J Neurooncol
DOI 10.1007/s11060-011-0644-z

LABORATORY INVESTIGATION - HUMAN/ANIMAL TISSUE

The genotoxic effect of radiofrequency waves on mouse brain

Emin Karaca · Burak Durmaz · Huseyin Altug · Teoman Yildiz · Candan Guducu · Melis Irgi · Mehtap Gulcihan Cinar Koksal · Ferda Ozkinay · Cumhur Gunduz · Ozgur Cogulu

Received: 22 February 2011 / Accepted: 18 June 2011

Abstract Concerns about the health effects of radiofrequency (RF) waves have been raised because of the gradual increase in usage of cell phones, and there are scientific questions and debates about the safety of those instruments in daily life. The aim of this study is to evaluate the genotoxic effects of RF waves in an experimental brain cell culture model. Brain cell cultures of the mice were exposed to 10.715 GHz with specific absorbtion rate (SAR) 0.725 W/kG signals for 6 h in 3 days at 25°C to check for the changes in the micronucleus (MNi) assay and in the expression of 11 proapoptotic and antiapoptotic genes. It was found that MNi rate increased 11-fold and STAT3 expression decreased 7-fold in the cell cultures which were exposed to RF. Cell phones which spread RF may damage DNA and change gene expression in brain cells.

Keywords Radiofrequency · Cell phone · DNA · Genotoxity

E. Karaca (✉) · B. Durmaz · F. Ozkinay · O. Cogulu
Department of Medical Genetics, Ege University Faculty of Medicine, Izmir, Turkey
e-mail: emin.karaca@med.ege.edu.tr

H. Altug
Department of Histology and Embryology, Ege University Faculty of Medicine, Izmir, Turkey

T. Yildiz
Department of Physics, Ege University Faculty of Science, Izmir, Turkey

C. Guducu · M. Irgi
High School of Science, Izmir, Turkey

M. G. C. Koksal
Department of Pharmacology, Ege University Faculty of Medicine, Izmir, Turkey

C. Gunduz
Department of Medical Biology, Ege University Faculty of Medicine, Izmir, Turkey

Introduction

Radiofrequency (RF) is used in almost all areas of daily life such as mobile phones, televisions, microwave ovens, base stations, etc. There are more than 3 billion cell phone users worldwide [1]. Concerns about different health problems have particularly focused on mobile phones because they are currently being used by individuals with a wide range of ages starting from the very young and therefore the gradual increase in usage of mobile phones has raised public concerns about their safety. On the other hand, controversy exists about the association between RF energy and cancer, particularly leukemia and brain tumors [2]. Because of serving high electromagnetic fields (EMFs) close to the brain, tumors of this region have received particular attention. An association between the use of mobile phones and brain tumors has been reported [3, 4]; however, no correlation was found between mobile phone use and glioma or meningioma [5]. The 13-nation Interphone study has reported no increased risk of glioma or meningioma with the use of mobile phones although they have not demonstrated that there is no risk as stated by The Interphone Study Group http://www.rfcom.ca/programs/interphone.shtml. There are many other studies under way and the possible effects of long-term use of mobile phones require further investigations. Therefore, the aim of this study focused on the effect of RF waves similar to daily life usage on brain cell cultures of a newborn mouse.

Published online: 06 July 2011

Materials and methods

Cell culture

Brain tissue was extracted from a 3-day-old prepubertal male Wistar albino mouse. The mouse was anesthetized with pentobarbital and decapitated. The brain was immediately removed and cut into small pieces mechanically and cultured in a 25-cm^2 polystyrene flask (main culture flask) containing bio-amf medium with 10% supplement, 1% L-glutamine, 1% penicillin–streptomycin for 14 days at 37°C and 5% CO_2 in an incubator. The cultures were re-fed with fresh medium once a week and the cell culture in the main flask was passaged four times to prevent confluency. Six Petri dishes were prepared at the end of 3 weeks. All Petri dishes were placed in a monitored room at 25°C. Three of them were in a RF field and the other three were outside the field in the same room. The absence of any interference between the control group cell culture and microwave source was confirmed by using a dipole receiver. RF exposure conditions are explained below. At the end of the exposure, the samples were simultaneously removed and processed for cell viability, micronucleus assay and total mRNA. For this purpose, all Petri dishes were trypsinized and cells were plated in MEM + 10% FCS on two 6-well plate at a density of 10^6 cells/ml. One contained the study group which included 3 wells with the RF-exposed cells, and the other contained a control group of 3 wells with the cells without RF exposure. The viability was tested by trypan blue exclusion test of cell viability protocol.

