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Microwaves from Mobile Phones Inhibit 53BP1 Focus Formation in Human Stem Cells Stronger than in Differentiated Cells: Possible Mechanistic Link to Cancer Risk

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Competing financial interests

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DNA repair

List of abbreviations:

DNA double strand breaks (DSB); electromagnetic field (EMF); extremely low frequency (ELF); Global System for Mobile Communication (GSM); phosphorylated histone H2AX (γ-H2AX); non-thermal microwaves (NT MW); specific absorbed rate (SAR); transverse electromagnetic transmission line cell (TEM-cell); tumor suppressor p53 binding protein 1 (53BP1).

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Abstract

Background. It is widely accepted that DNA double-strand breaks (DSB) and their misrepair in stem cells are critical events in the multistage origination of various leukemia and tumors including gliomas.

Objectives. We studied whether microwaves (MW) from mobile phone of Global System for Mobile Communication (GSM) and Universal Global Telecommunications System (UMTS) induce DSB or affect DSB repair in stem cells.

Methods. 53BP1 foci that are typically formed at the sites of DSB location (referred to as DNA repair foci) were analyzed by laser confocal microscopy.

Results. We report for the first time that MW from mobile phones inhibit formation of 53BP1 foci in human primary fibroblasts and mesenchymal stem cells (MSC). These data parallel our previous findings for human lymphocytes. Importantly, the same GSM carrier frequency (915 MHz) and UMTS frequency band (1947.4 MHz) was effective for all cell types. Exposure at 905 MHz did not inhibit 53BP1 foci in differentiated cells, both fibroblasts and lymphocytes, while some effects at 905 MHz were seen in stem cells. Contrary to fibroblasts, stem cell did not adapt to chronic exposure during two weeks.

Conclusions. Strongest MW effects were always observed in stem cells that may suggest both significant misbalance in DSB repair and severe stress response. Our findings that stem cells are most sensitive to MW exposure and react to more frequencies than differentiated cells may be important for cancer risk assessment and indicate that stem cells are most relevant cellular model for validating the safe mobile communication signals.

Introduction

The intensity levels of exposure to microwaves (MW) from mobile phones are lower than the ICNIRP standards that are based on the thermal effects of acute MW exposures (ICNIRP 1998). However, effects of prolonged exposure to non-thermal (NT) MW at intensities comparable with those of mobile phones have also been observed in many studies that indicate relationship of NT MW exposure with permeability of brain blood barrier (Nittby et al. 2008), cerebral blood flow (Huber et al. 2005), stress response (Blank and Goodman 2004), and neuronal damage (Salford et al. 2003). The data obtained by comet assay (Lai and Singh 1997; Diem et al. 2005) and micronuclei assay (d'Ambrosio et al. 2002; Zotti-Martelli et al. 2005; Trosic et al. 2002) implied possible genotoxic effects of NT MW while other studies did not support this genotoxicity (Meltz 2003). Experimental data have indicated that the NT MW effects occur depending on several physical parameters including carrier frequency, polarization, modulation, intermittence (Belyaev 2005a). Difference in these physical parameters and biological variables including genetic background and physiological state may explain various outcomes of studies with NT MW (Belyaev 2005b; Huss et al. 2007).

A recent review of available epidemiological studies concluded that the usage of mobile phones longer than 10 years is associated with increased risk of ipsilateral gliomas and acoustic neurinomas (Hardell et al. 2008). Stem cells have for long time been considered as an important cellular target for origination of cancer, both tumors and leukemia (Feinberg et al. 2006; Soltysova et al. 2005). It is believed that gliomas originate from brain stem cells (Altaner 2008). DNA double-strand breaks (DSB) and their misrepair are critical molecular events resulting in chromosomal aberrations (CA), which have often been associated with origination of various leukemia and tumors including gliomas (Fischer and Meese 2007). Only one study on possible MW-induced DSB in stem cells is available (Nikolova et al. 2005). Surprisingly, the data obtained therein by the neutral comet assay suggested that prolongation of the exposure time abolished the DSB formation observed at the shorter exposure time. Furthermore, neutral comet assay has limited applicability to detect DSB because similar increase in the comet tails

may be also caused by non-genotoxic effects that imply changes in chromatin conformation such as relaxation of DNA-loops (Belyaev et al. 1999).

Several proteins involved in DSB repair such as phosphorylated histone 2A family member X (γ-H2AX) and the tumor suppressor TP53 binding protein 1 (53BP1) have been shown to produce discrete foci that co-localize to DSB and referred to as DNA repair foci (Kao et al. 2003; Sedelnikova et al. 2002). Analysis of DNA repair foci is currently accepted as most sensitive and specific technique for measuring DSB in untreated cells as well as in cells exposed to cytotoxic agents (Bocker and Iliakis 2006; Bonner et al. 2008). By analysis of the DNA repair foci in normal human fibroblasts, we were able to detect DSB induced by very low dose of ionizing radiation, 1 cGy, that result in only 0.4 DSB/cell in average (Markova et al. 2007). We have also used this technique to analyze 53BP1/γ-H2AX foci in human lymphocytes exposed to MW from GSM/UMTS phones (Markova et al. 2005; Belyaev et al. 2005; Belyaev et al. 2009). We have found that MW exposure inhibited formation of endogenous 53BP1/γ-H2AX foci (Markova et al. 2005; Belyaev et al. 2005; Belyaev et al. 2009). This inhibition might be caused by a decrease in accessibility of DSBs to proteins because of stress-induced chromatin condensation (Belyaev et al. 2009). Inability to form DNA repair foci has been correlated to radiosensitivity, genomic instability and other repair defects (Bassing et al. 2002; Celeste et al. 2002; Kuhne et al. 2004; Olive and Banath 2004; Taneja et al. 2004). Inhibition of DSB repair may lead to CA by either illegitimate recombination events (Bassing and Alt 2004) or reduced functionality of non-homologous end-joining (Fischer and Meese 2007). Therefore, if similar effects on endogenous DNA repair foci would be detected in stem cells it might provide a direct mechanistic link to the epidemiological data showing association of MW exposure with increased cancer risk.

