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Original Article

The impact of 3G mobile phone base station radiation on 14-3-3 Family Proteins in hepatocellular carcinoma cell line

Baz istasyonundan yayılan 3G cep telefonu radyasyonunun hepatoselüler karsinoma hücre hattında 14-3-3 Protein Ailesine etkisi

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ABSTRACT

Aim: The widespread increase of mobile telecommunication services is mainly responsible for the amount of radiofrequency radiation (RFR) in public and residential areas. Third generation (3G) is different from second generation (2G) system technologies with respect to carrier frequency. 14-3-3 proteins are a family of highly conserved cellular proteins that plays a crucial role in the regulation of metabolic pathways. Besides 14-3-3 proteins regulate many cellular processes such as cell growth, differentiation and apoptotic cell death. The aim was to study the possible effects of third generation mobile phone base station like Radiofrequency Radiation exposure on the 14-3-3 protein expression in the hepatocellular carcinoma cells (HepG2).

Materials and Methods: HepG2 cells were exposed RFR in four different periods as 1 hour, 2, 3 and 4 hours and then western blot analysis was performed to analyse protein expression level.

Results: Expressions of 14-3-3 protein were found unaltered in the control, sham and exposure groups.

Conclusion: This report presents data about the effects of 3G system technologies mobile phone base station radiation on 14-3-3 protein expression. 14-3-3 isoenzyme analysis and long term exposure experiments should be done to understand the exact effects of 3G mobile phone radiation on HepG2 cell line.

Keywords: 14-3-3 protein family; Hepatocellular carcinoma; Radiofrequency.

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ÖZ

Amaç: ÇMobil telekomünikasyon servislerindeki yaygın artış, halka açık alanlar ve konutlardaki radyofrekans radyasyon (RFR) miktarının başlıca sorumlusudur. Üçüncü nesil (3G) sistem teknolojileri ikinci nesil (2G) teknolojilere göre taşıyıcı frekansları bakımından oldukça farklıdırlar. 14-3-3 protein ailesi metabolik yolların düzenlenmesinde önemli rollere sahip hücresel proteinleri içerir. Bununla beraber 14-3-3 protein ailesi hücre büyümesi, farklılaşması ve apoptotik hücre ölümü gibi birçok hücresel süreçte görev alırlar. Çalışmanın amacı 3G cep telefonu baz istasyonundan yayılan radyofrekans radyasyona maruz kalan hepatoselüler karsinoma hücrelerinde (HepG2) 14-3-3 protein ekspresyonunun incelenmesidir.

Gereç ve Yöntemler: HepG2 hücreleri 1, 2, 3, ve 4 saat olmak üzere dört farklı periyotta radyofrekans radyasyona maruz bırakılmış ve protein ekspresyonundaki farklılıkların incelenmesi için western blot analizi gerçekleştirilmiştir.

Bulgular: Kontrol, sham ve maruziyet gruplarında 14-3-3 protein ekspresyonunda değişime rastlanmamıştır.

Sonuç: Bu çalışmada üçüncü nesil cep telefonu baz istasyonundan yayılan radyasyonun 14-3-3 protein ekspresyonuna etkisi ile ilgili veriler elde edilmiştir. 14-3-3 izoenzim analizleri ve uzun süreli maruziyeti içeren çalışmaların yapılması ile üçüncü nesil cep telefonundan yayılan radyasyonun HepG2 hücre hattına etkisinin daha iyi anlaşılmasına olanak sağlanacaktır.

Anahtar Kelimeler: 14-3-3 protein ailesi; Hepatoselüler karsinom; Radyofrekans.

Introduction

The use of mobile phones is increasing dramatically. Although many researches have been carried out to date, there are still remaining questions unanswered about the side effects of mobile phone radiation [1]. Nevertheless, most of the research carried out was focused mainly on the side effects of Global System for Mobile Communication (GSM) phone radiation in the frequency band of 900 MHz and 1800 MHz. There have been few studies conducted to investigate the response of biological systems to 3G mobile phone radiaton in the frequency band of 2100 MHz. It has been reported that radiofrequency field exposure causes to change cell proliferation and the synthesis rate of different biomolecules [2-5]. Moreover, the role of cell phone exposure on tumor induction has also been proposed in an epidemiological research [6].