The definition of cell types

In order to reveal the types of the cultured cells, they were first embedded in paraffin blocks, than these were cut in 5-μm-thick hematoxylin eosin (HE) stained for light microscopy (Fig. 1). Screen shots were taken within selected non-overlapping areas with a Camedia Digital camera.

Microwave equipment and RF signal exposure

A microwave source was used to evaluate the effect of RF/microwave radiation on mouse brain cell cultures. The exposure system was established in an empty testing room without any other device which might deliver a magnetic field. Electromagnetic radiation originating from the field of the study room from electrical supplies was 0.021 W/kg. Magnetic field experiments were performed by Microwave Equipment which has three major components (Fig. 2). It consisted of a 2.8-cm Microwave Transmitter (C051-044.672), a 2.8-cm Microwave Receiver (C052-045.674) and a 12-V DC Power Supply (all UNILAB, Blackburn, UK). The signal was used at 10.715 GHz oscillator frequency with 8.0-mW power output [6, 7]. The beam width of the microwave was 6 cm and the exposure per cm^2 over the sample was 0.84 mW/cm^2. The average SAR was 0.725 W/kg. The SAR value of electromagnetic radiation was determined by using semcad-x package computer program. The SAR value chosen in our experiments corresponds to the value of those in mobile phone systems (Fig. 3). The exposure system was turned on continuously for 6 h per day and continued for 3 days. The temperature of the room was kept steady at 25°C to provide an appropriate cooling system, and the inside temperature of the Petri dishes was monitored.

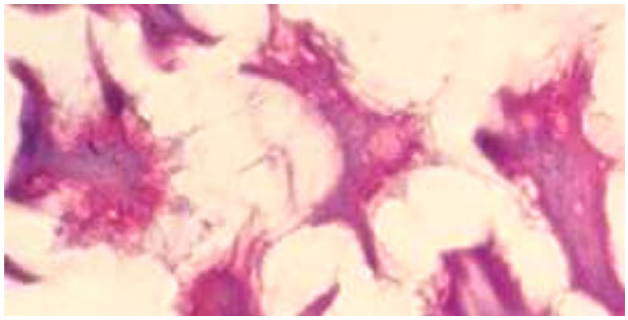

Fig. 1 Micrographs of 3-day-old primary cultures of primordial neurons from neonatal mouse brain with hematoxylin eosin staining

RNA processing and microarray

Apoptosis was evaluated by using Cell Death Detection ELISA Plus Kit (Roche Applied Science, Mannheim, Germany) according to the manual instructions. Expression levels of apoptosis-specific genes were carried out by oligoarray method (SuperArray, Frederick, MD, USA). The OligoGEArray Human Apoptosis microarray profiles the expression of 11 genes which have proapoptotic and anti-apoptotic effects. This array includes Bcl-2, Bcl-211, Bax, Bcl-212, caspase 3, Tert, FasL, TNF, NF-kb1, Tp53 and STAT3 genes.

Briefly, total RNA of 3 μg was prepared from brain culture samples in 6-well plates using an Array Grade Total RNA isolation kit (SuperArray). The integrity and quality of isolated RNA was determined by spectrophotometer (NanoDrop products, Wilmington, DE, USA). cDNA was labeled from total RNA with Biotin 16-dUTP and the GEArray TM Amp Labeling-LPR Kit (SuperArray) according to the manufacturer's instructions. The biotin-labeled cDNA was then added to the membrane and hybridized overnight to Human Apoptosis OligoGEArray as stated by the manufacturer. Signal detection was achieved by exposure to CDP-Star alkaline phosphatase chemiluminescent substrate (SuperArray). Images were