While γ-H2AX foci have been used to analyze endogenous and induced DSB in most studies, recent data have indicated that γ-H2AX foci may also be produced by chromatin structure alternations and may not contain DSB (Han et al. 2006; Suzuki et al. 2006; Banath et al. 2004; Yu et al. 2006). Accordingly, some γ-H2AX foci may not associate with DNA damage response proteins like 53BP1

(Markova et al. 2007; Markova et al. 2005; Belyaev et al. 2009; McManus and Hendzel 2005). High expression of endogenous γ-H2AX in pluripotent mouse embryonic stem (mES) cells - around 100 large γH2AX foci per cell), was not explained by DSB, DNA degradation, or apoptosis, but it was attributed to the unusual organization of chromatin in mES cells (Banath et al. 2009). The number of endogenous 53BP1 foci, fewer than 3 foci/nucleus, appeared normal in mES cells and is comparable with other cell types (Banath et al. 2009). In contrast to γ-H2AX foci, which may be produced by the DSB-relevant and DSB-unrelated mechanisms, 53BP1 is re-localized to the locations of DSB along with other DNA damage response proteins such as phosphorylated ATM, Rad50 and MRE11 and there is no indication that DSB-unrelated events would result in formation of the 53BP1 foci (Medvedeva et al. 2007; Yoshikawa et al. 2009). Therefore, in this study we analyzed only 53BP1 foci as a more relevant marker for DSB.

The differences in the DSB repair pathways between mouse and human stem cells have been described (Banuelos et al. 2008). In general, the comparisons of stem cells across species suggest that significant difference may be observed and thus extrapolation from animal stem cellular models to human health risk assessment should be done with care (Ginis et al. 2004; Brons et al. 2007). For this study, we have chosen human adipose-tissue derived mesenchymal stem cells (MSC). This cell type display multi-potency with the ability under the correct conditions to differentiate into lineages that cover wide range of organs and tissues such as bone, fat, cartilage, muscle, lung, skin, hepatocytes, and neurons (Porada et al. 2006; Bunnell et al. 2008; Sasaki et al. 2008). Of note, the mesenchymal stem cells are at higher risk of malignant transformation than embryonic stem cells (Soltysova et al. 2005).

In contrast to GSM exposure at the frequency of 915 MHz that consistently inhibited DNA repair foci in lymphocytes from 26 persons in total (Markova et al. 2005; Belyaev et al. 2005; Belyaev et al. 2009), GSM exposure at 905 MHz did not inhibit DNA repair focus formation thereby providing evidence that MW effects depend on carrier frequency. It would be of interest to compare the response of stem cells with response of differentiated primary human cells such as human lymphocytes and human

fibroblasts that are also usually exposed to MW from mobile phones. Therefore, in this study we exposed human stem cells and primary human fibroblasts to GSM/UMTS MW at the same frequencies as previously used in experiments with human lymphocytes.

Materials and Methods

Cells

Human diploid VH-10 fibroblasts from the foreskin of normal boy (a gift from Dr. Ada Kolman, Department of Molecular Biology and Genome Research, Stockholm University, Sweden) were maintained at 5 % CO_2 and 37^0C in a humidified incubator as previously described (Markova et al. 2007). Human MSC separated from adipose tissue of two healthy persons as described (Kucerova et al. 2007) (a gift from Dr. V. Altanerova, Cancer Research Institute, Bratislava, Slovakia) were cultivated in MEM Alpha Medium (low glucose, Gibco, Invitrogen) supplemented with 10% Mesenchymal stem cell stimulating supplement (human, StemCell Technologies) and 1% antibiotic - antimycotic mix (Gibco, Invitrogen, concentration per ml of medium: penicillin 100 units, streptomycin 100 µg, Fungizone 0.25 µg). The suspension of cells 1 x 10^5 (MSC) or 2 x 10^5 (VH-10) in volume 3ml of medium were seeded on cover slides in Petri dishes (Sarsted, Germany, 35x10 mm) and incubated at 37°C in 5% CO_2 humidified atmosphere for 36-40 h while 80% confluent layer of cells was reached.

