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ORIGINAL PAPER

Mobile Phone Radiation Alters Proliferation of Hepatocarcinoma Cells

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Abstract This study investigated the effects of intermittent exposure (15 min on, 15 min off for 1, 2, 3, or 4 h, at a specific absorption rate of 2 W/kg) to enhanced data rates for global system for mobile communication evolution-modulated radiofrequency radiation (RFR) at 900- and 1,800-MHz frequencies on the viability of the Hepatocarcinoma cells (Hep G2). Hep G2 cell proliferation was measured by a colorimetric assay based on the cleavage of the tetrazolium salt WST-1 by mitochondrial dehydrogenases in viable cells. Cell injury was evaluated by analyzing the levels of lactate dehydrogenase (LDH) and glucose released from lysed cells into the culture medium. Morphological observation of the nuclei was carried out by 4′,6-diamidino-2-phenylindole (DAPI) staining using fluorescence microscopy. In addition, TUNEL assay was performed to confirm apoptotic cell death. It was observed that cell viability, correlated with the LDH and glucose levels, changed according to the frequency and duration of RFR exposure. Four-hour exposure produced more pronounced effects than the other exposure durations. 1,800-MHz RFR had a larger impact on cell viability and Hep G2 injury than the RFR at 900 MHz. Morphological observations also supported the biochemical results indicating that most of the cells showed irregular nuclei pattern determined by using the DAPI staining, as well as TUNEL assay which shows DNA damage especially in the cells after 4 h of exposure to 1,800-MHz RFR. Our results indicate that the applications of 900- and 1,800-MHz (2 W/kg) RFR cause to decrease in the proliferation of the Hep G2 cells after 4 h of exposure. Further studies will be conducted on other frequency bands of RFR and longer duration of exposure.

Keywords Mobile phone · Radio frequency radiation · Hepatocarcinoma · Proliferation · DAPI, TUNEL

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Introduction

Use of wireless communication devices has increased the amount of radiofrequency radiation (RFR)—generated primarily by mobile phones and their base stations—in the environment. This uncontrollable increase has aroused public and scientific interest in the possible effects of RFR on human health.

There is a misunderstanding that RFR's biological effects are caused only by heat generation since the International Commission of Non-Ionizing Radiation Protection (ICNIRP) Guidelines indicated that, according to the available experimental evidence, exposure of resting humans for approximately 30 min to RFR at a whole-body specific absorption rate (SAR) of between 1 and 4 W/kg resulted in a body temperature increase of less than 1 °C (ICNIRP 1998). However, there are a number of studies reporting significant effects on various cellular activities in experimental systems under isothermal conditions and at the exposure levels below the limit stated in the guidelines.

There are concerns that RFR may alter cellular functions nonthermally. Changes in the kinetics of cell division and

in proliferation of cells play a crucial role in the generation of cancer. For this reason, there is a rapid increase in the number of reports on RFR-induced effects on various cellular processes such as cell proliferation, genotoxicity, and tumor growth and development in the nonthermal region. The reports include investigations carried out on various cell systems and under different exposure conditions such as frequency and exposure duration. The most investigated RF waveforms are those of the Global System for Mobile Communications (GSM)-modulated signals at 900 MHz [1–7] and 1,800 MHz [8–16], and the UMTS signals [17–24]. Studies have been carried out on different cancer cell types such as glioma cells [2, 25, 26] and human epidermoid cancer cells [18], and on the immune cells such as the peripheral blood mononuclear cells [1, 8, 9]. Most of the studies investigated cellular effects of RFR at a SAR of less than 2 W/kg under isothermal conditions [1, 6, 8, 9, 11–17, 21, 22, 24, 27, 28].

Although there are a number of studies related to cellular damages in the literature, there has been none published on the effects of mobile phone radiation on hepatocytes. Hepatocellular carcinoma (Hep G2), which is originated from hepatocytes, is the fifth most common malignant tumor worldwide, and caused more than 500,000 deaths annually [29]. Hepatocellular carcinoma, like any other cancer, develops when there is a mutation to the cellular machinery that causes the cell to replicate at a higher rate and/or results in the cell avoiding apoptosis. Apoptosis, also termed "programmed cell death," is the necessary mechanism complementary to proliferation that ensures homeostasis of all tissues. Studying induced apoptosis of tumor cells is an important field of research in tumor therapy and tumor molecular biology at present. Inducing apoptosis is a new therapeutic target of cancer research [30–32]. Beside several applications of low-intensity, extremely low-frequency electromagnetic fields (EMF) in diseases such as malaria and cancer [33], the use of RFR is emerging as an additional noninvasive approach for the therapy of human tumors, taking advantage of the generation of shock responses and apoptosis in human cancer cells [18, 34]. Costa et al. [36] reported that there is evidence that the growth of Hep G2 cells may be altered by very low levels of EMF modulated at specific frequencies. They also investigated the in vitro mechanism explaining the antiproliferative effect of very low-intensity EMF on Hep G2 cells [35, 36].

