Research Article

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Effects of radiofrequency radiation on colorectal cancer cell proliferation and inflammation

Radyofrekans radyasyonun kolorektal kanser hücre proliferasyonu ve inflamasyonu üzerine etkileri

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Abstract

Objectives: The aim of this study is to investigate the effects of radiofrequency radiation (RFR) on apoptosis, proliferation, stress response, and inflammation markers in colorectal cancer cells.
Methods: We tested the effects of intermittent exposure to RFR at different frequencies on two different colorectal cancer cell lines; HCT-116 and DLD-1. Protein levels were subsequently analyzed by ELISA.
Results: RFR led to a decrease in P53, p-P53, p-P38, and p-IkB levels in HCT-116 cells, while leading to an increase in BAD, p-BAD, p-STAT3,NF-κB levels. Two thousand one hundred Megahertz of RFR altered the P53, BAD, and NF-KB expression in HCT-116 cells. P53, p-P53, BAD, p-BAD, NF-κB, p-NF-κB, p-P38, p-SAPK/JNK, p-STAT3, and p-IkB levels increased after exposure to RFR at 900 and 2,100 MHz in DLD-1 cells. Unlike HCT-116 cells, 1,800 MHz of RFR was reported to have no effect on DLD1 cells.
Conclusions: RFR increased apoptosis and inflammatory response in HCT116 cells, while lowering the active P38 and active P53 levels, which are indicators of poor prognosis in several cancers. Genetic differences, such as *P53* mutation (DLD-1), are critical to the cell response to RFR, which explains the reason why scientific studies on the effects of RFR yield contradictory results.

Keywords: apoptosis; colorectal cancer; DLD1; HCT116; radiofrequency.

ÖZ

Amaç: Bu çalışmada, radyofrekans radyasyonun (RFR) kolorektal kanser hücrelerinde; apoptozis, proliferasyon, strese cevap ve inflamasyon belirteçlerine etkilerinin araştırılması amaçlandı.
Gereç ve yöntem: 900, 1800 ve 2100 MHz frekanslı RFR alanların,1 saat ve 4 saat süreyle (15 dk. açık/15 dk. kapalı), kolorektal kanser hücreleri (HCT-116 ve DLD-1) üzerindeki etkilerini analiz ettik. Sırasıyla; P53, p-P53, BAD, p-BAD, NF-κB, p-NF-κB, p-IκB, p-SAPK/JNK, p-P38, p-STAT3 protein seviyeleri ELISA yöntemi ile analiz edildi.
Bulgular: HCT-116 hücrelerinde 900 ve 1800 MHz RF maruziyeti, P53, p-P53, p-P38 ve p-IkB protein seviyelerinin azalmasına; BAD, p-BAD, p-STAT3 ve NF-κB protein

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seviyelerinin artışına sebeb oldu. 2,100 MHz RF maruziyeti ise, P53, BAD ve NF-κB ekspresyon seviyelerini değiştirmiştir. DLD-1 hücrelerinde, 900 ve 2100 MHz RF maruziyeti ile, P53, p-P53, BAD, p-BAD, NF-κB, p-NF-κB, p-P38, p-SAPK/JNK, p-STAT3 ve p-IkB protein seviyeleri artmıştır. HCT-116 hücrelerinin aksine, DLD-1 hücrelerinde 1800 MHz RFR maruziyeti etkisiz kalmştır.

Sonuç: RFR maruziyeti, HCT-116 hücreleri apoptoz ve inflamasyon yanıtta bir artış gösterirken bazı kanserler için kötü prognoz göstergesi olan aktif P38 ve P53 seviyelerinde bir azalma göstermiştir. Bir *P53* mutasyonuna sahip olmak (DLD-1) gibi genetik farklılıklar, hücrelerin RFR' e verdiği yanıt için kritik öneme sahiptir. Bu durum, sonuçları birbirleriyle çelişen RFR etkisinin bilimsel çalışmalarını açıklar.

Anahtar kelimeler: apopitoz; DLD-1; HCT-116; kolorektal kanser; radyofrekans.

Introduction

As a result of the advances in communication technology, today people are inevitably exposed to radiofrequency radiation (RFR). Billions of people may be affected by RFR emitted by mobile phones and their base stations. Although it concerns such a large population, the biological effects of RFR on mammalian cells are still unclear. Therefore, there is still a need for scientific studies to clarify the mechanisms of mammalian cells affected by RFR. Mobile phones emit electromagnetic radiation (EMR) within the frequency band of RFR, i.e., 3 kHz–300 GHz, while transmitting and receiving signals that are partly absorbed by the mobile phone user.

The biological effects of EMR have been studied *in vivo* and *in vitro* mammalian cells for genetic damage, tumorigenesis, cell proliferation, apoptosis, etc. [1–3]. Although the results of these studies are inconsistent and controversial, the risk of EMR to human health was assessed as a possible carcinogen for humans by the International Agency for Research on Cancer (IARC) and listed in group 2B in 2011 [4].

Colorectal cancer is the third most commonly diagnosed malignancy among both men and women in developed countries [5]. People with colon cancer are in general treated with surgery first, and then with chemotherapy. In this study, we aimed to analyze the effect of mobile phone-like RFR on colorectal cancer cells (HCT-116 and DLD-1).