Cell exposure

Cells were exposed to GSM/UMTS MW essentially as described previously (Belyaev et al. 2009; Sarimov et al. 2004). Briefly, exposures were performed using two specially designed installations, each based on a transverse electromagnetic line cell (TEM-cell) and a test mobile phone. The output of each phone was connected by the coaxial cable to the correspondent TEM-cell. Cells were exposed to either GSM (905 MHz or 915 MHz) or UMTS (1947.4 MHz, middle channel), output power being the same, 0.25 W, at least three times at each exposure condition. All exposures were performed at 37^oC in a 5% CO2-incubator, using Petri dishes containing 3 ml of media per dish. The specific absorption rate (SAR)

was 37 mW/kg for the frequency of 905/915 MHz and 39 mW/kg for the frequency of 1947 MHz. Taking into account all possible uncertainties, the SAR values at all locations within exposed samples were always well below thermal effects. Temperature was measured in the MW-exposed samples before, during and after exposure with a precision of 0.1^{o}C. No changes in temperature were induced in the samples during exposures.

Except for MW, mobile phones emit also electromagnetic fields (EMF) of extremely low frequency (ELF) that can also contribute to the exposure effects (Weisbrot et al. 2003). To avoid eventual effects of ELF exposure, the test-mobile phones were situated at the distance of 1 m from the CO_2-incubator with exposed samples. Accordingly, the ELF emission of our test-mobile phones did not increase background ELF field, which did not exceed 200 nT, root mean square, as measured with a three-dimensional microteslameter (Field dosimeter 3, Combinova, Bromma, Sweden) at the location of MW exposure.

Sham exposures were performed in the same TEM-cells with MW power off. The order of MW- and sham-exposures was randomized among sessions. In each experiment, the sham exposures were performed in duplicate, in the TEM-cell for GSM exposure and in the TEM-cell for UMTS exposure. No differences were observed between sham-exposed samples (sham-sham exposures). Therefore, the MW effects were generally analyzed with reference to combined sham-exposures. The heat treatment, 41^{o}C, was used as a positive control for stress response. As a positive control for genotoxic effect, the cells were irradiated with ^{137}Cs γ-rays, 3 Gy, using a Gammacell 1000 (Isomedix, Inc., USA) source. The dose rate was 10.6 Gy/min.

Immunostaining and foci analysis

Immediately after exposure, the cells were placed on ice for 1 hour to prevent repair of eventual DSB. The immunostaining was performed essentially as described previously (Markova et al. 2007). The images were recorded from 5-10 fields of vision that were randomly selected from two slides on the confocal laser scanning microscope Zeiss Axiovert 100M using the plan-apochromat 63x/1.4-numerical-

aperture oil immersion objective and the LSM 510 software. Through-focus maximum projection images were acquired from optical sections 1.00 μm apart and with a section thickness of 2.00 μm in the Z-axis. Resolutions in the X- and Y-axis were 0.20 μm. Eight optical sections were usually obtained for each field of vision and the final image was obtained by projection of all sections onto one plane. For each independent exposure experiment and for each exposure condition (type of cell, type of exposure, exposure duration), usually 300 cells were analyzed in double blind fashion.

Statistical analysis

Statistica 8.0 (StatSoft Inc, Tulsa, OK) and SPSS Statistics 17.0 (SPSS Inc., Chicago, Illinois) software were used according to the manuals. Using ANOVA for several means we set the statistical power to 0.80 based on estimates of sample variation and effect size obtained in our pilot experiments. The cell distributions of foci were analyzed using the Kolmogorov-Smirnov test. Most data did not fulfill the Poisson distribution. We analyzed two types of data arrays, either mean values from independent experiments or all raw data representing foci in each individual cell, using both non-parametric and parametric statistics. Bonferroni adjustment was used in multiple comparisons by ANOVA. In general, all methods provided similar results and conclusions. Results were considered as significantly different at $p<0.05$.

Results

Both in fibroblasts and MSC, irradiation with 3 Gy led to significant increase in 53BP1 foci caused by radiation-induced DSB. In accordance with previously published data (Markova et al. 2007), 26 foci/cell were found in fibroblasts 2 h post-irradiation. At this time point, slightly higher level, 32 foci/cell, was detected in stem cells (Supplemental Material, Figure 1). While approximately 1 endogenous 53BP1foci/cell was seen in sham-exposed fibroblasts (Supplemental Material, Figure 2), we observed a distinct MW-induced reduction in the level of these foci in response to 915 MHz (Figure 1). UMTS MW also consistently reduced formation of endogenous 53BP1 foci in fibroblasts (Figure 1). Of

note, the MW-induced reduction in 53BP1 foci was the same regardless the duration of exposure within 1-3 h showing that saturation in the effects occurred at 1-h exposure (Figure 1). Analysis with the factorial ANOVA has confirmed that the data did not depend on the exposure time. To verify the hypothesis that MW exposure at 1-3 h exposure affected formation of 53BP1 foci we compared the effects using the Kruskal-Wallis ANOVA by ranks, the Median test and ANOVA. All tests showed that MW affected formation of 53BP1 foci at high significant levels, $p<0.001$. Multiple comparisons have shown significant effects of 915 MHz ($p<0.003$) and UMTS ($p<0.01$) at 1-3 h exposure. On the other hand, exposure at 905 MHz did not affect fibroblasts. Statistically significant difference was observed between effects of 915 MHz and 905 MHz exposure, $p<0.01$. These data parallel our findings for human lymphocytes (Belyaev et al. 2009) and suggest that both lymphocytes and fibroblasts respond to MW at the same carrier frequencies while other carrier frequencies do not affect these cells. Heat shock significantly inhibited formation of 53BP1 foci similar to 915 MHz and UMTS MW ($p<0.001$). These data are in accordance with our previous findings for human lymphocytes (Markova et al. 2005; Belyaev et al. 2005; Belyaev et al. 2009) suggesting that NT MW exposure at specific carrier frequencies induces stress response similar to heat shock.

