Role of physical and biological variables in bioeffects of non-thermal microwaves

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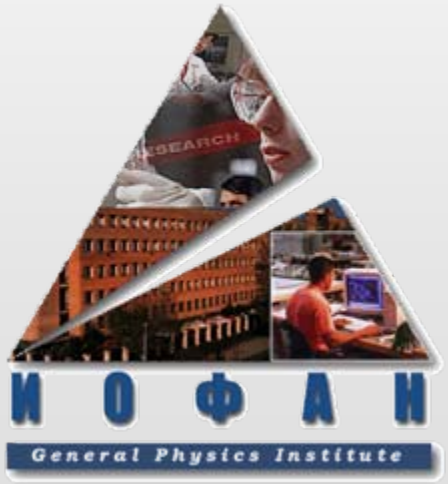

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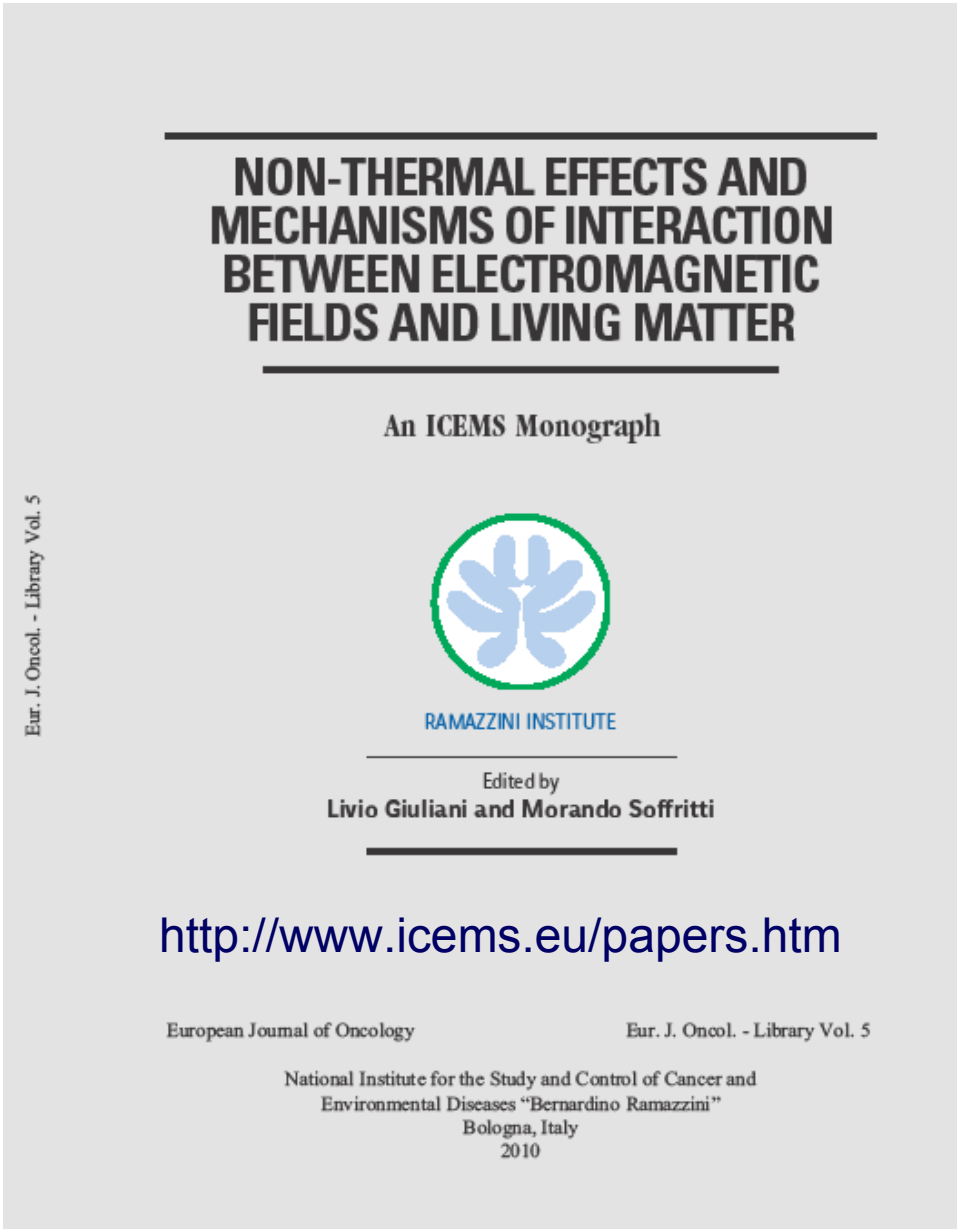
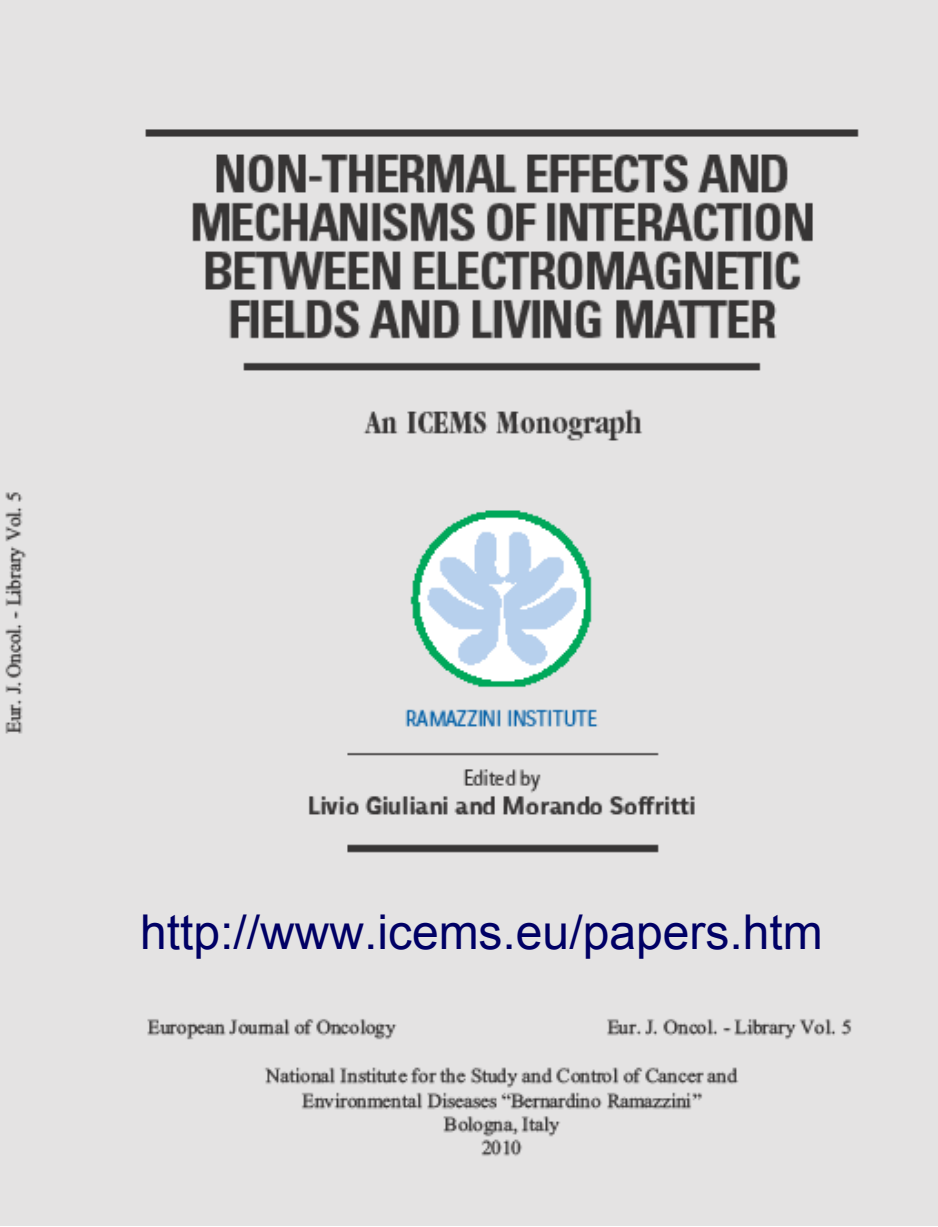
The is evidence for complex dependence of the effects of non-thermal (NT) microwaves (MW) on various physical and biological parameters, which are of key importance for the appearance NT MW effects and, therefore, should be controlled in replication studies. Dependencies on carrier frequency, modulation, polarization, genotype, physiological traits, presence of radical scavengers and antioxidants, have been consistently reported.



