

The effect of high-frequency electromagnetic field on molecular melting profile of human breast adenocarcinoma MCF-7 cell DNA.

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Abstract

The aim of the study was to evaluate the genotoxic effect of high-frequency electromagnetic field on the human adenocarcinoma cells using HRM analysis. Detecting genetic variations can contribute to the future perspective of anti-tumor strategies combining EMF and anti-cancer drugs. Our data suggest that high-frequency EMF with a frequency of 900 MHz (an input power of 8 W) after 3 h exposure may produce alterations in the melting temperature of DNA of MCF-7 cells from $76.4 \pm 0.9^\circ\text{C}$ to $82.6 \pm 1.0^\circ\text{C}$. DNA was extracted from control sample and EMF-exposed MCF-7 cells and MDR1 was sequenced. Comparing the ratio of G+C/A+T on the forward end as well as on the backward end: forward - MCF-7/wt (36.36%/38.67%), MCF-7/EMF (35.30%/39.04%); backward - MCF-7/wt (41.54%/44.62%), MCF-7/EMF (40.13%/44.20%); the decrease of the relative content of G+C/A+T of EMF-exposed MCF-7 cells was observed.

Keywords: High-frequency electromagnetic field, MCF-7 cell line, high-resolution melting analysis

Accepted August 06 2013

This article may be cited as:

Marianna T, Laputková G, Sabo J, Živčák J. The effect of high-frequency electromagnetic field on molecular melting profile of human breast adenocarcinoma MCF-7 cell DNA. Biomedical Research 2013; 24 (4): 469-475.

Introduction

The biological effects of electromagnetic fields (EMF) in the high-frequency region of the spectrum have been under thorough investigations during the last decades. Up till now, there has been reported a great many of studies documenting numerous biological effects due to high frequency radiation *in vivo* and/or *in vitro*. As a biological target, the DNA molecule has, as well, received attention with respect to the potential high-frequency EMF damage [1, 2], because of its relevance to cell function, proliferation, viability, mutation and carcinogenesis. Nevertheless, there have been conflicting results with respect to the potential hazard of high frequency radiation exposure. Regarding EMF effects on tumor promotion, some studies show a carcinogenetic effect to the induction of brain tumors [3], others [4] suggest that high frequency electromagnetic field could not contribute to the initiation stage of tumor formation. The possible disturbance produced by high-frequency EMF on physiology of cells remains controversial.

Among various drug transporters, P-glycoprotein, the MDR1 gene product, is one of the best studied and characterised. P-glycoprotein is expressed in normal human tissues such as liver, kidney, intestine and the endothelial cells of the blood-brain barrier. P-glycoprotein is an important prognostic factor in malignant diseases. A growing number of preclinical and clinical studies have demonstrated that polymorphism of the MDR1 gene may be a factor in the overall outcome of pharmacotherapy for numerous diseases [5]. In addition, a study of Kuo and Lu [6] has shown that an appropriate exposure of brain-microvascular endothelial cells to a low frequency EMF could effectively inhibit the expression of P-glycoprotein and multidrug resistance-associated protein on human brain-microvascular endothelial cells. On the other hand, according to Gigert et al. [7] electromagnetic field exposure could contribute to resistance observed in breast cancer after long-term treatment with tamoxifen.

The great interest stimulated by high resolution melting (HRM) methods explains its rapid expansion to cope with

broad spectrum of human genetic mutations [8] and polymorphisms [9]. HRM includes the precise monitoring of the variation in fluorescence initiated by the release of an intercalating DNA dye from a DNA duplex as it is denatured by increasing temperature. HRM approach proved to be very attractive showing considerable advantages over the conventional methods; it is an in-tube, rapid and low cost method of detecting genetic variations. The analysis is performed immediately after the amplification and is thus particularly suitable for medium to high-throughput applications.

The aim of this study was to evaluate the genotoxic effects of high-frequency electromagnetic field on the human adenocarcinoma cells to detect genetic variations using HRM analysis to contribute to the future perspective of anti-tumor strategies combining EMF and anticancer drugs.