MSC displayed approximately 2-fold increased level of endogenous 53BP1 foci as compared to fibroblasts (Figure 2, Supplemental Material; Figure 3). These data parallel the findings of others with mouse embryonic stem (mES) cells (Banath et al. 2009). Interestingly, almost no foci were detected in mitotic spreads of chromosomes of both stem cells and fibroblasts. The level of foci in mitotic cells was statistically significantly lower than in interphase cells (data are not shown). These results were in line with previously published data indicating that many endogenous 53BP1 foci may not pass mitosis (Markova et al. 2007).

Similar to our findings for fibroblasts, a distinct MW-induced reduction in the level of endogenous 53BP1 foci was observed in MSC exposed to 915 MHz and UMTS MW (Figure 2). However, these inhibitory effects of MW exposures in MSC were about 2-fold more pronounced than in

fibroblasts (Figure 1, 2). As it seen from Figure 2, prolongation of exposure did not result in increased inhibition providing evidence that effects of MW exposure saturated at 1-h exposure duration. Analysis with factorial ANOVA has confirmed that the data did not depend on the exposure time. The Kruskal-Wallis ANOVA by ranks, the Median test and ANOVA have shown that MW affected formation of 53BP1 foci at very high significant levels, $p<0.0001$. The effects of 915 MHz and UMTS at 1-3 h exposure were highly significant ($p<0.0005$). Contrary to fibroblasts, MSC had a proportion of cells, approximately 5%, with multiple foci, more than 10 foci/cell (Supplemental Material, Figure 4). The origin of these foci is unknown but they were completely inhibited by MW exposure (Figure 3). Heat shock at 41^0C also inhibited formation of 53BP1 foci in stem cells (Figure 2), albeit this inhibition was also stronger than in the heat-shocked fibroblasts (Figure 1). Altogether, the obtained data provide evidence that 915 MHz/UMTS MW exposure as well as heat shock result in stronger stress response in MSC as compared to fibroblasts. While some reduction in formation of foci was observed following exposure of stem cells to GSM MW at 905 MHz (Figure 2, 3), this effect was not statistically significant. However, the effects of 905 MHz and 915 MHz were not statistically different either. These findings indicate that MW may affect stem cells at more carrier frequencies as compared to differentiated cells.

We further tested whether MSC and fibroblasts can adapt to MW effects during chronic exposure. The cells were exposed during two weeks (5 days per week, 1 h exposure daily). Interestingly, MSC with multiple foci almost disappeared during two-week cultivation of untreated cells. Thus, the levels of endogenous foci did not differ between MSC and fibroblasts (Figure 4). Fibroblasts almost completely adapted to the chronic MW exposure (Figure 4). On the other hand, no such adaptation was seen in stem cells. All used statistical testes have shown that chronic MW exposure affected formation of 53BP1 foci in MSC ($p<0.05$, multiple comparisons by Kruskal-Wallis ANOVA by ranks and Median tests; $p<0.001$, ANOVA). Inhibitory effects of MW exposures at the frequencies of 915 MHz/GSM and 1947.4 MHz/UMTS were statistically significant during two-week exposure of stem cells (Kruskal-Wallis ANOVA, $p<0.05$; ANOVA, $p<0.005$). Comparison of arrays containing data from each individual cell

has confirmed that chronic MW exposure resulted in significant effects in MSC (Supplementary Material, Statistics). In addition, these comparisons revealed the effect of 905 MHz/GSM exposure and that the UMTS exposure affected MSC stronger than the GSM exposures.

Discussion

We report here for the first time that exposure of human MSC and human primary fibroblasts to MW from GSM/UMTS mobile phones inhibit formation of endogenous 53BP1 foci. Similar although not the same inhibitory effects of MW from GSM/UMTS mobile phones have previously been found in primary human lymphocytes (Belyaev et al. 2009). We used these cell types for two main reasons. First, the emerging data show that effects of low-intensity MW are cell cell-type dependent (Schwarz et al. 2008; Sanchez et al. 2006). In particular, immortalized and primary cells may respond differently to MW. Therefore, the data obtained with human primary cells would be of utmost relevance for assessment of possible health risks of MW exposure from mobile phones. Second, it now appears that most, if not all, adult tissues and organs including blood, skin and brain contain stem cells (Metcalfe and Ferguson 2008). Therefore, stem cells like blood cells and fibroblasts are always subjected to exposure from mobile phones.

Our data indicate that fibroblasts represent most resistant cell type as compared with both stem cells (this study) and human peripheral blood lymphocytes (Belyaev et al. 2009). Moreover, we show here that fibroblasts are able to adapt to MW during chronic exposure. These results are consistent with suggestion that adaptive cell behavior in response to MW exposure is unlikely to have adverse effects at the skin level (Sanchez et al. 2006). However, no adaptation was seen in stem cells (Figure 4). Thus, while our findings with chronic exposure of fibroblasts may suggest no health risks at the skin level, high sensitivity of stem cells may imply such risks.

No heating was induced in the samples exposed to MW. The SAR values at different locations of the exposed samples were always well below thermal effects. Therefore, the MW effects could not be attributed to the heating albeit the similar response was observed after both MW exposure and heat shock.

