycle control in human neuroblastoma cells. *J. Cell Physiol.* **213**, 759–767 (2007).
51. K-S. Lee, J-S. Choi, S-Y. Hong, T-H. Son and K. Yu, Mobile phone electromagnetic radiation activates MAPK signaling and regulates viability in *Drosophila*. *Bioelectromagnetics* **29**, 371–379 (2008).
52. T. Nikolova, J. Czyz, A. Rolletschek, P. Blyszczuk, J. Fuchs, G. Jovtchev, J. Schuderer, N. Kuster and A. M. Wobus, Electromagnetic fields affect transcript levels of apoptosis-related genes in embryonic stem cell-derived neural progenitor cells. *FASEB J.* **19**, 1686–1688 (2005).
53. H. Hirose, N. Sakuma, N. Kaji, T. Suhara, M. Sekijima, T. Nojima and J. Miyakoshi, Phosphorylation and gene expression of p53 are not affected in human cells exposed to 2.1425 GHz band CW or W-CDMA modulated radiation allocated to mobile radio base stations. *Bioelectromagnetics* **27**, 494–504 (2006).
54. J. R. Li, C. K. Chou, J. A. McDougall, G. Dasgupta, H-H. Wu, R. L. Ren, A. Lee, J. Han and J. Momand, TP53 tumor suppressor protein in normal human fibroblasts does not respond to 837 MHz microwave exposure. *Radiat. Res.* **151**, 710–716 (1999).
55. D. B. Allison, X. Cui, G. P. Page and M. Sabripour, Microarray data analysis: from disarray to consolidation and consensus. *Nat. Rev. Genet.* **7**, 55–65 (2005).
56. J. Vanderstraeten and L. Verschaeve, Gene and protein expression following exposure to radiofrequency fields from mobile phones. *Environ. Health Perspect.* **116**, 1131–1135 (2008).
57. C. Harvey and P. W. French, Effects on protein kinase C and gene expression in a human mast cell line, HMC-1, following microwave exposure. *Cell Biol. Int.* **23**, 739–748 (1999).
58. S. Pacini, M. Ruggiero, I. Sardi, S. Aterini, F. Gulisano and M. Gulisano, 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 (2002).
59. S. Lee, D. Johnson, K. Dunbar, H. Dong, X. Ge, Y. C. Kim, C. Wing, N. Jayathilaka, N. Emmanuel and S. M. Wang, 2.45 GHz radiofrequency fields alter gene expression in cultured human cells. *FEBS Lett.* **579**, 4829–4836 (2005).
60. D. Remondini, R. Nylund, J. Reivinen, F. Poulletier de Gannes, B. Veyret, I. Lagroye, E. Haro, A. M. Trillo, M. Capri and C.

Maercker, Gene expression changes in human cells after exposure to mobile phone microwaves. *Proteomics* **6**, 4745–4754 (2006).

61. A. Karinen, S. Heinavaara, R. Nylund and D. Leszczynski, Mobile phone radiation might alter protein expression in human skin. *BMC Genomics* **9**, 77 (2008).
62. T. Y. Zhao, S. P. Zou and P. E. Knapp, Exposure to cell phone radiation up-regulates apoptosis genes in primary cultures of neurons and astrocytes. *Neurosci. Lett.* **412**, 34–38 (2007).
63. R. Zhao, S. Zhang, Z. Xu, L. Ju, D. Lu and G. Yao, Studying gene expression profile of rat neuron exposed to 1800 MHz radiofrequency electromagnetic fields with cDNA microassay. *Toxicology* **235**, 167–175 (2007).
64. E. Gurisik, K. Warton, D. K. Martin and S. M. Valenzuela, An *in vitro* study of the effects of exposure to a GSM signal in two human cell lines: monocytic U937 and neuroblastoma SK-N-SH. *Cell Biol. Int.* **30**, 793–799 (2006).
65. Q. Zeng, G. Chen, Y. Weng, L. Wang, H. Chiang, D. Lu and Z. Xu, 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 (2006).
66. T. D. Whitehead, E. G. Moros, B. H. Brownstein and J. L. Roti Roti, Gene expression does not change significantly in C3H 10T½ cells after exposure to 847.74 CDMA or 835.62 FDMA radiofrequency radiation. *Radiat. Res.* **165**, 626–635 (2006).
67. T. D. Whitehead, E. G. Moros, B. H. Brownstein and J. L. Roti Roti, The number of genes changing expression after chronic exposure to code division multiple access or frequency DMA radiofrequency radiation does not exceed the false-positive rate. *Proteomics* **6**, 4739–4744 (2006).
68. S. S. Qutob, V. Chauhan, P. V. Bellier, C. L. Yauk, G. R. Douglas, A. Williams, L. Berndt, G. Gajda, A. Thansondote and J. P. McNamee, Microarray gene expression profiling of a human glioblastoma cell line exposed *in vitro* to 1.9 GHz pulse modulated radiofrequency fields. *Radiat. Res.* **165**, 636–644 (2006).
69. I. Y. Belyaev, C. B. Koch, O. Terenius, K. Roxström-Lindquist, L. O. Malmgren, W. H. Sommer, L. G. Salford and B. R. Persson, Exposure of rat brain to 915 MHz GSM microwaves induces changes in gene expression but not double stranded DNA breaks or effects on chromatin conformation. *Bioelectromagnetics* **27**, 295–306 (2006).
70. A. Paparini, P. Rossi, G. Gianfranceschi, V. Grugaletta, R. Falsaperia, P. De Luca and V. Romano Spica, No evidence of major transcriptional changes in the brain of mice exposed to 1800 MHz GMS signal. *Bioelectromagnetics* **29**, 312–323 (2008).