Materials and Methods

Cell Growth Conditions and Cell Passaging

The cell line MCF-7 was purchased from the American Type Culture Collection (ATCC, USA). Cells were grown according to the procedure of Třebuňová et al. 2012 [10]. Briefly, a monolayer of cells were cultured in 10% Dulbecco's Modified Eagle Medium (Invitrogen, USA) containing foetal bovine serum (FBS, Invitrogen), mixture of 5000 units of penicillin and 5000 µg of streptomycin/ml in 0.85% saline (PenStrep) (Invitrogen, USA). Cells were kept in the Heracell 150i incubator (Thermo Scientific, USA) at 37°C in 5% CO₂ and 95% humidity. All solutions used were of the cell culture grade quality. The equipment employed was commercially pre-sterilized and disposable.

High-frequency electromagnetic field exposure

Cells were irradiated for three hours with a high frequency electromagnetic field generated within the certified gigahertz TEM cell manufactured by Fischer Custom Communications (FCC-TEM-JM3, FCC, USA) providing uniform incident plane wave exposure conditions. A schematic diagram of the experimental setup is shown in Fig. 1. Signal generator was used to produce electromagnetic field frequency of 900 MHz (MG 3642A, Anritsu, Japan). The cell cultures, growing in sterile 96-well microtiter plate (Sarstedt, Germany) and residing as a thin monolayer at the flat bottom of the flat well, were placed so that the H-field vector was parallel to the plane of the culture medium. For the supplied plane wave signal 8 W input power was required to achieve a 465 V m⁻¹ electric field.

To compare the cell viability of a sham-exposed sample and a cage control sample that has no contact to exposure source (neither active nor inactive) compared to sham exposure, a check experiment was made. A shame-

exposed 96-well plate with the cell culture was placed into the TEM cell (electromagnetic field was switched off) and the other plate was located next to the TEM cell (a cage control) in the same cell incubator. Cell viability was measured using standard colorimetric test. Briefly, cell suspensions containing 4×10⁴ viable cells were cultivated in 96-well tissue culture plates in a final volume of 100 µl. The optical density of each well was measured spectrophotometrically at 480 nm in MRX Dynatech ELISA reader (UK).

DNA samples

DNA samples were extracted from both control MCF-7 cells (MCF-7/wt) and MCF-7 cells exposed to the electromagnetic field with ultra-high frequency (MCF-7/EMF). DNA was isolated from cells using SensiFAST™ SYBR, No-ROX Kit (Bioline, UK) according to the manufacturer's instructions.

Melting analysis

Primers (Sigma, USA) were chosen according to the study of Sung-Tsai et al. 2007 [11]. PCR amplification and melting analysis were performed on the Line Gene K (Bioer, China) using FastStart Universal SYBR Green Master (ROX) (Roche, Switzerland) as recommended by the manufacturer. The cycling protocol started with one cycle of 50°C for 2 min followed by 1 cycle of 95°C for 10 min followed by 40 cycles of 95°C for 15 s (1°C/cycle), a touch down of 95°C to 61.2°C for 1min. The melting analysis step started at 61.2°C to target temperature of 95°C for 15 s (0.5°C/cycle). Fluorescence data were converted into melting peaks by the aid of fluorescence quantitative PCR detection software (Line-Gene K, Bioer, China) to determine binding efficiency of DNA exposed to high-frequency electromagnetic field. The experiments were performed in duplicates.

Statistical analysis for the viability of cell

The results for the viability of cells evaluated by the MTT test were expressed as the mean from four independent experiments ± standard deviations (SD). Multiple comparisons between groups were assessed using one-way analysis of variance (ANOVA). Probability values of P>0.05 were accepted as statistically significant.

Sequencing DNA and Statistical Analysis

PCR product was sequenced in Sequencing Centre of Department of Molecular Biology (Faculty of Sciences, Comenius University, Bratislava, Slovak Republic). The concentration of DNA was 44.8 µg/µl and 40.0 µg/µl for MCF-7/wt and MCF-7/EMF respectively. The concentration of primer was 1 pmol/µl. The template accomplished the volume of 20 µl. The volume of primer was 10 µl. The resulting percentage of nucleotide bases and amino acid of MDR1 gene were analyzed with the aid of BioEdit Sequence Alignment Editor.

Results and Discussion

The control experiment confirmed that there is no significant difference in viability of cells grown simultaneously in TEM cell and outside the TEM cell in the same incubator. An absorbance of a shame-exposed sample and a cage control sample was 0.648 ± 0.070 and 0.651 ± 0.074 for $n=4$ of 96-well plates, respectively.