The aim of the present study was to investigate whether RFR exposure affected proliferation of human Hep G2 cells using a colorimetric assay based on the cleavage of the tetrazolium salt WST-1 by mitochondrial dehydrogenases in viable cells. In addition, cell damage was quantified by measuring release of the cytosolic enzyme lactate dehydrogenase (LDH) and glucose from lysed cells into the medium. In order to analyze the apoptosis, 4′,6-diamidino-2-phenylindole (DAPI), a DNA-binding fluorescent dye staining, was used as a morphological method to detect apoptosis. Also, fragmentation of DNA with TdT-mediated dUTP nick-end labeling (TUNEL) staining was studied in HepG2 cells.

The aims of this research were to compare, in the Hep G2 cells, the effects of (i) exposure to a 900- or 1,800-MHz digitally modulated GSM-EDGE (Enhanced Data rates for GSM Evolution) signals at 2 W/kg SAR, and (ii) different exposure durations (1, 2, 3, and 4 h) on cell viability and cell injury.

Materials and Methods

Cell Culture

Hep G2, the human hepatocellular carcinoma cell line, was purchased from ATCC (Wesel, Germany). Hep G2 cells were maintained in DME High Glucose media with 584 mg/l L-glutamine (Irvine Scientific, *Santa Ana, California,* USA) containing 10 % fetal bovine serum (Irvine Scientific, Santa Ana, California, USA) and 50 µg/ml Gentamycin sulfate solution (Irvine Scientific, Santa Ana, California, USA). Cells were grown in 75 cm^2 vented cap flasks (BD Falcon, *San Jose, California,* USA) in an incubator with humidified 5 % CO_2 at 37 °C, and the medium was changed every 2 days. Cells were then subcultured in a 1:4 ratio with trypsin/EDTA solution (Irvine Scientific, Santa Ana, California, USA).

Cells were counted with a Neubauer haemocytometer. To study the viability and injury, Hep G2 cells were seeded in flat-bottom 96-well cell culture plates (Greiner Bio One, *Frickenhausen, German*) at a density of 1×10^5 cells/well in 100 µl of culture medium. For morphological observation, 1×10^6 cells/well in 1 ml were seeded in two well-chamber slides (Lab-Tek II Chamber Slide) in order to obtain nicely stained cells. Same subculture cells were used, so that there would be no age effect on the cells studied.

RFR Exposure Standardization and Dosimetry

Digitally modulated GSM-EDGE signals at 900 and 1,800 MHz were produced by a vector signal generator (Rohde & Schwarz SMBV 100A, 9 kHz–3.2 GHz, *München*, Germany) and a horn antenna (Schwarzbeck, Doppelsteg Breitband Horn antenna BBHA 9120 L3F, 0.5–2.8 GHz, Schönau, Germany) in a temperature-controlled shielded room. The room temperature was set at 37 °C which was monitored continuously during the entire experiment, and the cells were kept at a temperature of

37 ± 0.1 °C during, before and after the exposure. The distance between the horn antenna and the culture plates was 20 cm for the exposure system of 1800 MHz, while it was 35 cm for the exposure system of 900 MHz in order to provide far-field condition. The incident power generated by the signal generator was 1 W, and the measured output E field values were 40 ± 2 V/m for 900 MHz, and 55 ± 2 V/m for 1,800 MHz. The direction of the RF propagation was perpendicular to the diameters of the wells. The average SAR was estimated to be 2 W/kg for both 900- and 1,800-MHz signals using the finite domain time difference method. The output power and the frequency were controlled by a spectrum analyzer (Rohde & Schwarz, FSH 18, 10 MHz–18 GHz, *München*, Germany) integrated to the signal generator. Measurements were taken during the entire experiment, and the data were saved in a computer which was connected to the device via a fiber optic cable.