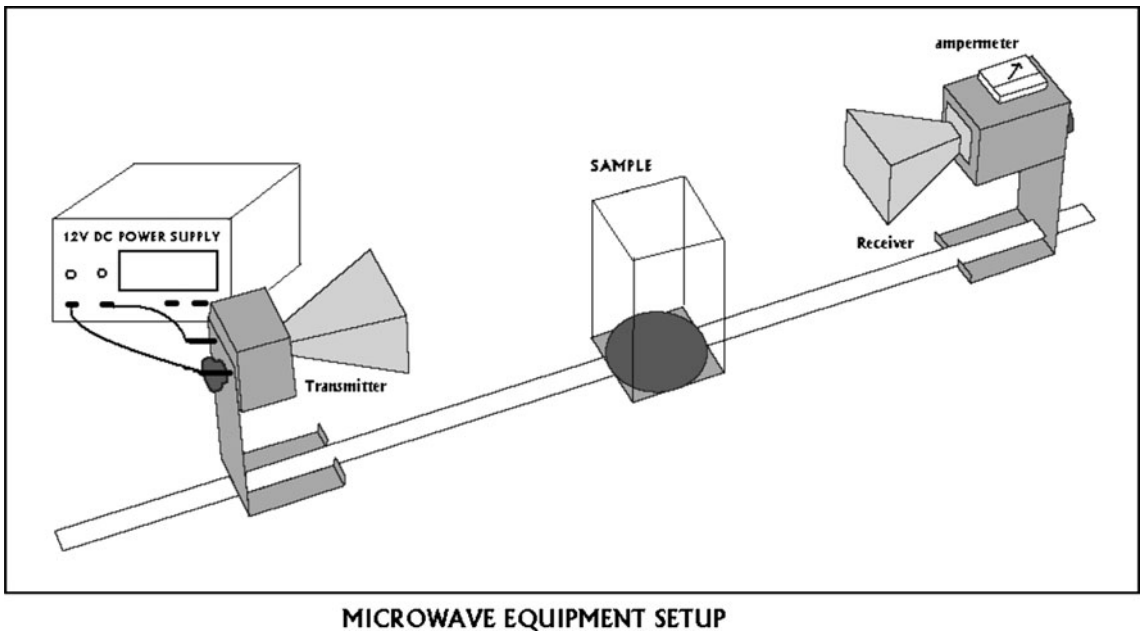

Fig. 2 Schematic representation of the experimental system

processed using Kodak Gel Logic 1500 Imaging System and analyzed with the GEArray Analyser Software.

Micronucleus assay

In order to determine the genotoxicity of RF, we performed micronucleus formation assay. Micronuclei (MN) were observed using cytochalasin B (Cyt-B) by the method of Fenech and Morley [8].

Cyt-B (6 μg/mL) was added into the mouse brain culture Petri dishes at the end of 5 days, incubated for the last 28 h of incubation and then cultures were harvested. The cultures were centrifuged at 1,100 rpm for 10 min. After the supernatant was removed, 5 ml of prewarmed hypotonic solution (0.5% KCl) was added to the pellet and incubated for 23 min at 37°C. The cultures were centrifuged at 1,100 rpm for 10 min and the supernatant was removed for methanol/glacial acetic acid (3:1v/v) fixation. Slides were prepared after three fixative changes and left for staining with 5% Giemsa for 1 h (Fig. 4).

The frequency of MNi in 1,000 cells and the proportion of apoptotic and necrotic cells were recorded. The micronucleated cell rate is the number of micronucleated cells scored per 1,000 observed binucleated lymphocytes.

All the slides were scored by the same person who did not know the type of the cells whether exposed to RF or not.

Statistical analysis

The comparison of the variation factors distribution in each group was performed by the χ^2 test.

Results

The inside temperature of the cell culture Petri dishes was 25°C at the beginning of the experiment and it did not show any change throughout the experiment period. The mean number of MNi in 1,000 cells was 92.7 ± 7.3 in the cell cultures which were exposed to RF and 8.3 ± 1.5 in the control cell cultures ($P < 0.05$). The proportion of apoptotic cells increased two times compared to the control group (Study group: 36/1,000; Control group: 18/1,000; $P < 0.05$). The results were confirmed by using Cell Death Detection ELISA Plus Kit. The mean number of cells undergoing necrosis in the group exposed to RF was 5, while it was 1 in 1,000 in the control cultures ($P < 0.05$).