We investigated the effects of mobile phone-like RFR on these cell lines with different genetic characteristics and selected the most critical proteins involved in apoptosis, proliferation, inflammation, and stress response (P53, p-P53, BAD, p-BAD, NF-κB, p-NF-κB, p-P38, p-SAPK JNK, p-STAT3, and p-IkB). Apoptosis is a programmed death mechanism that mammalian cells use in the event of any cell damage, ageing, and biological stress. The P53 tumor suppressor protein is one of the key regulators of the intrinsic pathway of apoptosis. When DNA damage or any other genomic aberration occurs, P53 is activated by phosphorylation, leading to cell cycle arrest and DNA repair or apoptosis [6]. Phosphorylation of P53 (p-P53) at Ser20 and Ser15 causes a weakened P53 – MDM2 interaction, which is a negative regulator of P53, leading to P53 accumulation and activation [7]. After P53 activation, the intrinsic pathway of apoptosis occurs. Alternatively, P53 may activate CD95 and the extrinsic pathway is initiated [8].

The BCL-2 family proteins are classified into three groups. The first group is anti-apoptotic BCL-2 family proteins, which include BCL-2, MCL-1 (myeloid leukemia cell differentiation protein), BCL-XL (B-cell lymphoma extra-large), BCL-W (BCL-2-like protein 2), BCL-B (closest in amino acid sequence homology to the Boo protein), and BCL-2A1 (BCL related protein A1) [9]. The second group is pro-apoptotic BCL-2 family proteins that are composed of BAX (BCL-2-associated protein X), BAK (BCL-2 homologous antagonist killer), and BOK (BCL-2-related ovarian killer). And the third group, which is also pro-apoptotic when overexpressed, is composed of BID (BH3 interacting domain death agonist), BIK (BCL-2-interacting killer), BIM (BCL-2-like protein 11), HRK (Harakiri), BAD (BCL-2-associated death promoter), PUMA (P53 upregulated modulator of apoptosis), NOXA (Phorbol-12-myristate-13-acetate-induced protein 1), and BMF (BCL-2 modifying factor) [8]. BAD is regulated primarily by phosphorylation [10]. When BAD is phosphorylated, it is set apart in the cytosol, but if BAD is unphosphorylated, it is translocated to the mitochondria to initiate the release of cytochrome *c* [11].

Inflammation is a biological response to indicate that the body is struggling with a detrimental agent and trying to heal itself. Inflammation induced by environmental factors, such as tissue injury, may develop into chronic inflammation. Due to a long term exposure to stimuli such as RFR, inflammation may turn into an inflammatory disease and may cause tumor formation [12, 13]. Several signaling cascades are linked to these inflammatory responses; i.e., numerous intracellular and extracellular signal elements, such as antigen receptors and pro-inflammatory cytokines, may activate phosphatidylinositol 3-kinases (PI3K)/Akt, Janus kinase (JAK)/signal transducer and activator of transcription (STAT),

mitogen-activated protein kinase, stress-activated protein kinase (SAPK)/(JNK), and nuclear factor-kappa B (NF-κB) signaling pathways.

NF-κB is a transcription factor that orchestrates important biological and physiological processes such as proliferation, inflammation, immune response, drug resistance, cancer development, and metastasis. While healthy cells express NF-κB transiently and in a strictly modulated way, cancer cells express highly active NF-κB [14]. NF-κB activation is controlled by the polyubiquitination and phosphorylation of IκB members, which are inhibitory molecules of NF-κB, and then degradation occurs. In resting cells, NF-κB interacts with an IκB family member, such as IκBα.

An initiating signal leads to phosphorylation of IκBα that induces its polyubiquitination and degradation, as a result. The released NF-κB is translocated to the nucleus and binds to the specific DNA sequences of the genes regulated by NF-κB; such as immunoreceptors, cytokines, chemokines, and regulators of proliferation and apoptosis genes. Phosphorylation of NF-κB subunits may up or downregulate the transcription of these target genes [15].

MAP kinases are composed of four signaling cascade subgroups: (1) Stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK), (2) the P38 group of protein kinases, (3) big MAP kinase 1 (BMK1), and (4) extracellular signal-regulated kinases (ERKs).

SAPK/JNK is activated by many types of cellular stress and extracellular signals; such as UV, gamma radiation, inflammatory cytokines, ceramides, and growth factor agonists etc. It plays a significant role in regulating cell survival, apoptosis, and proliferation [16]. P38 group MAP kinases are also active in inflammation, cell cycle, senescence, apoptosis, cell differentiation, development, and tumorigenesis in specific cell types [17]. JAK-STAT pathway plays a critical role in the immune system and it can regulate the polarization of T helper cells [18].