14-3-3 proteins are a family of eukaryotic proteins acting as a regulator in various processes ranging from mitosis to apoptosis. They are key regulators of cellular processes such as signal transduction events and inhibition, activation or structural stabilization of different enzymes and proteins [7, 8].

The aforementioned mammalian brain protein family named as 14-3-3 due to its particular migration pattern and elution characteristics on respective techniques namely starch gel electrophoresis two-dimensional and DEAE-cellulose chromatography [9].

14-3-3 protein family members are 30 kDa acidic proteins expressed in a wide range of organisms and tissues exhibiting highly conserved character. Seven major 14-3-3 isoforms are named beta (β), epsilon (ε), eta (η), gamma (γ), tau (τ), sigma (σ) and zeta (ζ) respectively [10, 11]. These proteins have significant role in considerable cellular actions such as cell-cycle control, signal transduction, stress response, malignant transformation and apoptosis. To date, more than 100 different interacting partners for the 14-3-3 proteins have been reported. They are act as an adaptor molecules that stimulating protein-protein interactions, regulating the protein localization at subcellular level and activating or inhibiting enzymes [12].

14-3-3 regulates members of the mitochondrial apoptotic machinery, as well as an interfering many of the signaling molecules that regulate the survival and death signals to the mitochondrial death pathway [13]. There is some evidence to suggest that the antiapoptotic activity of 14-3-3 proteins applied by direct interference on the critical function of the mitochondrial core pro-apoptotic machinery [14].

Thus, investigating the expression level of 14-3-3 family may give a clue about the altered level of different proteins which is modulated by mobile phone radiation [1, 5]. The aim of the present study was to evaluate the possible effect of non-ionizing 3G mobile phone radiation on expression of 14-3-3 proteins in hepatocellular carcinoma cell line HepG2.

Material and Methods

Cell Culture

Human hepatocellular carcinoma cell line HepG2 was purchased from ATCC (cat.HB-8065). HepG2 cells were maintained in DME High Glucose media with 584mg/l L-Glutamine (cat.9031,Irvine Scientific, USA) containing

10% Fetal Bovine Serum (Irvine Scientific, USA) and 50µg/ml Gentamycin sulfate solution (Irvine Scientific, USA). The cells were grown in 75cm2 vented cap flasks (BD Falcon, USA) in humidified 5% CO2 37ºC incubator (Sanyo, Japan) and medium was renovated every 2 days.

Exposure System

3G base station modulated UMTS signals at 2100 MHz were produced by using a vector signal generator (Rohde &Schwarz SMBV 100A, 9 kHz - 3.2 GHz, Germany) a horn antenna (Schwarzbeck, Doppelsteg Breitband Horn antenna BBHA 9120 L3F, 0.5 - 2.8 GHz, Schönau, Germany) in a temperature controlled shielded environment. The temperature was kept at 37 °C throughout the experiment.

The generated power was controlled by a spectrum analyzer (Agilent Technologies N9320A, 9 kHz - 3 GHz, Santa Clara, United States) connected to the signal generator. The produced signals were controlled by means of the spectrum analyzer connected to the signal generator, and a NARDA EMR 300 meter and a type 26.1 probe (Pfullingen, Germany) were used for the measurement of the output radiation. Measurements were taken throughout the entire experiment and the data was recorded to a computer bounded by a fiber optic cable. The average whole body specific absorption rate (SAR) was estimated to be 0.2 W/kg using the Finite Domain of Time Difference (FDTD) method.

Treatment Cells with RF

The cells were incubated in the flasks 24 hours before the experiment to allow binding. Dead or unbound cells were removed by rinsing twice with Phosphate Buffer Saline (Irvine Scientific, USA) before assay. The flasks were coded as control, sham and 2100 MHz, continuous RFR exposed for 1 hour, 2, 3 and 4 hours. Control flasks were left in the incubator for the entire period of the experiment. Sham exposed flasks were in the same conditions as with the RFR exposed, with the only difference, having signal generator turned off.

Experimental Design

Control Groups (Group I to Group IV)

Group I. Control for the one-hour-exposed cells. Cells left in the incubator for 1 hour.

Group II. Controls for the two-hour-exposed cells. Cells left in the incubator for 2 hours.

Group III. Controls for the three-hour-exposed cells. Cells left in the incubator for 3 hours.