Experimental Design and Exposure Protocol

Cells were incubated in plates 24 h before the start of an experiment in order to enable attachment. Dead or unattached cells were washed out two times with a phosphate buffer saline (Irvine Scientific, USA) before exposure. Plates were coded as control, sham, 900- or 1,800-MHz RFR used for an exposure periods of 1, 2, 3, and 4 h. RF-exposure samples were exposed for 1, 2, 3, or 4 h to 900- or 1,800-MHz RFR. Exposure was intermittent with the signal 15 min ON and 15 min OFF. Sham exposures were done in the same condition as the RFR-exposed plates, but the signal generator was off. Control plates were left in the incubator.

Therefore, the experiment contained the following 16 treatment groups:

Groups I–IV, groups of cells taken out from the incubator and assayed simultaneously with the 1, 2, 3, and 4 h exposed samples, separately.

Groups V–VIII, cells sham-exposed for 1, 2, 3, and 4 h, respectively.

Groups IX–XII, cells intermittently exposed to the 900-MHz RFR for 1, 2, 3, and 4 h, respectively.

Groups XIII–XVI, cells intermittently exposed to the 1,800-MHz RFR for 1, 2, 3, and 4 h, respectively.

The experiments were done under single-blind condition.

Cell Viability Assay

A colorimetric assay was used for the quantification of cell proliferation and cell viability, based on the cleavage of the tetrazolium salt, WST-1 into formazine by mitochondrial dehydrogenases in viable cells (The Quick Cell Proliferation Assay Kit, Biovision, California, USA). The formazan dye produced by viable cells could be quantified spectrophotometrically. The assay was performed according to the manufacturer's protocol. In brief, cells were seeded in 96-well microtiter plates in a final volume of 100 µl medium and incubated for 24 h before the experiment. WST-1/Elecrocoupling solution of the amount of 10 µl/well was added in all control, sham, and RF-exposure groups. After 8 h, incubation absorbances were measured at 420–650 nm reference wavelengths in a microtiter plate reader. The results were presented as percent of cell viability of the exposure groups to the respective control samples.

Assessment of Cell Injury

Injury of Hep G2 cells was quantified by measuring LDH and glucose released from lysed cells into the medium. Concentrations of supernatant LDH and Glucose levels were determined using commercially available colorimetric assay kit from TML Medical (Turkey). A clinical chemistry analyzer (ERBA XL 600, Germany) was used for the analysis. Total LDH and glucose release corresponding to the complete Hep G2 death were determined.

DAPI Nuclear Staining

The blue–fluorescent 4,6-diamidino-2-phenylindole (DAPI) nucleic acid preferentially stains double-strained (dsDNA). Apoptotic cells were identified by the condensation and fragmentation of nuclei. It appears to associate with AT clusters in the minor groove. Binding of DAPI to dsDNA produces a ∼ 20-fold fluorescence enhancement, apparently due to the displacement of water molecules from both DAPI and the minor groove.

Changes in cell morphology and characteristics of apoptosis were examined by fluorescence microscopy of DAPI-stained cells. The monolayer-adherent cells were grown in two-well chamber slides. Cells were washed twice with PBS. DAPI stain (Gerbu, Germany) was prepared in a 1:1000 dilution in water for injection grade water, and added to the cells, covering them completely. Incubation was performed for 5 min at room temperature. At the end of the incubation period, slides were washed three times with PBS. The apoptotic nuclei (intensely stained, fragmented nuclei, and condensed chromatin) were examined at 400× magnification using a fluorescent microscope with a 340/380-nm excitation.

TUNEL Assay

Terminal transferase dUTP nick-end labeling of DNA strand breaks was carried out as described as per the

Table 1 Effect of radiofrequency radiation (RFR) exposure on proliferation, supernatant levels of lactate dehydrogenase (LDH) (IU/L), and glucose (mg/dl) levels of Hep G2 cells