Materials and methods

Cell culture and cell viability assay

DLD-1 and HTC-116 colon cancer cell lines were provided from Ankara University, Faculty of Veterinary Medicine, Department of Biochemistry. The cells were grown in RPMI 1640 medium with 10% Fetal bovine serum (FBS) (Sigma–Aldrich, St Louis, MO, USA) and 50 μg/mL gentamicin sulfate solution (Millipore, USA). The cells were grown in an incubator with 5% CO_2 at 37 °C, cultured in flasks and the medium was replaced every two days. Cell viability and growth rates were examined under microscope. The effect of RF on the cytotoxicity of human colorectal carcinoma cells was determined by measuring mitochondrial dehydrogenase activity using methyl thiazolyl tetrazolium (MTT) as a substrate. The cells were seeded into 96-well plates for the MTT assay, each well containing 5×10^5 cells. Then, the cells were exposed to RF as mentioned in the RF exposure method, adding 100 μL of MTT (5 mg/mL) into the wells. Sodium dodecyl sulfate (10%, 100 μL) was used to solubilize the formazan salt formed after 4 h of incubation and a microplate reader (Sunrise, Tecan GmbH, Austria) was used to measure the amount of formazan salts at 570 nm. Each experiment was repeated three times.

RF exposure system

A horn antenna (Schwarzbeck, Germany) and a vector signal generator (Rohde & Schwarz, Munchen, Germany) were used to produce GSM-EDGE modulated signals at 900, 1,800, and 2,100 MHz in a shielded room. Temperature of the room was kept at 37 ± 0.1 °C throughout the entire experiment. During the exposure, far-field conditions were ensured by arranging the distance between the cell culture plates and the antenna at 10, 20, and 35 cm for 900, 1,800, 2,100 MHz respectively. Reference levels measured by spectrum analyzer were 40 ± 2 V/m for 900 MHz, 55 ± 2 V/m for 1,800 MHz and 137 V/m for 2,100 MHz. Specific Absorption Rate (SAR) was calculated using the finite element method and found as 2 W/kg approximately [3].

RFR exposure of DLD-1 and HCT-116 colon cancer cells

We analyzed the effects of intermittent radiofrequency exposure of RFR (15 min on, 15 min off) for 1 and 4 h at 900, 1,800, and 2,100 MHz frequencies on DLD-1 and HCT-116 cells.

ELISA analysis of apoptosis and inflammation-related markers

After exposure, DLD1 and HCT116 cells were harvested with a cell scraper and washed three times with PBS. Using the cell lysis buffer, whole-cell lysates were collected (Bio-Rad Laboratories, USA). Total protein concentration was determined by using Pierce™ 660 nm Protein Assay Reagent (Thermo, USA). An equal amount (20 μg) of protein obtained from the whole-cell lysates were used for each sample. The protein levels of P53, p-P53, Clv-PARP, BAD, p-BAD, NF-κB, p-NF-κB, p-SAPK/JNK, p-P38, p-STAT3, p-IkB in DLD-1, and HCT-116 cells were identified with PathScan® Sandwich ELISA Kits (Cell Signaling Technology Inc., Beverly, MA, USA Cat. 7276S). Following the RFR exposure of DLD-1 and HCT-116 cells at 900, 1800, 2,100 MHz frequencies for 1 h and 4 h, cell lysates were obtained and then quantified by using the bicinchoninic acid (BCA)™ protein assay kit (Pierce, Rockford, Illinois, USA) according to the manufacturer's protocols. At the end of this procedure, the absorbance levels of apoptotic and inflammatory markers were measured at 450 nm with Spectramax M3 microplate reader (Molecular Devices, USA).

Statistical analysis

The experiments were performed in triplicates. All the data were presented in mean values ± standard derivation (SD). Statistical analysis was performed by using GraphPad Prism Software (Graphpad

Software, Inc., San Diego, CA). Statistical comparisons of exposure frequencies (900, 1,800, 2,100 MHz) were made with one-way analysis of variance (ANOVA) followed by multiple comparisons. $p<0.05$ was considered as significant ($p\leq0.05$*; $p\leq0.01$**; $p\leq0.001$***).

Results

Effects of radiofrequency radiation on inflammatory response, apoptotic cell death, and cell survival proteins expression

1 h of exposure at 900, 2,100 MHz, and 4 h of exposure at 900 and 1,800 MHz led to a statistically significant increase in P53 expression levels, and statistically significant increase was also reported in p-P53 expression levels at 900, 2,100 MHz of exposure for 1 h in DLD1 cells. On the other hand, significant decrease was observed in P53 and p-P53 expressions in all exposure groups of 1 and 4 h in HCT116 cell line (Figure 1A, B).

BAD and p-BAD expression levels were significantly elevated at 900 and 2,100 MHz of exposure for 1 h. While BAD levels were significantly lowered with 4 h of exposure at all frequencies, P-BAD levels did not change in any group after 4 h of exposure in DLD1 cells. In addition, **BAD** and **p-BAD** expression levels were significantly elevated in all groups after 1 and 4 h of exposure in HCT116 cell line (Figure 1C, D).

In DLD1 cells, **NFkB** and **p-NFkB** expression levels were significantly elevated at 900 and 2,100 MHz of exposure to RFR for 1 and 4 h. While **NFkB** levels were significantly affected, **p-NFkB** levels did not change in any exposure group in HCT116 cells (Figure 2A, B). Significant increase was reported in p-IkB expression levels at 900 and 2,100 MHz of exposure for 1 h in DLD1, whereas significant decrease was observed at 900 and 1,800 MHz of exposure for 4 h in HCT116 cells (Figure 2C).

p-SAPK/JNK expression levels were significantly elevated at 900 and 2,100 MHz of exposure for 1 and 4 h in DLD1 cells, however only the 1,800 and 2,100 Mhz groups presented significant increase after 1 h of exposure in HCT116 (Figure 2D).