The influence of high-frequency EMF exposure on the generation of DNA strand alterations derived from human adenocarcinoma MCF-7 cells was evaluated using the HRM. Fig. 2 illustrates the melting curves analysis and the derivative melting curves of MCF-7/wt and MCF-7/EMF. One clear melting transition for both amplicon duplexes melted at 65-90°C was observed. Based on 12 measurements, one melting peak was displayed at $76.4 \pm 0.9^\circ\text{C}$ for MCF-7/wt and $82.6 \pm 1.0^\circ\text{C}$ for MCF-7/EMF, respectively. The elevation was significant at 3 h of exposure with $p < 0.01$.

Using MDR1 primer pairs: forward 5'-CCCATCATTGCAATAGCAGG-3', backward 5'-GTTCAAACCTTCTGCTCCTGA-3' it was possible to amplify fragments of genomic DNA and detect PCR products. Fig. 3 and Table I give the representation of the molecular profile of DNA strand extracted from human adenocarcinoma MCF-7/wt and MCF-7/EMF. Comparing

of the relative content G+C/A+T in MCF-7/wt (36.36%/38.67%) and MCF-7/EMF (35.30%/39.04%) on the forward end and on the backward end (MCF-7/wt (41.54%/44.62%) and MCF-7/EMF (40.13%/44.20%)) the decrease of the relative content of G+C/A+T after the exposure of cells to high-frequency EMF was observed. As can be seen from Table II, the alterations in amino acid composition coded by fragments of DNA from MCF-7/wt in comparison with MCF-7/EMF were detected. Thus, the sequencing results did not confirm identical nucleotide sequences of 180 bp for both fragments of DNA.

However, a number of studies have reported that high-frequency EMF exposure had no genotoxic effects on cells, or that a relation between radiofrequency radiation and cancer was weak to nonexistent [12, 13], this study showed that exposure of MCF-7 cells in vitro to continuous high-frequency EMF can lead to weak changes of MDR1 gene. As regards the reasons for DNA damage, data from the authors of another study [14] indicate that exposure of lymphoblastoid cells in vitro to two different radiofrequency signals under a thermal conditions altered the amount of DNA single-strand breaks detected by the alkaline comet assay. Depending on the signal and the time of exposure, DNA damage was observed to both increase and decrease. Thus, free radicals may play a part in radiofrequency-induced DNA damage.

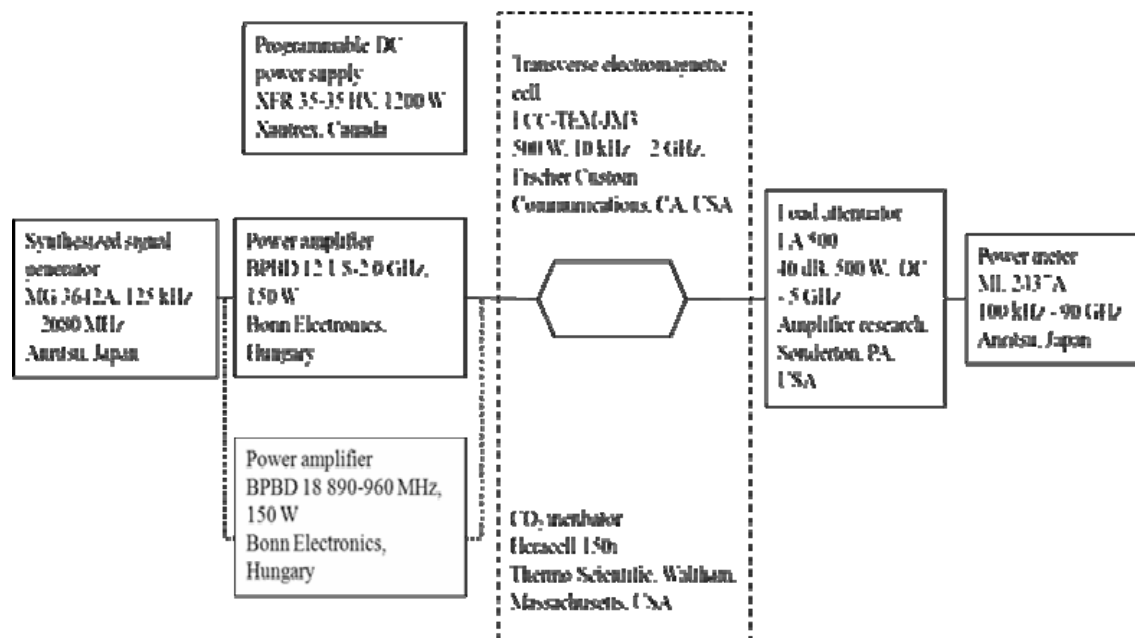


Figure 1. Schematic diagram of the experimental set-up.