This similarity indicates that MW exposure at 915 MHz/1947.4 MHz is a stress factor for fibroblasts and especially for human stem cells where stronger effects were seen.

Modifications of 53BP1, such as phosphorylation, are needed for repair of DSB (Ward et al. 2006). Thus, our finding on the inhibition of DNA repair foci can be accounted for inhibition of phosphorylation of 53BP1 protein. Experimental evidence for such mechanism has recently been reported (Leszczynski et al. 2002). Alternatively, MW exposure can result in chromatin condensation that prevents accessibility of DSB to the DNA repair proteins (Markova et al. 2005; Belyaev et al. 2005; Sarimov et al. 2004). Regardless molecular mechanism, inhibition of DSB repair in stem cells may result in CA by either illegitimate recombination events (Bassing and Alt 2004) or reduced functionality of non-homologous end-joining (Fischer and Meese 2007).

We have found that the constitutive level of 53BP1 foci in human MSC is significantly higher than in differentiated primary human cells such as fibroblasts (this paper) and lymphocytes (Belyaev et al. 2009). Importantly, we did not observe adaptation to non-thermal GSM/UMTS MW chronic exposure in stem cells. Altogether, our findings show that human stem cells are more sensitive to MW exposure from mobile phones than differentiated primary cells. Thus, inhibition of 53BP1 foci in stem cells may account for higher risks than in differentiated cells with lower constitutive 53BP1 level.

We have found in this study that inhibitory effect of MW on the 53BP1 foci leveled off at 1h-exposure. No further increase in effects was observed both in MSC and fibroblasts at prolongation of exposure to 3 h. This data are in agreement with previous results that MW effects were the same at 1-h and 2-h exposures obtained of human peripheral blood lymphocytes (Markova et al. 2005; Belyaev et al. 2005). Our preliminary data indicate that saturation in the MW effect is observed even at shorter exposure time, 30 min, while almost linear dependence on exposure time was seen within shorter exposure times (to be published elsewhere).

Both 1947.4 MHz UMTS frequency band and 915 MHz GSM signal affected all tested human cell types: stem cells and fibroblasts (this paper) and lymphocytes (Belyaev et al. 2009). On the other

hand, MW exposure at another GSM frequency (905 MHz) did not result in statistically significant effects in lymphocytes and fibroblasts. Thus, GSM MW exposure may either inhibit or do not inhibit DNA repair foci in dependence on carrier frequency. Neither SAR nor the SAR measurement uncertainty depended on carrier frequency in the range of 905-915 MHz. Therefore, the difference in the effects at 905 and 915 MHz could not be attributed to the differences in the MW absorption. The "frequency" and "intensity" windows have often been reported for the non-thermal MW effects, see for review (Belyaev 2005b; Blackman 1992; Grundler 1992). Correspondently, there may be "effective" and "ineffective" carrier GSM frequencies that either affect human cells or induce no effect. Several physical mechanisms have been suggested to account for the frequency-dependent effects of NT MW (Matronchik and Belyaev 2008; Kaiser 1995; Binhi 2002; Belyaev et al. 1996). Our previous findings indicated that the intensity windows may not coincide for various carrier frequencies (Belyaev et al. 1996; Shcheglov et al. 1997). Correspondently, the SAR value of 39 mW/kg as used here may be optimal for the effects at 915 MHz but not at 905 MHz. Alternatively but less likely, it is possible that the cells have molecular components that have different electrical properties, thus altering the effective intensity (Joines and Blackman 1980). In either case, future testing of the cell response as a function of exposure intensities at 905 and 915 MHz should help to resolve this issue. Regardless physical mechanism, our findings suggest that specific carrier frequencies/bands that do not induce adverse effects can be validated in laboratory studies with primary human cells as the prerequisite for the development of safe wireless technologies.

While no statistically significant effects were seen in stem cells under exposure to 905 MHz by comparison of mean values, a trend to inhibition of the DNA repair foci was observed in these cells both under acute and chronic exposures (Figure 2, 4). Moreover, the MW effects at 905 MHz and 915 MHz were not statistically significantly different in stem cells and analysis of the individual cell arrays revealed effects of exposure to 905 MHz. These findings indicate that stem cells may react to more frequencies than differentiated primary human cells. Higher biological significance of MW effects in stem cells and

apparently wider range of effective frequencies suggest that stem cells are the most relevant cellular model for assessment of health risks from mobile communication.

Endogenous 53BP1 foci are typically considered as sensitive markers for endogenous DSB resulting in intrinsic genomic instability (Banath et al. 2009; Adams and Carpenter 2006; Sedelnikova et al. 2008), There is no evidence that 53BP1 play not any role in the repair of endogenous DSB. However, 53BP1 foci represent only indirect DSB measurements. If unrelevance of the endogenous 53BP1 foci to DSB would be proven, the MW effects described here should solely be interpreted as a manifestation of stress response. This alternative interpretation is supported by the data that MW exposure inhibits 53BP1 foci similar to heating of cells (Figures 1, 2). Stress response has previously been suggested as a criterion for adverse effects of electromagnetic fields (Blank and Goodman 2004). In fact, the currently accepted safety standards assume that MW exposure is harmful only if it acts similar to heating (ICNIRP 1998). Stress may be especially important for stem cells because it is believed to be an important factor in the multistage origination of cancer from human stem cells (Feinberg et al. 2006; Tez 2008). Both interpretations of the obtained data, either disruption of the balance between cellular repair systems and DNA damage or stress response, are not mutually exclusive and both may provide a mechanistic link to the epidemiological data showing association of prolonged MW exposure with brain cancer risk (Hardell et al. 2008). It should also be mentioned that stress can reduce neurogenesis (Sohur et al. 2006).