While significant changes in p-P38 expression levels were observed at 900 and 2,100 MHz of exposure for 1 and 4 h in DLD1 cells, **p-P38** expression levels were significantly lowered at 900 and 1,800 MHz of exposure for 1 and 4 h in HCT116 (Figure 2E).

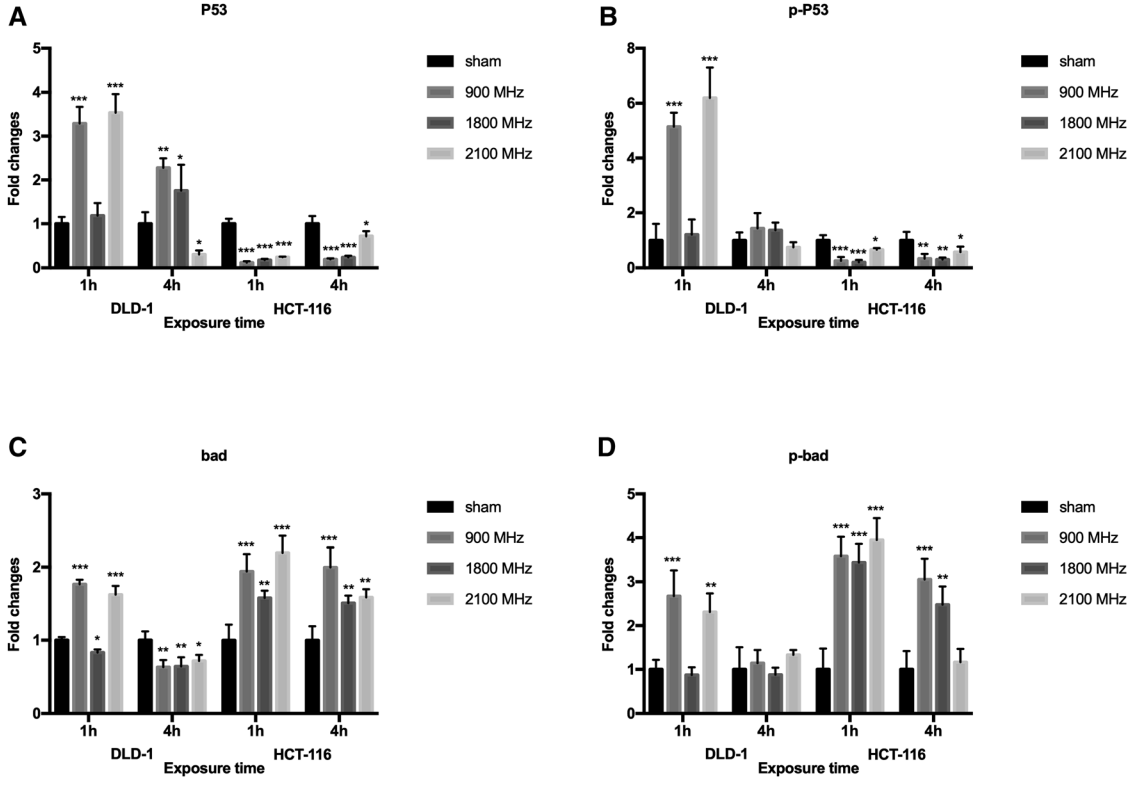


Figure 1: Relative fold changes of tested.
(A) P53, (B) p-P53, (C) BAD, (D) p-BAD protein levels measured by ELISA in HCT-116 and DLD-1 colon cancer cells after 900, 1,800 and 2,100 MHz of radiofrequency radiation exposure for 1 and 4 h. Data are presented in mean ± S.D. (n=3; $p\leq0.05$*; $p\leq0.01$**; $p\leq0.001$*** vs. control group).