Group IV. Controls for the four-hour-exposed cells. Cells left in the incubator for 4 hours.

Sham-Exposed Groups (Group V to Group VII)

Group V. Sham-exposed cells for the one-hour-exposed cells. Cells in the flask were kept in the same experimental conditions without the RFR -exposed cells for 1 hour.

Group VI. Sham-exposed cells for the two-hour-exposed cells. Cells in the flask were kept in the same experimental conditions without the RFR -exposed cells for 2 hours.

Group VII. Sham-exposed cells for the three-hour-exposed cells. Cells in the flask were kept in the same experimental conditions without the RFR -exposed cells for 3 hours.

Group VIII. Sham-exposed cells for the four-hour-exposed cells. Cells in the flask were kept in the same experimental conditions without the RFR -exposed cells for 4 hours.

RFR-Exposed Groups (Group VIII to Group XII)

Group IX. RFR-exposed cells for the one-hour cells. Cells in the flask were exposed to 2100 MHz RFR for 1 hour.

Group X. RFR-exposed cells for the two-hour cells. Cells in the flask were exposed to 2100 MHz RFR for 2 hours.

Group XI. RFR-exposed cells for the three-hour cells. Cells in the flask were exposed to 2100 MHz RFR for 3 hours.

Group XII. RFR-exposed cells for the four-hour cells. Cells in the flask were exposed to 2100 MHz RFR for 4 hours.

Western Blot Analysis

The adherent cells in the T75 flask were harvested with EDTA immediately after the end of the exposure and then washed three times with PBS. Following the removal of PBS cells were lysed with buffer containing 7M Urea, 2M Thiourea, 10 mM DTT and 1 mM PMSF. The Bradford protein assay was used to determine the protein content of the lysate.

Semi quantitative Western Blotting technique was used to compare the expression level of 14-3-3 proteins. The proteins were resolved in %10 Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). An equal amount (40 µg) of protein originated from the cell-lysates was applied to each well. Following electrophoresis the proteins were transferred to Nitrocellulose membrane and blocked with 3% non-fat dry milk. 14-3-3 Rabbit IgG affinity purified anti-Human antibodies (IBL, Japan) against to the types β, γ, ε, ζ, η, τ of the protein is used as a primary antibody. Polyclonal Anti Rabbit IgG Horseradish Peroxidase (HRP) conjugated (Abcam, United States) was used as a secondary antibody. β-actin primary antibody (Genscript, USA) and Polyclonal Anti Mouse IgG Horseradish Peroxidase (HRP) secondary antibody was used in loading control analysis for normalization. The signal produced by Luminol reagent (Santa Cruz, USA). was visualized by a radiography film (Kodak, USA). Developed films

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were scanned using Bio-Rad GS-800 densitometer and signal intensity was determined by BioRad-Quantity One 4.6 to compare expression levels among groups. Normalized three independent experiment data were statistically analyzed with student t-test at the confidence level of % 95.

Results

Western Blot analysis results are shown in Figure 1 and Figure 2. Expressions of 14-3-3 protein were found unaltered in among the groups.

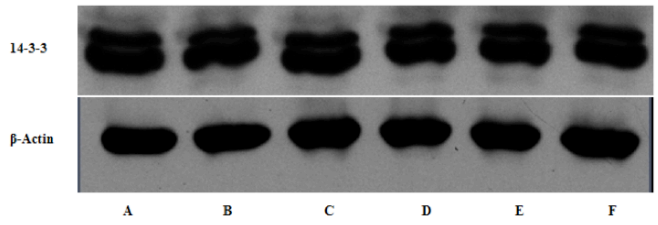

Figure 1. A.Control for the one-hour-exposed cells. B.Controls for the two-hour-exposed cells. C.Sham-exposed cells for the one-hour-exposed cells. D.Sham-exposed cells for the two-hour-exposed cells. E.RFR-exposed cells for the one-hour cells. F.RFR-exposed cells for the two-hour cells.