Parameters	Groups		Mean ± SEM	Statistical significance
Cell proliferation, OD	1 h	Control	0.57 ± 0.01	$\boldsymbol{p < 0.05}$
		Sham	0.54 ± 0.02	
		900 MHz	0.51 ± 0.05	
		1,800 MHz	0.69 ± 0.06*	
	2 h	Control	0.57 ± 0.01	$\boldsymbol{p < 0.05}$
		Sham	0.55 ± 0.03	
		900 MHz	0.49 ± 0.02	
		1,800 MHz	0.59 ± 0.06	
	3 h	Control	0.57 ± 0.03	$\boldsymbol{p < 0.05}$
		Sham	0.54 ± 0.03	
		900 MHz	0.48 ± 0.02	
		1,800 MHz	0.47 ± 0.06	
	4 h	Control	0.57 ± 0.02	$\boldsymbol{p < 0.05}$
		Sham	0.55 ± 0.02	
		900 MHz	0.41 ± 0.04*	
		1,800 MHz	0.35 ± 0.03*	
Lactate dehydrogenase (LDH), IU/l	1 h	Control	201.2 ± 10.1	$p > 0.05$
		Sham	208.7 ± 7.4	
		900 MHz	203.1 ± 6.9	
		1,800 MHz	197.7 ± 8.7	
	2 h	Control	200.4 ± 7.1	$p > 0.05$
		Sham	202 ± 6	
		900 MHz	195.3 ± 14.4	
		1,800 MHz	206.1 ± 9.7	
	3 h	Control	223.5 ± 6.1	$p > 0.05$
		Sham	221.5 ± 3.4	
		900 MHz	227.9 ± 10.8	
		1,800 MHz	219.6 ± 7.2	
	4 h	Control	223.6 ± 6.1	$\boldsymbol{p < 0.05}$
		Sham	224 ± 4.3	
		900 MHz	248.4 ± 6.8*	
		1,800 MHz	254.1 ± 7.8*	
Glucose, mg/dl	1 h	Control	446.1 ± 11	$p > 0.05$
		Sham	438.2 ± 21.8	
		900 MHz	459.2 ± 8,4	
		1,800 MHz	449.3 ± 6.4	
	2 h	Control	444.9 ± 11.5	$p > 0.05$
		Sham	447.1 ± 12.8	
		900 MHz	476.7 ± 8.8	
		1,800 MHz	457.3 ± 13.1	
	3 h	Control	467.2 ± 10.7	$p > 0.05$
		Sham	470 ± 8.8	
		900 MHz	484.3 ± 10.2	
		1,800 MHz	477.4 ± 7.7	
	4 h	Control	474.9 ± 17.9	$p < 0.05$
		Sham	490.1 ± 16	
		900 MHz	527.2 ± 9.5*	
		1,800 MHz	574.2 ± 8.5*	

Cells were exposed to 900- or 1,800-MHz RFR for 1, 2, 3, and 4 h. Data presented are mean ± standard error of mean (SEM). The number of samples for each group was 8

Significant difference from the sham-exposed group at $*p < .05$ from Mann–Whitney U test

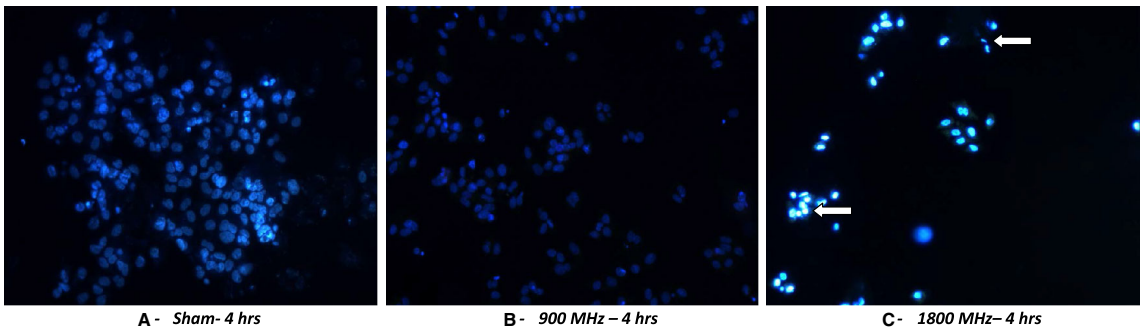

Fig. 1 Changes in cell morphology and the characteristic of apoptosis were examined by fluorescence microscopy of DAPI-stained cells. DAPI-stained Hep G2 cells were examined at 400× magnification using a fluorescent microscope with a 340/380-nm excitation. *Arrows* in **c** indicate that nuclear condensation was observed (40× magnification)