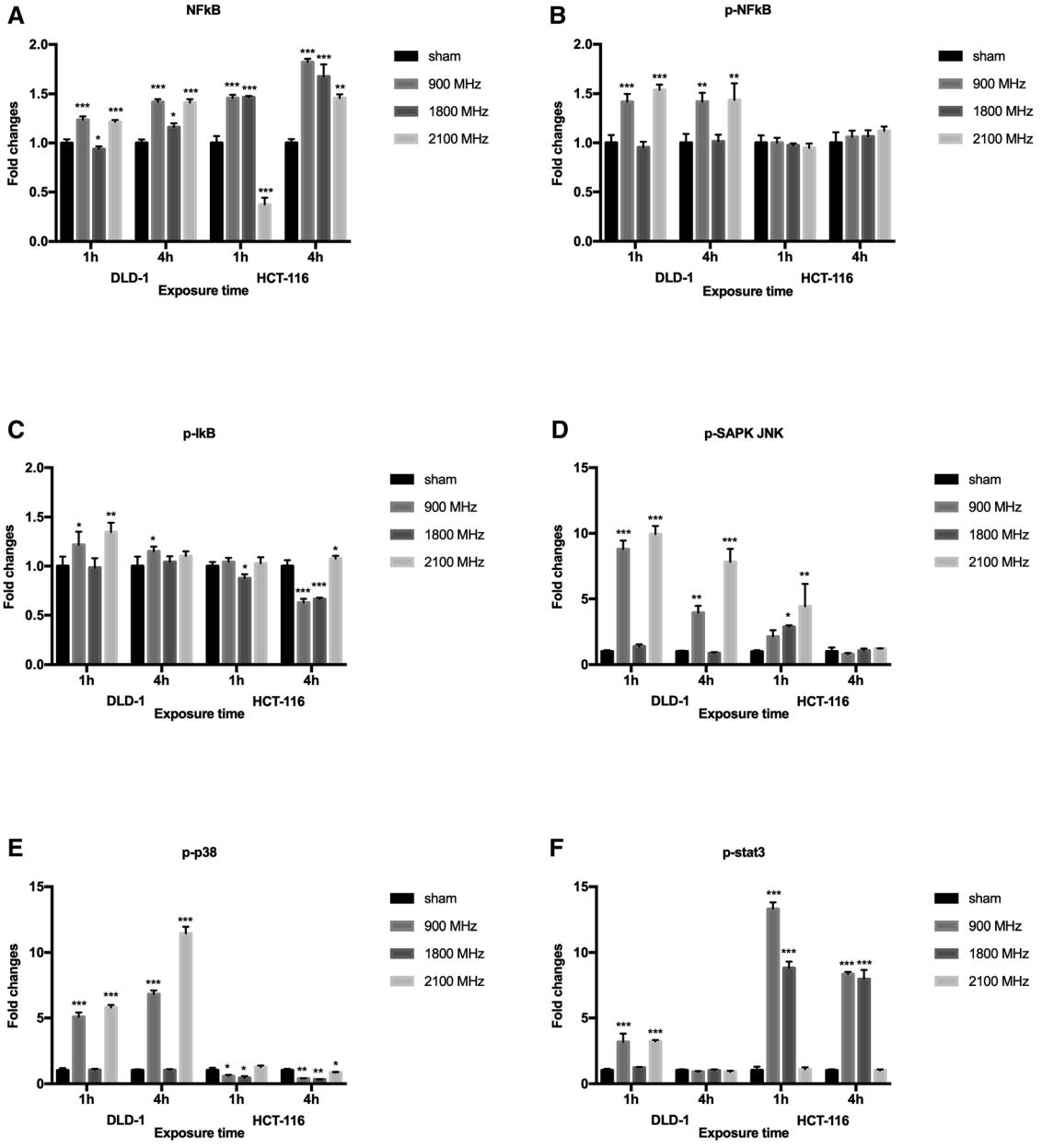


Figure 2: Relative fold changes of tested.
(A) NFkB, (B) p-NFkB, (C) p-IkB, (D) p-SAPK JNK, (E) p-P38, (F) p-STAT3 protein levels measured by ELISA in HCT-116 and DLD-1 colon cancer cells after 900, 1,800, and 2,100 MHz of radiofrequency radiation exposure for 1 and 4 h. Data are presented in mean ± S.D. (n=3; p≤0.05*; p≤0.01**; p≤0.001*** vs. control group).

STAT3 mediates the expression of a variety of genes in response to cell stimuli, playing a key role in many cellular processes such as cell growth and apoptosis. Statistically significant increase was observed in the activated form of STAT3 (**p-STAT3**) expression levels at 900 and 2,100 MHz of exposure after 1 h, whereas RFR waves were ineffective in the exposure group of 4 h in DLD1 cell line. Furthermore, in HCT116 cells **p-STAT3** expression levels were elevated at 900 and 1,800 MHz of exposure for 1 and 4 h (Figure 2F).

Effects of radiofrequency radiation on cell proliferation

RF exposure at 900, 1,800 and 2,100 MHz did not suppress the proliferation of colorectal carcinoma cell lines in either dose-dependent or time-dependent manner (Figure 3).

The results indicate that RF exposure at 900 and 1,800 MHz, in particular, causes a decrease in P53, p-P53, p-P38, and p-IkB levels; while leading to an increase in

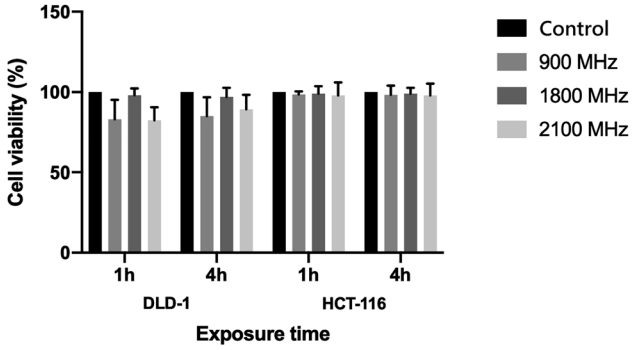


Figure 3: MTT cell proliferation assay results. HCT-116 and DLD-1 colon cancer cells after 900, 1,800 and 2,100 MHz of radiofrequency radiation exposure for 1 and 4 h.