Aitken et al. [15] exposed mice to 900 MHz EMF at a specific absorption rate of approximately 90 mW/kg inside a waveguide for 7 days at 12 h per day. A detailed analysis of DNA integrity in spermatozoa using QPCR

revealed statistically significant damage to both the mitochondrial genome ($p < 0.05$) and the nuclear beta-globin locus ($p < 0.01$). Moreover, Sekeroğlu et al. [16] showed significant differences in chromosome aberrations (in-

crease), the micronucleus frequency (increase), mitotic index (decrease) and the ratio of polychromatic erythrocytes (decrease) in bone marrow cells of all exposure groups compared with the control group of rats. Additionally, the cytotoxic and genotoxic damage was more remarkable in immature rats than in mature rats. However, some authors have stressed [1] that only the amplitude

modulated signals induced a significant increase in the comet assay parameters in trophoblast cells, while the unmodulated continuous wave exposure was ineffective. On the other hand, an increase in both types of DNA breaks was observed after exposure to either the pulsed or continuous-wave radiation, but no significant difference was observed between the effects of the two forms of radiation in brain cells of rat [17].

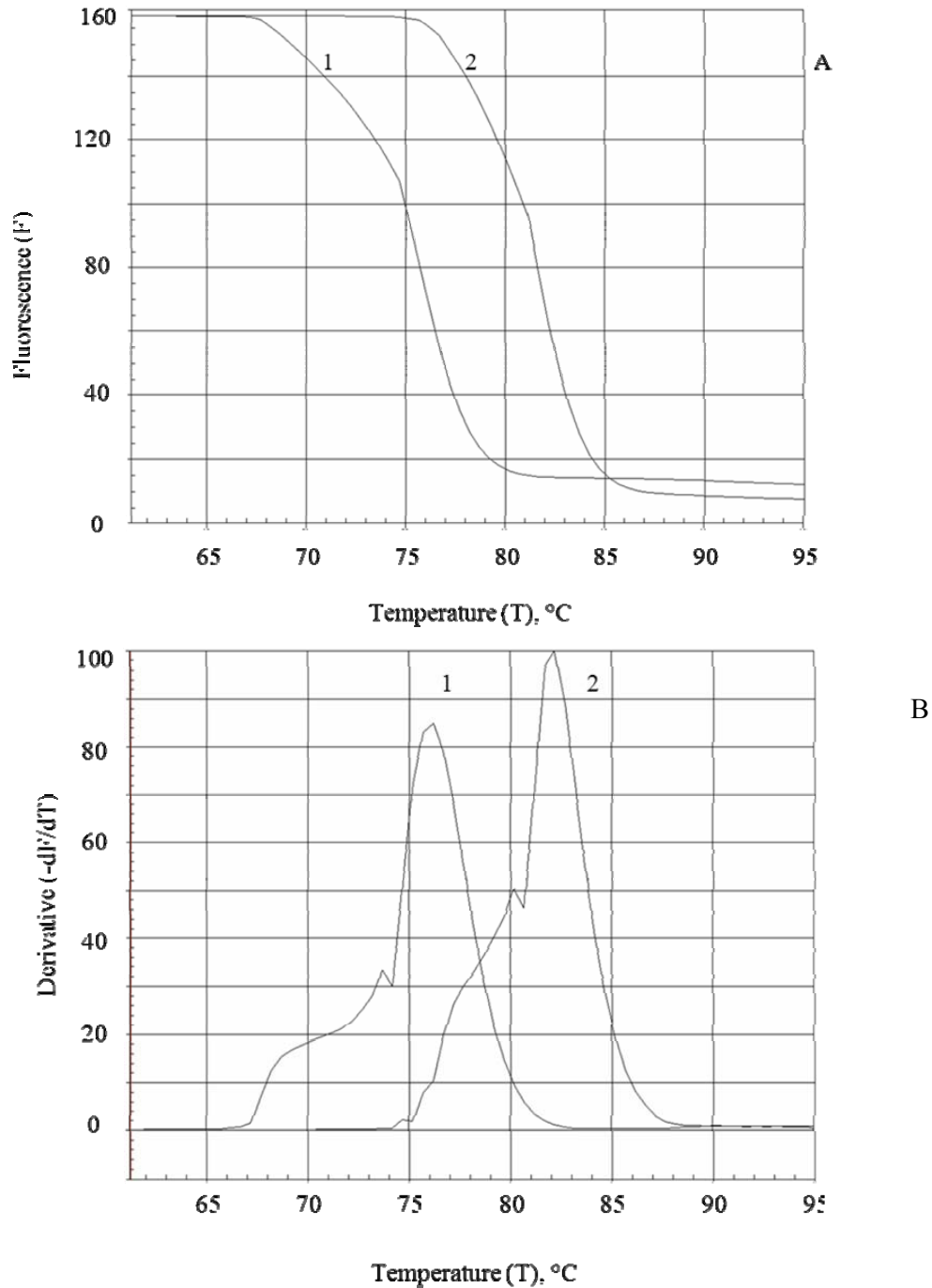


Figure 2. Representative melting curves (A) and derivative melting curves (B) of MCF-7/wt (curves 1) and MCF-7/EMF (curves 2).