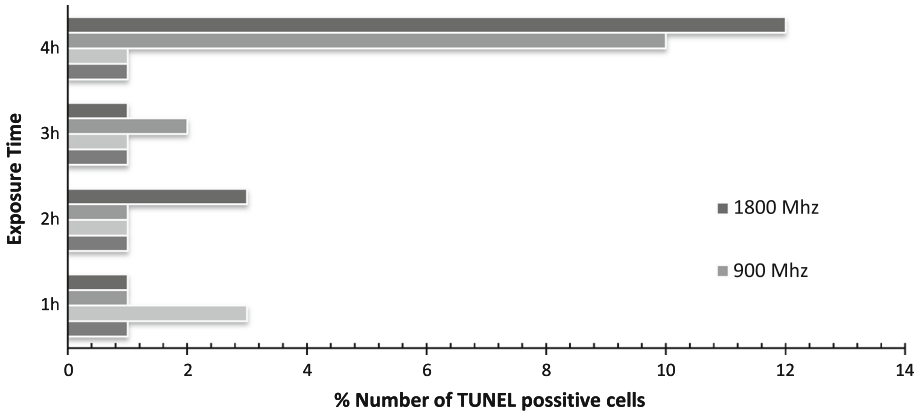

Fig. 2 The in situ staining of DNA strand breaks detected by the TUNEL assay and subsequent visualization by light microscopy give biologically significant data about DNA damage, as it is related to the apoptotic process. TUNEL-stained Hep G2 cells were examined at 400× magnification using a light microscope, and then counted as positive-stained cells

protocol provided in In Situ Cell Death Detection Kit (Roche, Germany).

TUNEL POD is an antibody that is used to convert fluorescence-based TUNEL assays into colorimetric assays suited for light microscopy. The antibody is labeled with peroxidase (POD). The POD is visualized with precipitating substrates such as DAB Substrate (Cat. No. 11 772 465 001). Assay was performed according to the manufacturer's procedure. In brief, cells were grown on chamber slides and treated with RFR as mentioned in the "Materials and Method" section. Cells were air dried, fixed, and then washed with PBS. After blocking, step cells were incubated in permeabilization solution. TUNEL reaction mixture was added and incubated for 1 h at 37 °C. After washing with PBS, TUNEL-POD is added to the samples. Then, slides were rinsed three times with PBS. Substrate was added and visualized under light microscope.

Data Analysis

Data analysis was carried out using the SPSS 11.5 statistical package (SPSS, Chicago, IL, USA). The Kruskal–Wallis test was applied to evaluate the differences among all the groups, while differences between pairs of groups were evaluated by the Mann–Whitney *U* test. Eight samples from each group were used for the entire analysis. Four independent experiments were performed. A difference at $p < .05$ was considered to be statistically significant.

Result

The results and the assessment of significance are presented in Table 1, Figs. 1 and 2. Results are expressed as mean ± standard error of mean (SEM).

There was no significant difference among all the sham-exposed groups (*Group V–VII)* with respect to untreated controls (*Group I–IV*) ($p > 0.05$). Therefore, the exposure procedure did not significantly affect the cellular parameters studied. For this reason, comparisons were made only between the sham and the RF-exposed treatment groups.

Cell Proliferation

Effects of mobile phone radiation on the proliferation of Hep G2 cells were investigated using the WST-1 assay. Cell viability changed according to the frequency and the duration of RFR exposure (Table 1).

When compared with their respective sham-exposed groups, a significant increase in cell proliferation was observed after 1 h of exposure to the 1,800-MHz RFR. However, a significant decrease in proliferation was observed in both the 900- and 1,800-Mhz-exposed cells after 4 h of exposure. No significant changes were observed in any other comparisons between the RF- and the sham-exposed groups.

Assessment of Cell Injury

LDH and glucose levels were measured in the supernatant released from lysed cells after exposure. Increases in LDH and glucose levels in the culture medium indicate cell injury. Significant increases in LDH and glucose levels were observed after 4 h of exposure to either the 900- or 1,800-MHz RFR, whereas no significant changes were observed after 1, 2, or 3 h of exposure (Table 1).

Morphological Observation

DAPI staining using fluorescence microscopy and TUNEL assay under light microscopy were used to show damaged cells with condensed and fragmented chromatin. Representative photographs are shown in Figs. 1 and 2, respectively.