BAD, p-BAD, p-STAT3, and NF-κB levels in HCT-116 cells. RF exposure at 2,100 MHz seems to be less effective than the other two frequencies. On the other hand; P53, P-P53, BAD, p-BAD, NF-κB, p-NF-κB, p-P38, p-SAPK/JNK, p-STAT3, and p-IkB levels were increased after RF exposure in DLD-1 colon cancer cells. Unlike HCT116 cells, 1,800 MHz of exposure in DLD1 cells was less effective than the other two frequencies (Figures 1 and 2.). RF exposure, at 900, 1,800 and 2,100 MHz had no effect on cell viability either after 1 or 4 h (Figure 3).

Discussion

The results of this study indicate that RF exposure affects the most important signaling pathways such as; P53, P38, SAPK/JNK, STAT, PlkB, NF-κB, and apoptosis that are critical in colorectal cancer cells. This study provides invaluable data to simultaneously demonstrate the effect of RFR on all these signaling pathways.

There are contradictory data about the biological effects of electromagnetic fields at different frequencies in the literature. These contradictions may be caused by the theory of resonance or by the characteristics of tissues with very different genetic properties. Therefore, studies on cancer treatment with RFR should focus not only on the impact of the frequency on the tissue itself but also on the mechanism of a genetic effect.

Some experimental studies have reported genotoxicity by RF field exposure. Ozgur et al. previously reported that the proliferation of Hep G2 hepatocarcinoma cells was increased after 1 h of exposure at 1,800 MHz, whereas a decrease was observed after 4 h of exposure [3]. In addition to this, Xing et al. reported that 1,800 MHz RFR could stimulate P53-mediated caspase-3 activation and decrease cell viability in human brain glioblastoma cell line U-87 MG under nonthermal conditions [19]. On the other hand, A172 human glioblastoma cells were exposed to wideband code division multiple access (W-CDMA) RFR at 2.1425 GHz at SARs of 80, 250, and 800 mW/kg, and continuous wave (CW) RFR at 80 mW/kg for 24 and 48 h, and no significant difference in the percentage of apoptotic cells was observed between the groups [20]. In addition, 1800 MHz RF-EMF exposure did not influence embryonic neural stem cells (eNSC), proliferation, cell cycle-related genes P21, P53 and apoptosis-related genes caspase-3, BAX, BCL-2. However, RF-EMF exposure inhibits neurite outgrowth in eNSC differentiated neurons [21]. In our study, similarly, the exposure of DLD cells at 1,800 MHz did not cause any difference in the studied protein expressions. However, 1 and 4 h of 1,800 MHz exposure in HCT116 cells led to significant effects. RFR exposures at all frequencies (900, 1,800, and 2,100 MHz) caused an increase in the pro-apoptotic BAD molecule in HCT116 cells.

For cell death to occur, first of all, the cells must generate a response to the RFR applied. Since the exposure durations are short, namely 1 and 4 h, cell death does not occur yet, but the cellular response begins as the protein levels are measured 1 and 4 h after the application. High levels of both p53 and active form of pP53 are indicative of initiation of a cellular response. Unlike P53 expression in DLD1 cells, that of HCT116 cells is decreased. The RFR exposure of DLD cells caused an increase in the apoptotic P53 proteins. The elevated level of the mutant P53 in DLD-1 cells may be the consequence of the extension of the *p53* gene half-life [22]. Phosphorylation of p53 occurs when the cells are undergoing apoptosis. Therefore, we can understand that DLD1 cells are undergoing apoptosis, as the p-BAD level is elevated. These cells also present a strong stress response. In response to the stress in DLD-1- cells, which is a P53 mutant caused by the RFR application, reactivation of wild type p53 and downregulation and/or restoration of mutant p53 may occur [23].

A similar contradictory effect between the cells was observed for p-P38. The P38 MAPK signal transduction pathway plays a role in the regulation of many cellular functions, such as cell differentiation, cell growth, inflammation, and death [24]. After exposure, P38 level increased in DLD-1 cells while decreasing in HCT116 cells. Elevated p-P38 levels have been associated with poor prognosis in some cancers, such as chronic lymphocytic leukemia and breast cancer [25, 26]. Nevertheless, Lee et al. reported that 1,763 MHz of RF radiation at an average SAR of either 2 or 20 W/kg for 30 min or 1 h on rat primary astrocytes and Human T-lymphocyte Jurkat cells did not cause any changes in the phosphorylation status of MAPKs, c-Jun N-terminal protein kinases (JNK1/2),

extracellular signal-regulated kinases (ERK1/2) or P38. This means that they were not activated because of 1,763 MHz of RF radiation [27].

Why does RFR have contradictory effects? The answer may lie in the different mutation profile of the cells. Although DLD-1 and HCT116 are both colorectal cancer cells, they have different genetic properties. DLD-1 has an S241F mutation in the *P53* gene, while HCT116 has a wild type *P53* gene [28]. There is mutant P53 protein expression in DLD-1 cells and anticancer drugs that restore wild-type p53 activity in cell lines expressing the mutant p53 [29]. This mutation explains not only the significant change in P53 expression in HCT116 cells, but it also helps explaining the expression differences in DLD-1 cells. Furthermore, the increased active BAD and decreased active P53 levels in RFR exposed HCT116 cells indicate that the cells in any case undergo apoptosis, but not by the P53 mediated apoptosis pathway. DLD-1 cells, on the other hand, undergo apoptosis with the increase in P53 and pP53 levels.

