Epigenetic changes are induced following exposure of peripheral blood cells to CW 800MHz radiation

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ABSTRACT

Genetic and epigenetic changes are involved in initiation and progression of cancer. We exposed peripheral blood cells from young male volunteers to CW 800MHz radiation at SAR values of 2.9 and 4.1 W/kg for 24 hours (n=5) and 72 hours (n=10). Control experiments were carried out in the temperature range of 33.5-40ºC. Epigenetic changes were monitored by analysis of FISH patterns of centromeres of chromosomes 11 and 17 reflecting alteration in their replication timing and synchrony. Our results demonstrate radiation induced non-thermal effect on the level of asynchronous replication in both chromosomes following exposure to the higher SAR level.

INTRODUCTION

The large exposure of the world population to cellular phones has led to a growing concern in the public about their health effects – mainly cancer. One of the hallmarks of cancer is the loss of control of the replication machinery in the cell leading to genomic instability through epigenetic mechanisms as evidenced by changes in replication timing. Replication of the genetic material needs to occur under a very tightly controlled temporal schedule, where each section has a specific time domain during the s-phase of the cell cycle at which it replicates. Replication timing is closely linked to the activity status (transcribed or not) of the DNA sequence in the tissue studied, where expressed sequences replicate early while unexpressed sequences replicate late [1].

MATERIALS AND METHODS

We exposed peripheral blood cells (PBLs) from young male volunteers to CW 800MHz radiation at SAR values of 2.9 and 4.1 W/kg. The exposure system consisted of a custom-made waveguide resonator. The electromagnetic-wave propagation in the exposure chamber loaded with the test samples was simulated by 3D numerical electromagnetic-field simulation software (Ansoft HFSS, version 10.0) and the SAR distribution in the exposure chamber and in each test tube was calculated after measuring the specific dielectric properties of the cultures used in the study. We lowered the temperature of the incubator containing the exposure set up to 33.5^{o}C in order to compensate for the increased temperature during exposure. We also determined the dependence of the replication timing and synchrony on temperature by culturing PBLs obtained from four donors at different temperatures in this e range of 33.5–40°C for 72 h.

. Peripheral blood cells from 10 male volunteers were exposed for the whole 72 hours of culturing and then harvested according to standard cytogenetic procedure. The mean age of the volunteers was 30.0±5.5 (23-39). In addition, **w**e set up 5 independent exposure experiments (mean age of the volunteers was 30.2±3.1 (28-33)) in which cells were exposed for 24 hours, cultured for 72 and harvested according to standard cytogenetic procedure. While series one (I) was exposed for the first 24 hours of culturing; series two (II) was exposed during the middle of the culturing period and series three (III) was exposed for the final 24 hours of culturing.

We used FISH for the centromeres of chromosomes 11 and 17 to analyze changes in replication timing using the FISH replication assay. Manual analysis of about 600 interphase nuclei was performed on the automatically acquired FISH image galleries using the Metacyte image analysis system by coding as to their replication status according to the signal shapes. In the FISH replication analysis the signals are designated as belonging to one of four replication stages [2,3] in accordance with their progression through the cell cycle. In the resting (G_0) cells as well as

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in the G_1 stages, the signals are dense and rounded single dots (A). At the start of the S phase the signals become less dense, exhibiting some side branching (B). Both the A and B stages are representative of prereplicated sequences (S). As the cell cycle progresses to the G_2 stage, these develop into a long thin signal resembling beads on a string (C) which then resolve into double dots representing DNA sequences that have already replicated (D). Both types of post replication status were designated D. The nuclei harboring signals of centromeres that have both not been replicated were marked as SS (late replicating); Nuclei with both replicated signals were marked as DD (early replicating); nuclei with one replicated and one unreplicated signal were marked as SD (asynchronous replication). As there is inter individual variation in the level of asynchronous replication, we looked at the folds of induction of the effect and used the one sample t-test for analysis.

RESULTS

Our results indicate that there is a statistically significant effect on the level of asynchronous replication in both chromosomes following 72 hr exposure to the higher SAR level of 4.1W/kg ($p=0.014$ and 0.027 for CEN 11 and 17, respectively). The centromere of chromosome 11 was more sensitive to the radiation and exhibited increased asynchronous replication even at the lower SAR level of 2.9W/kg ($p=0.012$).

Following 24 hrs of exposure no increased asynchromous replication was induced by the lower SAR (2.9W/kg) for either chromosome 11 or 17 at either of the three exposure periods. However, at the higher exposure level (4.1W/kg) we observed increased levels of asynchronous replication for centromeres 11 and 17 following exposure during period II ($p=0.03$ and 0.014, respectively) and III ($p=0.02$ and 0.014, respectively).

We found no statistically significant differences in the replication timing between cells incubated at 33.5°C, 37°C and 38.5°C. However, incubation at 40°C led to a significant increase in th level of asynchronous replication..

SUMMARY AND CONCLUSIONS

The present study suggests the existence of genetic instability, reflected by increased levels of asynchronous replication of centromeric loci in lymphocytes due to *in vitro* exposure to CW 800MHz radiation. These epigenetic changes are accompanied by genetic ones as reflected by previously observed increased aneuploidy of the same two chromosomes following identical exposure conditions for 72 hours [4]. Furthermore, the study suggests the possible existence of an athermal effect of RF radiation leading to increased levels of genomic instability.

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REFERENCES

[1] A. Korenstein-Ilan, A. Barbul, P. Hasin, A. Eliran, A.Gover and R. Korenstein Terahertz radiation increases genomic instability in human lymphocytes. *Radiat Res.* 170 (2):224-34, 2008.

[2] T. Litmanovitch, M. M. Altaras, A. Dotan, and L.Avivi. Asynchronous replication of homologous α-satellite DNA loci in man is associated with nondisjunction. Cytogenet. Cell Genet. 81(1):26-35, 1998.

[3] A. B. Mukherjee, VVVS Murty, Chaganti RSK Detection of cell-cycle stage by fluorescence in situ hybridization: its application in human interphase cytogenetics. Cytogenet Cell Genet 61(2):91-94, 1992.

[4] R. Mazor, A. Korenstein-Ilan, A. Barbul, Y. Eshet, A. Shahadi, E. Jerby, and R. Korenstein. Increased levels of numerical chromosome aberrations after in vitro exposure of human peripheral blood lymphocytes to radiofrequency electromagnetic fields for 72 hours. Radiat. Res. 169(1) 28-37, 2008.