Morphological changes were observed after 4 h of exposure to either 900- or 1,800-MHz RFR. After exposure to the 1,800-MHz radiation for 4 h; Hep G2 cells shrank and retracted from their neighbors, accompanied by floating apoptotic cells in the culture medium. With DAPI, most of the cells showed irregular nuclei pattern after 4 h of exposure. Furthermore, condensed chromatin formations which occur during apoptosis were observed. TUNEL assay was correlated to these results.

Discussion

The World Cancer Report stated that cancer rates were set to increase at an alarming rate globally. Cancer rates could further increase by 50 % to 15 million new cases in the year 2020, according to the report, which is the most comprehensive global examination of the disease to date [37]. A possible correlation between the increase in cancer rates and the increasing man-made sources generating RFR such as mobile phones and their base stations has raised the concern about the carcinogenic effect of RFR.

This study is related to the in vitro effect of intermittent exposure (15 min ON/15 min OFF) to mobile phone-like RFR of 900- and 1,800 MHz (2 W/kg) for 1, 2, 3, and 4 h on proliferation of the cells of hepatocellular carcinoma which is in the third place among cancer-related deaths. The results show that cell viability changed according to the frequency of the applied field and the exposure duration. Hep G2 cell proliferation increased after 1 h of exposure, whereas a decrease was observed after 4 h of exposure to the 1,800-MHz radiation. The 900-MHz radiation had no significant effect on cell proliferation after 1 h of exposure, but a decrease was observed after 4 h of exposure.

The relation between cancer and RFR has been studied for more than 20 years. Clearly et al. [38] have reported that exposure to a 2,450-MHz continuous-wave RFR for 2–27 h at a high SAR (50 W/kg) increased cell proliferation of brain glioma cells. They also observed similar effect of the same exposure conditions on human lymphocytes and cytolytic T lymphocytes [39]. Although temperature levels were well controlled, the SAR levels were high in these studies.

On the other hand, there are some studies which show that there is a biological effect caused by RFR already at low SAR levels—below 4 W/kg SAR, the ICNIRP limit for adverse health effect. Capri et al. [1] reported that peripheral blood mononuclear cells exposed to a GSM-modulated 900-MHz RFR for 3 days for 1 h/day showed a decrease in cell proliferation. Diem et al. [11] studied the effect of intermittent and continuous RFR exposures at 1,800 MHz with different SARs and different mobile phone modulations on DNA strand breaks in the cultured human diploid fibroblasts and cultured rat granulosa cells. They concluded that the induced DNA damage could not be based on thermal effects [11]. It was also reported that in vitro exposure of human peripheral blood lymphocytes to RFR as a continuous wave caused losses and gains of chromosomes; in other words, aneuploidy, a major ‘‘somatic mutation’’ leading to genomic instability and thereby to cancer via a nonthermal pathway [40, 41]. In addition, Palumbo et al. [7] found apoptosis in human jurkat cells exposed to a 900-MHz RFR (GSM signal) at 1.35 W/kg average SAR. Furthermore, various researchers have reported that cellular functions changed in different cancer cell lines due to exposure to RFR, in the mobile phone frequencies [2, 12, 13, 42]. Kwee and Raskmark [43] showed that exposure of transformed human epithelial amnion cells to a modulated RFR at 960 MHz at different power levels (SAR values of 0.021, 0.21, and 2.1 mW/kg) and exposure times, resulted in significant changes in cell proliferation [43]. In spite of these studies, it may be seen with the help of a literature survey that there is no study about the effects of RFR in mobile phone modulations on Hep G2 cells. This study may be one of the very initial studies in this area. Since most of the in vitro studies in the literature investigated the effect of 2 W/kg [1, 3, 6, 8, 9, 11–16, 21, 22, 24, 27, 28], which is the partial body standard of ICNIRP, the effects of 2 W/kg SAR in 900-

and 1,800-MHz frequencies on Hep G2 cells were investigated in this study.