Some *in vitro/in vivo* studies investigated whether RFR may affect transcriptional regulator or not. These studies showed that 8.2 GHz pulsed RF radiation at an SAR of 10.8 +− 7.1 W/kg for 90 min exposure increased DNA binding activity of NF-κB in normal human monocyte cells (MM-6) [30]. On the other hand, 835.62 MHz frequency-modulated CW with 0.6 W/kg SAR value did not affect the NF-κB binding activity of DNA [31]. STAT3 signaling is another critical pathway that plays an important regulatory role in the expression of inflammatory genes in cancer [32]. Phosphorylation of STAT3 was induced at 2.45 GHz exposure with 6 W/kg SAR value on microglia cells [33]. The inflammatory pathways' transduction mechanisms after RFR exposure remain largely unknown. Based on our data, it can be said that RFR exposure leads to an inflammatory response in both cell lines, as both NF-κB and p-STAT3 levels were elevated after exposure. However, active NF-κB levels were not elevated in HCT116 cells as they were in DLD-1 cells. This result supports our findings suggesting an improvement in HCT116 cells, as cancer cells express highly active NF-κB. NF-κB is normally found in inactive form by binding to the NF-κB (IkB) inhibitor in the cytoplasm. Stimuli such as pro-inflammatory cytokines can activate ubiquitination and proteasomal degradation of IkB and this degradation activates NF-κB, which in result enters the nucleus to induce gene expression, including the genes of pro-inflammatory cytokines. Active NF-κB can stimulate proliferation and antiapoptotic effects, leading to malignant transformation [34].

Activation of NF-κB, which has a well-known function in the regulation of immune response and inflammation, may result from the activation of tumor necrosis factor. SAPK/JNK member of the mitogen-activated protein kinase family is activated by a variety of environmental stresses. SAPK/JNK expression was elevated after 900 and 2,100 MHz of RFR exposure in DLD-1 cells, while it did not change in HCT116 cells except for the group exposed at 2,100 MHz for 1 h.

In conclusion, RFR exposure has an effect on the inflammation and apoptosis related proteins in HCT116 cells, and DLD-1 cells. Following the exposure, HCT116 cells showed an increase in apoptosis related proteins and inflammatory response, while presenting a decrease in active P38. Elevated P53 expression levels correlate with tumor progression and poor prognosis. This decrease in P53 in HCT116 cells indicates that apoptosis is not mediated by the P53 pathway. DLD-1 cells undergo apoptosis via the P53 pathway. Treatment methods using radiofrequency waves are quite common in medicine. Radiofrequency ablation is one of the surgical methods that produces a thermal effect on the target tissue by RF delivery generated by rapidly alternating currents from a probe tip to the tissue. During the RF procedure, probe tip-tissue interface temperature increases over 60 °C (70–90 °C) and this causes collateral thermal damage [35]. As mentioned above, radiofrequency ablation thermally affects the cancer cells by inducing necrosis. However, low-power RF fields may induce apoptotic pathways by non-thermal effects on the cancer cells [3].

Conclusion

In this study, we concluded that the presence of mutation profile differences, such as *P53* mutation between DLD-1 and HCT116 cells, may lead to significantly different results of the RFR effect. This explains the reason why RFR studies yield seemingly contradictory results.