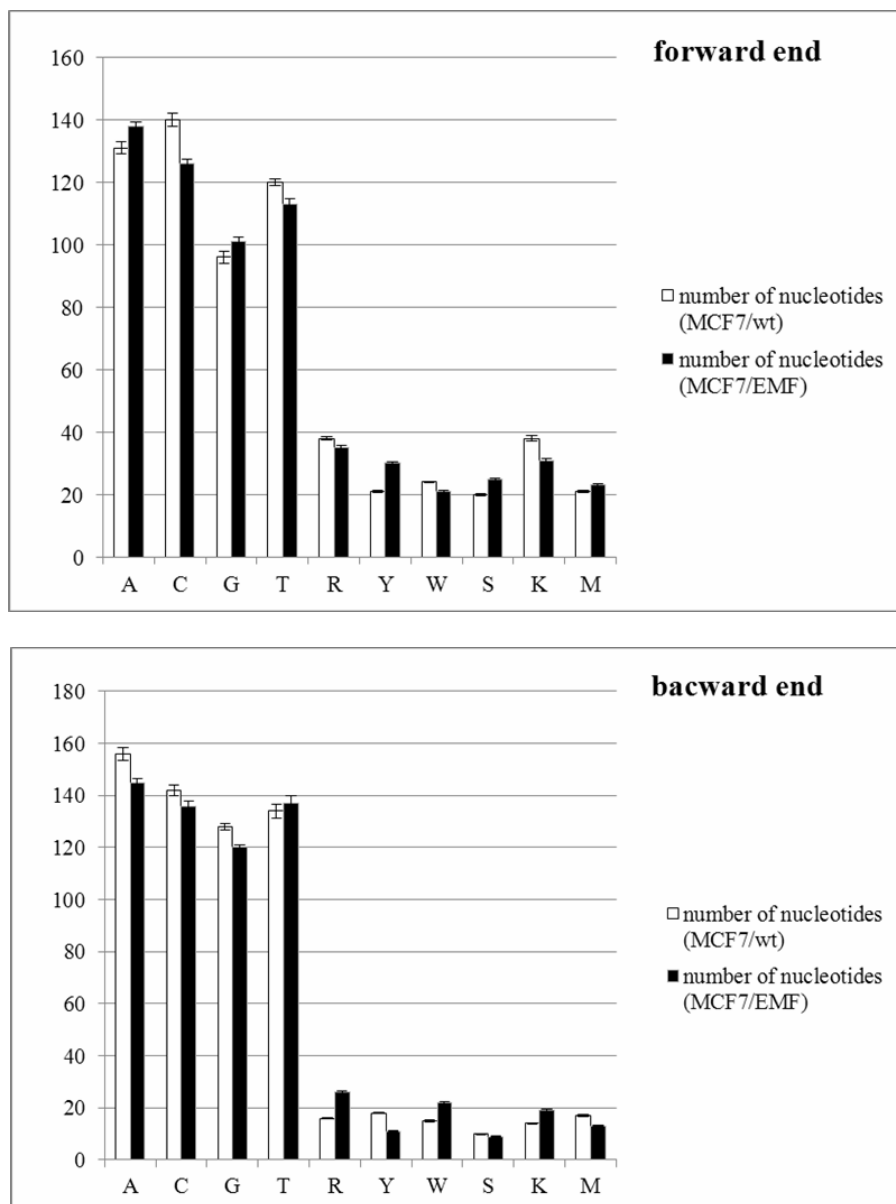


Figure 3. Nucleotide composition of DNA MCF-7/wt and MCF-7/EMF on the forward and the backward end - the mean \pm SD for $n=4$. (Nucleic acid codes: A \rightarrow adenosine, C \rightarrow cytidine, G \rightarrow guanine, T \rightarrow thymidine, R \rightarrow GA (purine), Y \rightarrow TC (pyrimidine), W \rightarrow AT (weak), S \rightarrow GC (strong), K \rightarrow GT (keto), M \rightarrow AC (amino)).

Table I. The basic characteristics of DNA of MCF-7/wt cells and MCF-7/EMF cells on the forward and the backward end - the mean \pm SD for $n=4$.

| DNA molecule | MCF-7/wt | MCF-7/EMF | MCF-7/wt | MCF-7/EMF |
|---------------------------------------------|-------------------|-------------------|-------------------|-------------------|
| | forward end | | backward end | |
| Length (base pairs) | 649 \pm 3 | 643 \pm 4 | 650 \pm 5 | 638 \pm 3 |
| Molecular weight (single stranded, Daltons) | 198558 \pm 918 | 196297 \pm 1218 | 197991 \pm 1526 | 194459 \pm 914 |
| Molecular weight (double stranded, Daltons) | 394401 \pm 1823 | 390746 \pm 2430 | 394959 \pm 3039 | 387545 \pm 1822 |
| G+C content (%) | 36.36 \pm 0.44 | 35.30 \pm 0.34 | 41.54 \pm 0.38 | 40.13 \pm 0.37 |
| A+T content (%) | 38.67 \pm 0.35 | 39.04 \pm 0.31 | 44.62 \pm 0.55 | 44.20 \pm 0.53 |

Table 2. Amino acid composition of DNA of MCF-7/wt cells and MCF-7/EMF cells on the forward and the backward end - the mean of four independent experiments.