Among the genes which have important roles in the apoptotic and anti-apoptotic pathways and cancer, only the expression of STAT3 was found to be decreased, by sevenfold, compared to the control cultures ($P < 0.05$). No statistical difference was found regarding the expressions of the other 11 genes between the study and control groups. The mean values of MNi and the expression levels of the genes studied in both study and control groups are summarized in Table 1 and Fig. 5.

Discussion

In this study, we tried to provide information about the hazardous effects of RF particularly on the brain by using mouse brain cell cultures, although these results do not necessarily reflect the human brain status. The EMF

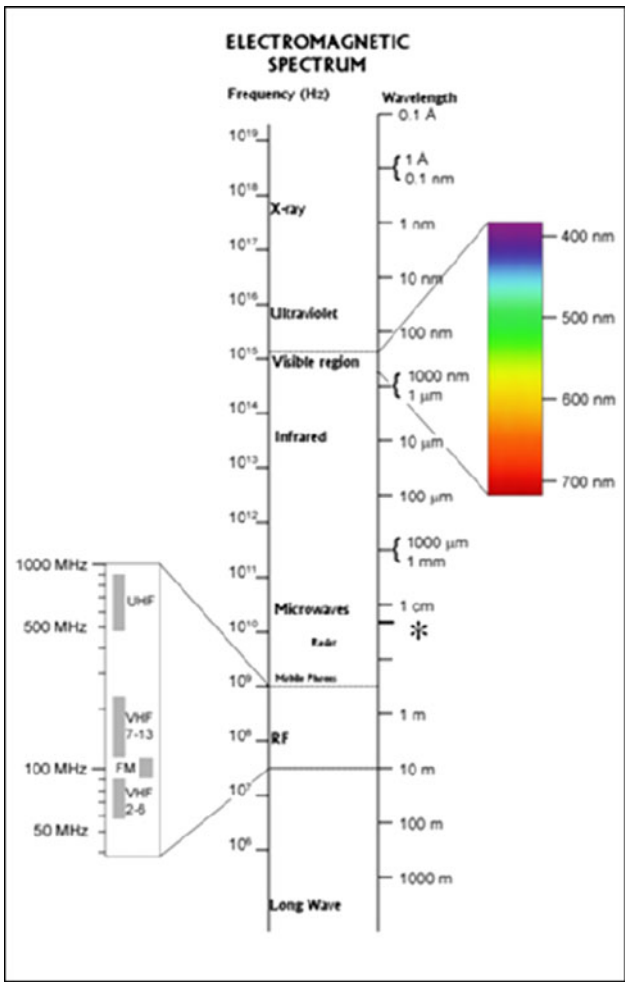

Fig. 3 Overview of the electromagnetic spectrum and electromagnetic source in the present study (Reprinted from Louis E. Keiner, Coastal Carolina University) (http://en.wikipedia.org/wiki/Electromagnetic_spectrum) .*EM source in the present study

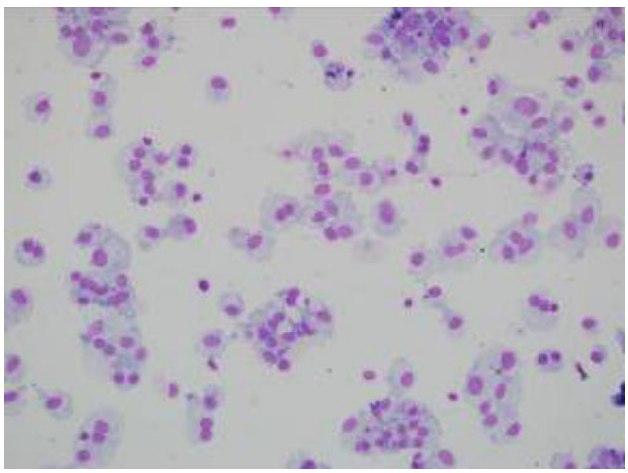

Fig. 4 Cells after radiofrequency exposure

Table 1 Mean values of MNi frequency and necrosis of study and control group

	Mean ± SD		
	Study group	Controls	*P*
MNi frequency	92.7 ± 6.8	8.3 ± 1.5	<0.05
Necrosis	5.0 ± 1.0	1.0 ± 1.0	<0.05
Apoptosis	36 ± 5.29	18 ± 2.0	<0.05