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References

1. Eghlidospour M, Ghanbari A, Mortazavi SMJ, Azari H. Effects of radiofrequency exposure emitted from a GSM mobile phone on proliferation, differentiation, and apoptosis of neural stem cells. Anat Cell Biol 2017;50:115–23.
2. Kayhan H, Esmekaya MA, Saglam ASY, Tuysuz MZ, Canseven AG, Yagci AM, et al. Does MW radiation affect gene expression, apoptotic level, and cell cycle progression of human sh-sy5y neuroblastoma cells? Cell Biochem Biophys 2016;74:99–107.
3. Ozgur E, Guler G, Kismali G, Seyhan N. Mobile phone radiation alters proliferation of hepatocarcinoma cells. Cell Biochem Biophys 2014;70:983–91.
4. Gaudin N. World Health Organisation-The International Agency for Research on Cancer; 2011. Press Release No; 208.
5. Siegel RL, Miller KD, Fedewa SA, Ahnen DJ, Meester RGS, Barzi A, et al. Colorectal cancer statistics, 2017. Ca – Cancer J Clin 2017; 67:177–93.
6. Levine AJ. p53, the cellular gatekeeper for growth and division. Cell 1997;88:323–31.
7. Shieh SY, Ikeda M, Taya Y, Prives C. DNA damage-induced phosphorylation of p53 alleviates inhibition by MDM2. Cell 1997; 91:325–34.
8. Grilo AL, Mantalaris A. Apoptosis: a mammalian cell bioprocessing perspective. Biotechnol Adv 2019;37:459–75.
9. Ke N, Godzik A, Reed JC. Bcl-B, a novel Bcl-2 family member that differentially binds and regulates Bax and Bak. J Biol Chem 2001; 276:12481–4.
10. Condorelli F, Salomoni P, Cotteret S, Cesi V, Srinivasula SM, Alnemri ES, et al. Caspase cleavage enhances the apoptosis-inducing effects of BAD. Mol Cell Biol 2001;21:3025–36.
11. Elmore S. Apoptosis: a review of programmed cell death. Toxicol Pathol 2007;35:495–516.
12. Soffritti M, Giuliani L. The carcinogenic potential of non-ionizing radiations: the cases of S-50 Hz MF and 1.8 GHz GSM radiofrequency radiation. Basic Clin Pharmacol Toxicol 2019;125: 58–69.
13. Wertheimer N, Leeper E. Electrical wiring configurations and childhood cancer. Am J Epidemiol 1979;109:273–84.
14. Wu J, Ding J, Yang J, Guo X, Zheng Y. MicroRNA roles in the nuclear factor kappa B signaling pathway in cancer. Front Immunol 2018; 9:546.
15. Christian F, Smith EL, Carmody RJ. The regulation of NF-κB subunits by phosphorylation. Cells 2016;5:12.
16. Nishina H, Wada T, Katada T. Physiological roles of SAPK/JNK signaling pathway. J Biochem 2004;136:123–6.
17. Zarubin T, Han J. Activation and signaling of the p38 MAP kinase pathway. Cell Res 2005;15:11–8.
18. Seif F, Khoshmirsafa M, Aazami H, Mohsenzadegan M, Sedighi G, Bahar M. The role of JAK-STAT signaling pathway and its regulators in the fate of T helper cells. Cell Commun Signal 2017; 15:23.
19. Xing FQ, Zhan QQ, He YD, Cui JS, He SL, Wang GY. 1800MHz microwave induces p53 and p53-mediated caspase-3 activation leading to cell apoptosis in vitro. PloS One 2016;11. https://doi.org/10.1371/journal.pone.0163935.
20. Hirose H, Sakuma N, Kaji N, Suhara T, Sekijima M, Nojima T, et al. 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 2006;27:494–504.
21. Chen C, Ma Q, Liu C, Deng P, Zhu G, Zhang L, et al. Exposure to 1800 MHz radiofrequency radiation impairs neurite outgrowth of embryonic neural stem cells. Sci Rep 2014;4:5103.
22. Cui D, Xiong X, Shu J, Dai X, Sun Y, Zhao Y. FBXW7 confers radiation survival by targeting p53 for degradation 2020. Cell Rep 2020;30:497–509.
23. Yogosawa S, Yoshida K. Tumor suppressive role for kinases phosphorylating p53 in DNA damage-induced apoptosis. Canc Sci 2018;109:3376–82.
24. Ono K, Han J. The p38 signal transduction pathway: activation and function. Cell Signal 2000;12:1–13.
25. Esteva FJ, Sahin AA, Smith TL, Yang Y, Pusztai L, Nahta R, et al. Prognostic significance of phosphorylated P38 mitogen-activated protein kinase and HER-2 expression in lymph node-positive breast carcinoma. Cancer 2004;100:499–506.
26. Hallek M, German CLLSG. Prognostic factors in chronic lymphocytic leukemia. Ann Oncol 2008;19(4 Suppl):iv51–3.
27. Lee JS, Huang TQ, Kim TH, Kim JY, Kim HJ, Pack JK, et al. Radiofrequency radiation does not induce stress response in human T-lymphocytes and rat primary astrocytes. Bioelectromagnetics 2006;27:578–88.
28. Ahmed D, Eide P, Eilertsen I, Danielsen S, Eknaes M, Hektoen M, et al. Epigenetic and genetic features of 24 colon cancer cell lines. Oncogenesis 2013;2:e71.
29. Wiegering A, Matthes N, Bettina M, Koospal M, Quenzer A, Peter S, et al. Reactivating p53 and inducing tumor apoptosis (RITA) enhances the response of RITA-sensitive colorectal cancer cells to chemotherapeutic agents 5-fluorouracil and oxaliplatin. Neoplasia 2017;19:301–9.
30. Natarajan M, Vijayalaxmi, Szilagyi M, Roldan FN, Meltz ML. NF-kappaB DNA-binding activity after high peak power pulsed microwave (8.2 GHz) exposure of normal human monocytes. Bioelectromagnetics 2002;23:271–7.
31. Goswami PC, Albee LD, Parsian AJ, Baty JD, Moros EG, Pickard WF, et al. Proto-oncogene mRNA levels and activities of multiple transcription factors in C3H 10T 1/2 murine embryonic fibroblasts exposed to 835.62 and 847.74 MHz cellular phone communication frequency radiation. Radiat Res 1999;151:300–9.
32. Vlahopoulos SA. Aberrant control of NF-kappaB in cancer permits transcriptional and phenotypic plasticity, to curtail dependence on host tissue: molecular mode. Cancer Biol Med 2017;14: 254–70.
33. Yang XS, Hao YT, He GL, Chen CH, Wang Y, Zhang GB, et al. Relationship between activation of microglia and Jaks phosphorylation induced by microwave irradiation. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi 2010;28:401–4.
34. Zhang MH, Li GZ, Xu H, Zhang J, Cao J. Effect of oxymatrine on NF-kappaB and other cell factors in rats lung tissue with septic shock. Zhongguo Zhongyao Zazhi 2008;33:2390–4.
35. Kim DH, Hyun DJ, Piquette R, Beaumont C, Germain L, Larouche D. 27.12 MHz radiofrequency ablation for benign cutaneous lesions. BioMed Res Int 2016;2016:6.