The best indications of the role of stem cells in cancer arise from hematological disorders such as leukemia. In several epidemiological studies, ELF exposure has been associated with the increased children leukemia. On the other hand, no association of ELF exposure with leukemia was found in adults. This discrepancy has not yet been clarified at the mechanistic basis while ELF has been classified as a possible carcinogen based on these studies (IARC 2002). Recent study suggested a possible association between electric transformers and power lines and the *XRCC1* Ex9 + 16A allele in patients with childhood acute leukemia (Yang et al. 2008). ELF exposure has been often reported to result in similar biological effects as exposures to NT MW (Blackman 1992; Adey 1981). Of note, ELF and MW

exposures similarly inhibited formation of DNA repair foci in human lymphocytes (Belyaev et al. 2005). It is known, that stem cells are more active in children as compared to adults (Williams et al. 2006). This increased activity of stem cells may clarify the differences between results obtained in ELF-leukemia studies with children and adults and call for studies on possible cancer risks of MW exposure of children.

Conclusions

In summary, we have demonstrated that GSM/UMTS MW from mobile phone inhibit formation of endogenous 53BP1 foci in human primary fibroblasts and stem cells. In contrast to fibroblasts, stem cells do not adapt to MW during chronic exposure. All together, our results indicate that stem cells are more sensitive to MW exposure than differentiated human primary cells while fibroblasts are least sensitive. Inhibitory effects of MW exposure on DSB repair in stem cell may result in formation of CA and therefore origination of cancer. Alternatively, MW effects may be accounted for stress response. Both possible interpretations provide a mechanistic link to increased cancer risk. Our finding that stem cells may react to more carrier frequencies as compared to differentiated cells may indicate that stem cells are most relevant cellular model for validating the safe mobile communication signals. As far as almost all organs and tissues possess stem cells and stem cells are more active in children the possible relationship of chronic MW exposure and various types of tumors and leukemia especially in children should be investigated.

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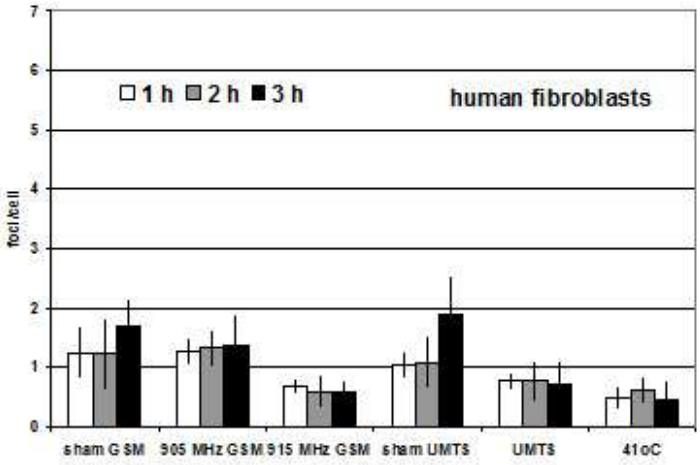
Figure legends

Figure 1 53BP1 foci in human normal fibroblasts VH-10 following 1 h, 2 h and 3 h exposure to GSM MW at 905 MHz or 915 MHz, UMTS MW at 1947.4 MHz, and heat shock at 41^{o}C, as measured by immunostaining and confocal laser microscopy. Mean values for cells from three-five experiments and standard deviations are shown in each data point.

Figure 2 53BP1 foci in human lipoaspirate mesenchymal stem cells following 1 h, 2 h and 3 h exposure to GSM MW at 905 MHz or 915 MHz, UMTS MW at 1947.4 MHz, and heat shock at 41^{o}C. Mean values for cells from three-five experiments and standard deviations are shown in each data point.

Figure 3 Distribution of 53BP1 foci among mesenchymal stem cells exposed to GSM (A) or UMTS (B) microwaves. Normalized frequency of cells with foci is given versus number of foci per cell.

Figure 4 53BP1 foci in human normal fibroblasts VH10 and human lipoaspirate mesenchymal stem cells following chronic exposure during 10 days (5 days per week, 1 h exposure daily) to GSM MW at 905 MHz or 915 MHz, and UMTS MW at 1947.4 MHz. Mean values for cells from three experiments and standard deviations are shown in each data point.