originating from cell phones are supposed to be safe because of having a lower frequency or longer wave lengths compared to ionizing radiation sources. Moreover, the literature mostly indicates that radiofrequency waves do not cause genotoxicity on human health. However, case–control studies, cell culture studies and animal studies regarding the effect of cell phone systems have caused public concern. There are also many results supporting their hazardous effects as well as negative findings in the literature [9–12]. Studies about the health effects of RF can be grouped as experimental cell culture studies, animal studies and studies of observations of the effects of RF in life by a number of methods, such as geographical correlation studies, studies in individuals from different occupations exposed to RF energy and studies of mobile phone users [2]. Laboratory studies have been reported to be critical for risk evaluation since the epidemiological studies lack a strong relationship between RF energy and cancer [13] . Gene expressions and micronucleus tests which show DNA damage were mostly performed in those studies. Because there is methodological standardization problem for the assessment of RF energy hazards on human health, and in vitro studies on cells and animals provide valuable data in supplementing epidemiological evidence about human disease, a brain cell culture system was used in this study. DNA damage has been found to be increased by 10 times compared to the control cell cultures which were not exposed to RF waves. The influences of RF on biological systems have been assessed in a number of different ways including animal studies and cell cultures; however, very few studies have been reported that have investigated the effects of RF on brain cell cultures [14, 15], and no study which assesses the rate of DNA damage by micronucleus assay has been performed. In the literature, DNA damage was shown in rat granulose cells after exposure to intermittent radiofrequency electromagnetic fields [16]. Lai and Singh have reported a similar damage in brain cells of rats exposed for 2 h to a 2,450-MHz field at 0.6–1.2 W/kg [17]. Yang et al. [18] have shown that the EMF of high voltage power lines enhances susceptibility to leukemia. Therefore, the results of our study support the findings of those previous studies.

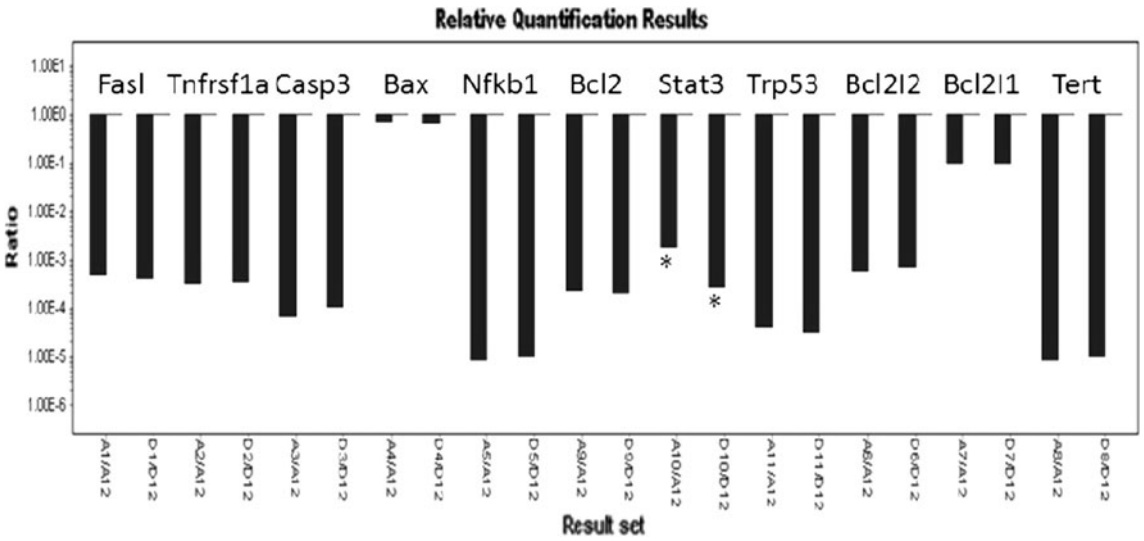

Fig. 5 For relative quantification, GADPH (A12, D12) was used as housekeeping gene. *D* Control group, *A* Study group. *STAT3 expression decreased sevenfold in the study group