There are also studies on the medical applications of EMF on cancer cells in the literature. Kirson et al. [44] demonstrated that low-intensity, intermediate-frequency (100–300 kHz), alternating electric fields, delivered by means of insulated electrodes, had a profound inhibitory effect on the growth rate of a variety of human and rodent tumor cell lines [44]. Furthermore, there is a study on the therapeutic effect of high-frequency radiation for inhibiting the proliferation of Hep G2 cells. Hernandez-Bule et al. studied the in vitro effects of capacitive–resistive electric transfer (CRET), a noninvasive electrothermal therapy using a 0.57-MHz electric current on the cell cycle and the proliferation of hepatocarcinoma cells in vitro. The aim of these researchers was to test the hypothesis that the effects of CRET therapies were partly due to the response of the biological system to the electrical stimulus itself. They reported that exposure to CRET-like currents could result in decreased proliferation and partial cytostasis of Hep G2 cells via electrically induced hyperthermia [45]. However, Velizarv et al. [46] reported that the changes in cell proliferation due to exposure to RFR was not a result of heat generation and that there was another mechanism related to the stress proteins initiating the cell-cycle reactions. The recent study published by Costa et al. [36] revealed that there is clinical evidence that very low and safe levels of amplitude-modulated 27.12-MHz RFR administered via an intrabuccal spoon-shaped probe may elicit therapeutic responses in patients with liver cancer. In addition, they studied the in vitro effect of 27.12-MHz RFR in order to show the mechanism explaining the antiproliferative the effect of very low-intensity RFR [35]. Hep G2 cells were exposed to tumor-specific modulation frequencies previously identified by biofeedback methods in patients with a diagnosis of cancer to replicate in vivo conditions in order to understand the mechanism of this novel approach. They reported that the growths of Hep G2 and breast cancer cells were significantly decreased by Hep G2-specific and breast cancer-specific modulation frequencies, respectively, while the same frequencies did not affect proliferation of non-malignant hepatocytes or breast epithelial cells [35].

There is also a hypothesis that the mechanism of cell damage caused by EMF is via an enhancement of free radical formation in cells and iron has an important role in the process via the Fenton Reaction [33]. They reported that cancer cells had high rates of iron intake due to their rapid rate of division and expressed a high cell surface concentration of transferrin receptors which maintain cellular iron homeostasis [33, 47]. Thus, cancers cells are more susceptible to the cell damaging effects of EMF. Toyokuni [48] also described the role of iron in carcinogenesis as a double-edged sword, noticing that its excess amounts posed a risk of cancer, presumably via generation of reactive oxygen species. Lai and Singh [33] concluded that EMF might be useful for the treatment of cancer, since cancer cells use high amount of iron. Moreover, liver has another specialty of producing hepcidin which appears to be the master regulator of iron homeostasis in humans and other mammals. Decreasing cell-proliferation rate via RFR may be caused by the iron content of the Hep G2 cells. This iron content might lead to increased free radical production via the Fenton reaction and cause molecular damages and cell death.

The way in which cell death occurs is an important parameter in order to understand the mechanism of the cell damage. Cell death may occur in either of two ways, apoptosis or necrosis. Apoptosis is a form of self-regulated cell death, which differs from necrosis [49], caused by factors external to the cell or tissue, such as infection, toxins, or trauma. It is in contrast to apoptosis, which is a naturally occurring cause of cellular death. In the present study, LDH, a stable cytoplasmic enzyme present in cells, was released into the culture medium upon damage of the plasma membrane [50], and glucose levels in the bathing medium were determined beside the morphological analysis. Supernatant levels of LDH and glucose were significantly higher in Hep G2 cell culture after 4 h of exposure to RFR, while the cell proliferation decreased. Although LDH release in cell culture does not necessarily imply necrosis, plasma membrane damage is one feature of necrotic cells, which could serve among other parameters to determine necrosis [50]. However, morphological observations indicated very few necrotic cells—mostly anisonucleoic apoptotic-like cells are formed. Since the apoptotic cells cannot undergo rapid phagocytosis under the in vitro cell culture conditions as they would do in vivo in the intact tissue, it might also be that LDH release is a feature of late apoptotic cells [51]. Furthermore, we observed several condensed chromatin formations in our present study. It is known that chromatin condensation occurs during apoptosis but not in necrosis.

Apoptosis has an essential role in controlling cell number in many developmental and physiological settings and in chemotherapy-induced tumor cell killing. Disturbance of this process is associated with the development of many severe diseases and disorders including cancer. Selective induction of tumor-cell apoptosis may become the basic strategy in the treatment of malignant tumors. Most of the available chemotherapy drugs work through destroying tumor cells by inducing apoptosis [52, 53]. This study shows that RFR exposure increases the rate of cell death in Hep G2 cells. This application may be improved and used in clinics as a noninvasive and nonthermal method for tumor retardation. In order to better understand the apoptotic pathways influenced by RFR exposure,

additional gene expression analysis, and immunohistochemical and biochemical tests have to be conducted for further assesment.

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Conflict of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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