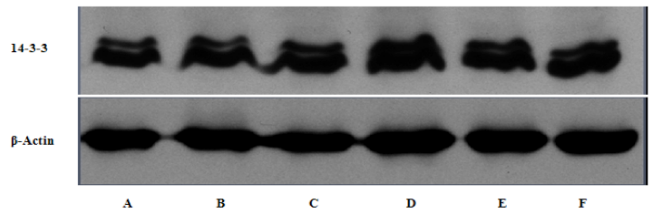

Figure 2 A.Controls for the three-hour-exposed cells. B.Controls for the four-hour-exposed cells. C.Sham-exposed cells for the three-hour-exposed cells. D.Sham-exposed cells for the four-hour-exposed cells. E.RFR-exposed cells for the one-hour cells. F.RFR-exposed cells for the one-hour cells.

Disscussion

A number of studies were conducted into the expression pattern of the cells exposed to mobile phone radiation by two dimensional electrophoresis [15-17]. In these studies proteins showing altered expression were verified with an alternative technique such as Western Blotting and RT-PCR assay. After validation assays the changes in expression profile were not found to be significant.

Hirose et al. [18] examined phosphorylation of Hsp27 as it occurs rapidly following exposure to various stresses. They induced the human glioblastoma A172 and IMR-90 fibroblast cell lines with continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) RF fields. Neither expression of hsp27 nor the phosphorylation of the protein was affected following exposure.

Nylund et al. [16] analysed the proteome response of brain and umbilical vein endothelial cells to the mobile phone radiation. In the aforementioned study some of the proteins expression profile was found to be changed following exposure. When the data was corrected with false discovery rate analysis, the results were not to be significant. These findings contradicted the result of their previous study which was conducted using human endothelial cell line EA.hy926 exposed to GSM 900 MHz radiation. Nylund et al. [16] clarified the discrepancy as (i) different exposure frequencies (900 MHz vs. 1800 MHz), (ii) differences in SAR distribution in cell culture dishes in the used exposure set-ups, (iii) differences in used cell types (primary cells vs. cell line), and (iv) differences in the 2DE proteomics methodology (silver stain vs. DIGE).

Röösli et al. [19] systematically reviewed the recent articles regarding the health effects of exposure to mobile phone base station (MPBS) radiation and concluded that while the data in the 17 published article was scarce, the absence of evidence of harm should not necessarily be interpreted as evidence that no harm exists.

Studies showed that overexpression of 14-3-3β in NIH3T3 cells induces cell growth and promotes tumor formation in nude mice. Also 14-3-3β found to be over expressed in lung cancer. These findings suggest that 14-3-3 β has an oncogenic potential [20].

Even though the over expression of 14-3-3 proteins has not been yet associated with specific pathologies; there are some reports indicating increased 14-3-3 expression in specific cancer types. Increased expression of 14-3-3 ζ was reported in lung cancer, breast cancers, oral squamous cell carcinomas and stomach cancer. 14-3-3β levels are increased in papillomavirus induced carcinomas, α and τ expression in lung cancer biopsies and of 14-3-3γ in chemo-resistant melanomas were published [21].

Lodygin et al. [22] found high expression levels of 14-3-3σ in normal prostate epithelial and benign prostate hyperplasia cells in immunohistochemical studies, whereas prostate cancer cells have either low expression profile or lacking of 14-3-3σ.

Down-regulation of 14-3-3σ was reported in breast cancer cells by SAGE analysis by Nacht et al. [23]. However, any genetic modification which could be detected in the 14-3-3σ locus may explain the decreased expression.

The large number of 14-3-3 targets plays a key role in various cellular functions. In the present study protein expression

profile of 14-3-3 protein family in 3G mobile phone exposed HepG2 cell line was examined by Western Blotting and no change in protein expression was observed. Our results were in line with the previous published articles.

In conclusion, the laboratory findings should be verified with an orthogonal analytical technique such as gene expression analysis (RT-PCR). Also 14-3-3 isoform analysis should be done to understand the exact effects of 3G mobile phone radiation in HepG2 cell line. Research should be carried out to investigate the long term effects of mobile phone radiation in-vivo and in-vitro.

Declaration of conflict of interest

The authors received no financial support for the research and/or authorship of this article. There is no conflict of interest.