Hazardous effects of mobile phones have been attributed to thermal (heat) and nonthermal effects (oscillatory similitude) [18]. While thermal effects have been well established, there is a lack of sufficient information concerning nonthermal effects. Cell phones have been reported to break DNA or to modulate it structurally in an experimental model [19]. In view of the fact that DNA alteration can also occur without any DNA damage, we also evaluated the expression of particular genes which play important roles in the malign transformation of the cell. Among those genes, interestingly only STAT3 showed down-regulation in the cell cultures exposed to RF, and the expression of other genes did not change compared to control cultures. It has been found that apoptotic or cell death pathways were dysregulated in primary neuron cultures [20, 21]. The risk of developing glioma or acustic neuroma doubles as a result of long-term cell phone usage [19]. Lagalla et al. have shown that 2-h exposure to magnetic fields (0.5 T) and radiofrequency caused an increase in the spontaneous release of a number of cytokines including IL2, IL4, IL10, TNF alpha and INF gamma [22]. Interestingly, Hao et al. have reported evidence that EMF exposure can initiate the activation of microglia cells and STAT3 signaling is involved in EMF-induced microglial activation [23]. It is well known that STAT3 plays a critical role in the development of many cancers. Downregulation of STAT3 in our study is inconsistent with those reported in the findings of Hao et al. and cannot explain the initiation and progression of central nervous system (CNS) insult to any extent. Although it has anti-apoptotic and proliferative effects which are associated with a number of human cancers, de la Iglesia et al. have recently reported that it also plays opposing roles in glial transformation depending on the genetic background of the tumor [24]. They found that STAT3 also shows tumor suppressive activity linked to PTEN function. For that reason, it may be speculated that inhibition of STAT3 activity due to RF waves may lead to the contribution of hazardous effects of mobile phones. It has also been shown that in vitro exposure to RF signals induces reversible DNA damage which could explain why cancer does not necessarily occur in all mobile phone users [25].

One of the greatest obstacles about the evaluation of the studies is RF energy exposure assessment because of different types of usages [26]. On the other hand, harmful effects of cell phones depend on a number of factors such as type of phone, side of the head which is exposed to the phone, the power output of the phone, and the distance between the head and the phone. Therefore, studies about the effects of mobile phones have a standardization problem which causes conflicting results [19]. The overall conclusion after all the studies about the safety of RF energy can be summarized as not having an increased risk of any brain tumor within approximately 10 years of phone use [26].

The limitation of our study is that this experiment may not be directly applicable to the use of cell phones because there is a significant depth of penetration of the microwave into the body tissues. In other words, in vitro conditions may not directly represent in vivo conditions. The thickness of adult human skin varies from 1.3 mm on the scalp to 4 mm on the back [27]. The penetration varies according to the frequency of microwaves and the previous studies showed that microwaves penetrate <0.8 mm deep so that the energy will be absorbed within the skin [28, 29, 30]. Therefore, the depth of penetration into the body's tissues

should be taken into account when considering the hazardous effects of cell phones. In conclusion, the results of our study support the proposition that cell phones may have a potential to cause hazardous effects on the genome; however, in in vivo conditions, the duration of exposure and the capacity of DNA repair may prevent the development of cancer to an extent.