| DNA molecule: MDR1 | Forward end | | Backward end | |
|--------------------|-------------|-----------|--------------|-----------|
| | MCF-7/wt | MCF-7/EMF | MCF-7/wt | MCF-7/EMF |
| Alanine | 131 | 138 | 156 | 145 |
| Cysteine | 140 | 126 | 142 | 136 |
| Aspartic acid | 0 | 0 | 0 | 0 |
| Glutamic acid | 0 | 0 | 0 | 0 |
| Phenylalanine | 0 | 0 | 0 | 0 |
| Glycine | 96 | 101 | 128 | 120 |
| Histidine | 0 | 0 | 0 | 0 |
| Isoleucine | 0 | 0 | 0 | 0 |
| Lysine | 38 | 31 | 14 | 19 |
| Leucine | 0 | 0 | 0 | 0 |
| Methionine | 21 | 23 | 17 | 13 |
| Asparagine | 0 | 0 | 0 | 0 |
| Proline | 0 | 0 | 0 | 0 |
| Glutamine | 0 | 0 | 0 | 0 |
| Arginine | 38 | 35 | 16 | 26 |
| Serine | 20 | 25 | 10 | 9 |
| Threonine | 120 | 113 | 134 | 137 |
| Valine | 0 | 0 | 0 | 0 |
| Tryptophan | 24 | 21 | 15 | 22 |
| Tyrosine | 21 | 30 | 18 | 11 |

Conclusions

Our data suggest that high-frequency EMF with a frequency of 900 MHz (an input power of 8 W) after 3 h of exposure produces alterations in the melting temperature of DNA of MCF-7 cells. Furthermore, high-frequency EMF exposure enhances the frequency of changes in the molecular profile of DNA.

Acknowledgements

This study was supported by the Agency of the Slovak Ministry of Education for the Structural Funds of the EU under projects ITMS: 26 220 120 039 (50 %), ITMS: 26 220 220 143 (25 %), ITMS: 26 220 120 067 (25 %).

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