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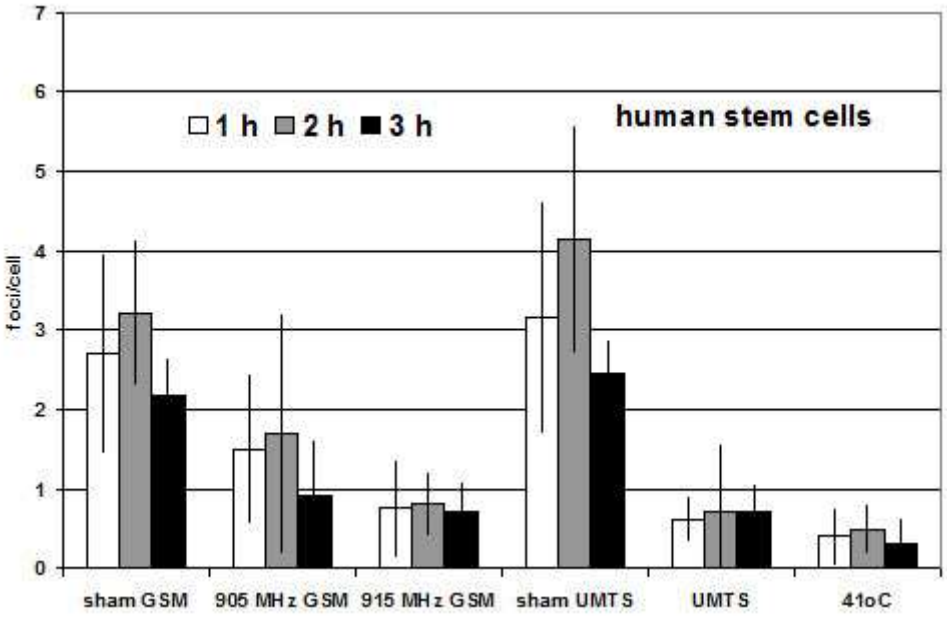