References

1. Hoskote SS et al (2008) An epidemiological review of mobile telephones and cancer. J Assoc Phys India 56:980–984
2. Moulder JE et al (2005) Mobile phones, mobile phone base stations and cancer: a review. Int J Radiat Biol 81:189–203
3. Hardell L et al (2006) Pooled analysis of two case-control studies on use of cellular and cordless telephones and the risk for malignant brain tumours diagnosed in 1997–2003. Int Arch Occup Environ Health 79:630–639
4. Hardell L, Carlberg M (2009) Mobile phones, cordless phones and the risk for brain tumours. Int J Oncol 35:5–17
5. Takebayashi T et al (2008) Mobile phone use, exposure to radiofrequency electromagnetic field, and brain tumour: a case-control study. Br J Cancer 98:652–659
6. Akleman F, Sevgi L (1998) FDTD analysis of human head—mobile phone interaction in terms of specific absorption rate (SAR) calculations and antenna design. In: Proceedings of the conference on antennas and propagation for wireless communication, Waltham, USA, pp 85–88
7. Paker S, Sevgi L (1998) FDTD evaluation of the SAR distribution in a human head near a mobile cellular phone. Turk J Electron Commun 6:14–19
8. Fenech M, Morley AA (1985) Measurement of micronuclei in lymphocytes. Mutat Res 147(1–2):29–36
9. Kim JY et al (2008) In vitro assessment of clastogenicity of mobile-phone radiation (835 MHz) using the alkaline comet assay and chromosomal aberration test. Environ Toxicol 23: 319–327
10. Maes A et al (2006) Cytogenetic investigation of subjects professionally exposed to radiofrequency radiation. Mutagenesis 21:139–142
11. Scarfì MR et al (2006) Exposure to radiofrequency radiation (900 MHz, GSM signal) does not affect micronucleus frequency and cell proliferation in human peripheral blood lymphocytes: an inter laboratory study. Radiat Res 165:655–663
12. Schwarz C et al (2008) Radiofrequency electromagnetic fields (UMTS, 1, 950 MHz) induce genotoxic effects in vitro in human fibroblasts but not in lymphocytes. Int Arch Occup Environ Health 81:755–767
13. Committee on Man, Radiation (COMAR) (2009) COMAR technical information statement: expert reviews on potential health effects of radiofrequency electromagnetic fields and comments on the bioinitiative report. Health Phys 97:348–356
14. Campisi A et al (2010) Reactive oxygen species levels and DNA fragmentation on astrocytes in primary culture after acute exposure to low intensity microwave electromagnetic field. Neurosci Lett 473:52–55
15. Thorlin T et al (2006) Exposure of cultured astroglial and microglial brain cells to 900 MHz microwave radiation. Radiat Res 166:409–421
16. Diem E et al (2005) Non-thermal DNA breakage by mobile-phone radiation (1800 MHz) in human fibroblasts and in transformed GFSH-R17 rat granulosa cells in vitro. Mutat Res 583: 178–183
17. Lai H, Singh NP (2004) Magnetic-field-induced DNA strand breaks in brain cells of the rat. Environ Health Perspect 112:687–694
18. Yang Y et al (2008) Case-only study of interactions between DNA repair genes (hMLH1, APEX1, MGMT, XRCC1 and XPD) and low-frequency electromagnetic fields in childhood acute leukemia. Leuk Lymph 49:2344–2350
19. Khurana VG et al (2009) Cell phones and brain tumors: a review including the long-term epidemiologic data. Surg Neurol 72:205–214
20. Nylund R, Leszczynski D (2006) Mobile phone radiation causes changes in gene and protein expression in human endothelial cell lines and the response seems to be genome and proteome dependent. Proteomics 6:4769–4780
21. Zhao TY et al (2007) Exposure to cell phone radiation up-regulates apoptosis genes in primary cultures of neurons and astrocytes. Neurosci Lett 412:34–38
22. Lagalla R et al (1996) An experimental in vitro evaluation of membrane antigen expression and of interleukin production by monocytes exposed to magnetic resonance. Radiol Med 91: 292–296
23. Hao Y et al (2010) STAT3 signalling pathway is involved in the activation of microglia induced by 2.45 GHz electromagnetic fields. Int J Radiat Biol 86:27–36
24. de la Iglesia et al (2008) Identification of a PTEN-regulated STAT3 brain tumor suppressor pathway. Genes Dev 22:449–462
25. Tiwari R et al (2008) Combinative exposure effect of radio frequency signals from CDMA mobile phones and aphidicolin on DNA integrity. Electromagn Biol Med 27:418–425
26. Rothman KJ (2000) Epidemiological evidence on health risks of cellular telephones. Lancet 356:1837–1840
27. Snyder WS et al (1975) Report of the task group on reference man. Pergamon, Oxford
28. Erwin DN, Hurt WD (1981) Assessment of possible hazards associated with applications of millimeter-wave systems. Aeromedical review USAF-SAM 2-81. USAF School of Aerospace Medicine, Aerospace Medical Division, Brooks AFB, TX
29. Gandhi OP, Riazi A (1986) Absorption of millimeter waves by human beings and its biological implications. IEEE Trans Microw Theory Tech MTT 34:228–235
30. Durney C (1986) Radiofrequency radiation dosimetry handbook, USAFSAM-TR 85-73. USAF School of Aerospace Medicine, Aerospace Medical Division, Brooks AFB, TX