References

1. Nylund R, Tammio H, Kuster N, Leszcynski D. Proteomic analysis of response of human endothelial cell line EA.hy926 to 1800 GSM mobile phone radiation. J Proteomics Bioinform 2009; 2: 455-62.
2. Fitzsimmons RJ, Farley JR, Adey WR, Baylink DJ. Frequency dependence of increased cell proliferation in vitro in exposures to a low-amplitude, low-frequency electric field: evidence for dependence on increased mitogen activity released into culture. J Cell Physiol 1989; 139: 586–91.
3. Marjanovic Cermak AM, Pavicic I, Tariba Lovakovic B, Pizent A, Trosic I. In vitro non-thermal oxidative stress response after 1800 MHz radiofrequency radiation. Gen Physiol Biophys 2017; 36: 407-14.
4. Manna D, Ghosh R. Effect of radiofrequency radiation in cultured mammalian cells: A review. Electromagn Biol Med 2016; 35: 265-301.
5. Nylund R, Leszczynski D. Proteomics analysis of human endothelial cell line EA.hy926 after exposure to GSM 900 radiation. Proteomics 2004; 4: 1359-65.
6. Hardell L, Mild KH, Carlberg M, Söderqvist F. Tumour risk associated with use of cellular telephones or cordless desktop telephones. W J Surg Onc 2006; 4: 74.
7. Ferl RJ, Manak MS, Reyes MF. The 14-3-3s. Genome Biol 2002; 3(7): reviews3010.1-reviews3010.7.
8. Rosenquist M, Sehnke P, Ferl R, Sommarin M, Larsson C. Evolution of the 14-3-3 protein family: does the large number of isoforms in multicellular organisms reflect functional specificity? J Mol Evol 2005; 51: 446-58.
9. Carlson FD. Physiological and biochemical aspects of nervous integration. 1st ed. Englewood Cliffs: New Jersey; 1967.
10. Aghazadeh Y, Papadopoulos V. The role of the 14-3-3 protein family in health, disease, and drug development. Drug Discov Today 2016; 21: 278-87.
11. Hartman AM, Hirsch AKH. Molecular insight into specific 14-3-3 modulators: Inhibitors and stabilisers of protein-protein interactions of 14-3-3. Eur J Med Chem 2017; 136: 573-84.
12. Van Hemert MJ, Steensma HY, Van Heusden GP. 14-3-3 proteins: key regulators of cell division, signalling and apoptosis. BioEssays 2001; 23: 936-46.
13. Wu YJ, Jan YJ, Ko BS, Liang SM, Liou JY. Involvement of 14-3-3 Proteins in Regulating Tumor Progression of Hepatocellular Carcinoma. Cancers (Basel) 2015; 7: 1022-36.
14. Danial NN, Korsmeyer SJ. Cell death: critical control points. Cell 2004; 116: 205–19.
15. Karinen A, Heinävaara S, Nylund R, Leszczynski D. Mobile phone radiation might alter protein expression in human skin. BMC Genomics 2008; 9: 77.
16. Nylund R, Kuster N, Leszczynski D. Analysis of proteome response to the mobile phone radiation in two types of human primary endothelial cells. Proteome Sci 2010; 8: 52.
17. Kim KB, Byun HO, Han NK et al. Two-dimensional electrophoretic analysis of radio-frequency radiation-exposed MCF7 breast cancer cells. J Radiat Res 2010; 51: 205-13.
18. Hirose H, Sakuma N, Kaji N et al. Mobile phone base station-emitted radiation does not ınduce phosphorylation of hsp27. Bioelectromagnetics 2007; 28: 99-108.
19. Röösli M, Frei P, Mohler E, Hug K. Systematic review on the health effects of exposure to radiofrequency electromagnetic fields from mobile phone base stations. Bull World Health Organ 2010; 88: 887–96.
20. Takihara Y, Matsuda Y, Hara J. Role of the beta isoform of 14-3-3 proteins in cellular proliferation and oncogenic transformation. Carcinogenesis 2000; 21: 2073–77.
21. Tzivion G, Gupta SV, Kaplun L, Balan V. 14-3-3 proteins as potential oncogenes. Sem Canc Bio 2006; 16: 203–13.
22. Lodygin D, Diebold J, Hermeking H. Prostate cancer is characterized by epigenetic silencing of 14-3-3σ expression. Oncogene 2004; 23: 9034-41.
23. Nacht M, Ferguson AT, Zhang W et al. Combining serial analysis of gene expression and array technologies to identify genes differentially expressed in breast cancer. Cancer Res 1999; 59: 5464–70.