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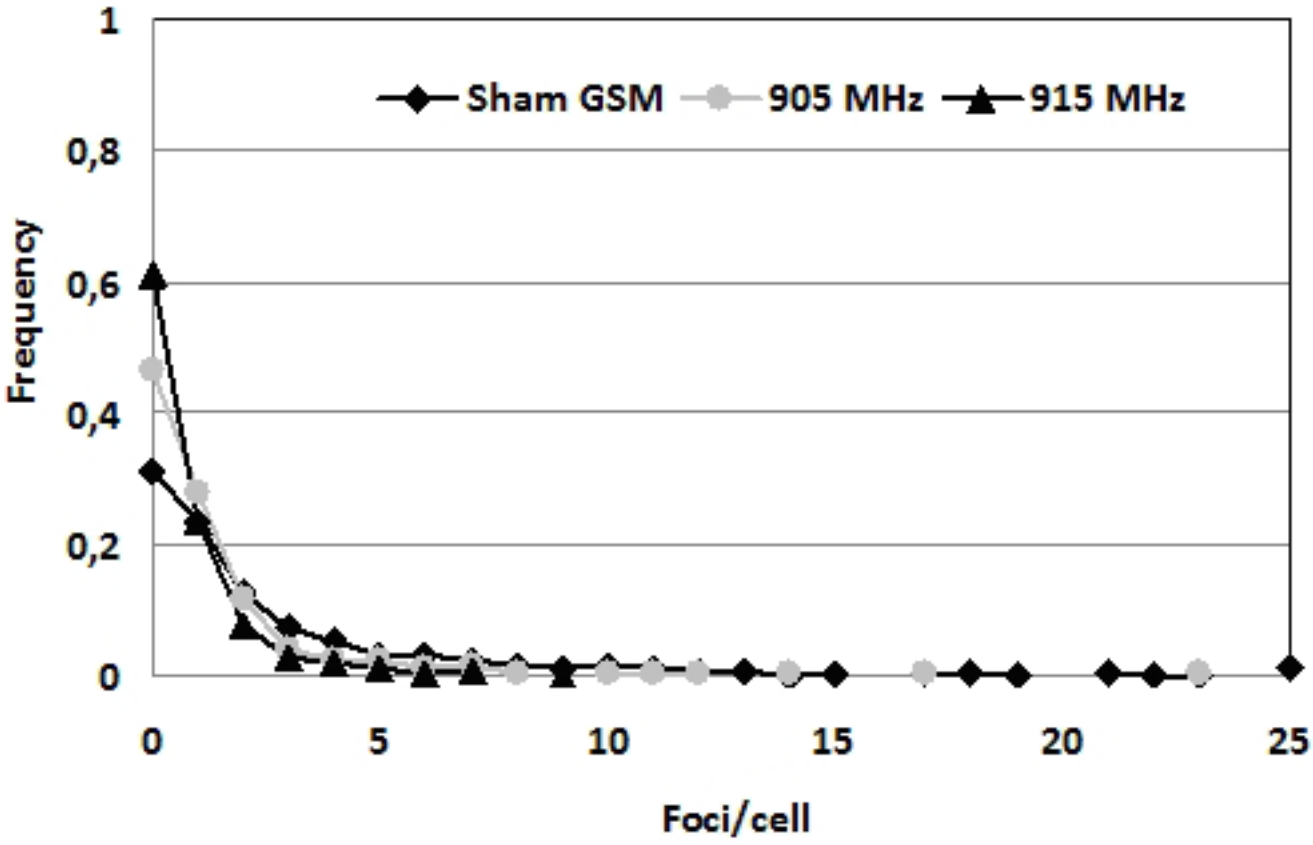
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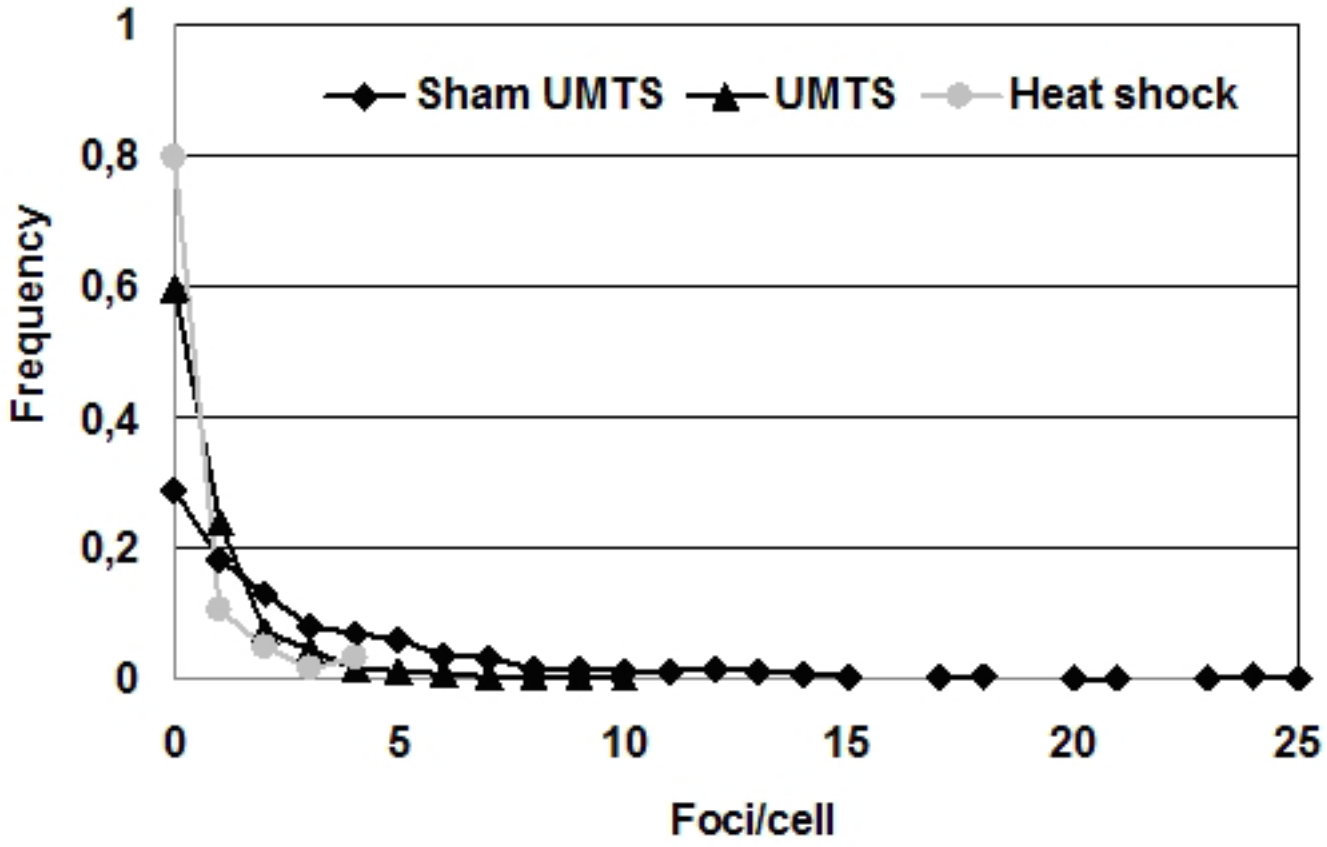
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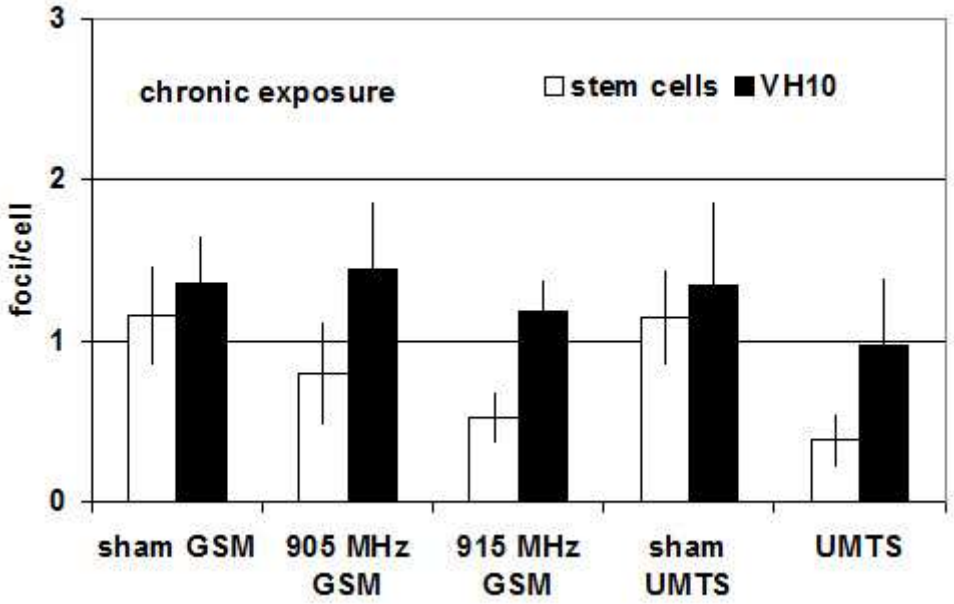

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