The emerging data suggest dependencies of the NT MW effects on, intermittence and coherence time of exposure, static magnetic field and ELF stray fields at the place of exposure, sex, age, individual traits, and cell density during exposure.

All these parameters should be taken into account when comparing results from different studies



Markova, E., L. O. G. Malmgren, I.Belyaev (2010). Environmental Health Perspectives **118**(3): 394-399.

Research

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

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BACKGROUND: It is widely accepted that DNA double-strand breaks (DSBs) and their misrepair in stem cells are critical events in the multistage origination of various leukemias and tumors, including gliomas.

OBJECTIVES: We studied whether microwaves from mobile telephones of the Global System for Mobile Communication (GSM) and the Universal Global Telecommunications System (UMTS) induce DSBs or affect DSB repair in stem cells.

METHODS: We analyzed tumor suppressor TP53 binding protein 1 (53BP1) foci that are typically formed at the sites of DSB location (referred to as DNA repair foci) by laser confocal microscopy.

RESULTS: Microwaves from mobile phones inhibited formation of 53BP1 foci in human primary fibroblasts and mesenchymal stem cells. These data parallel our previous findings for human lymphocytes. Importantly, the same GSM carrier frequency (915 MHz) and UMTS frequency band (1947.4 MHz) were effective for all cell types. Exposure at 905 MHz did not inhibit 53BP1 foci in differentiated cells, either fibroblasts or lymphocytes, whereas some effects were seen in stem cells at 905 MHz. Contrary to fibroblasts, stem cells did not adapt to chronic exposure during 2 weeks.

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

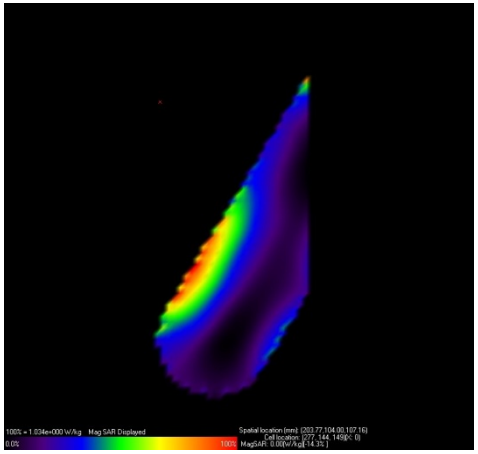
KEY WORDS: 53BP1 foci, DNA double-strand breaks, microwaves, mobile phones, stem cells. *Environ Health Perspect* 118:394–399 (2010). doi:10.1289/ehp.0900781 available via *http://dx.doi.org/* [Online 23 October 2009]

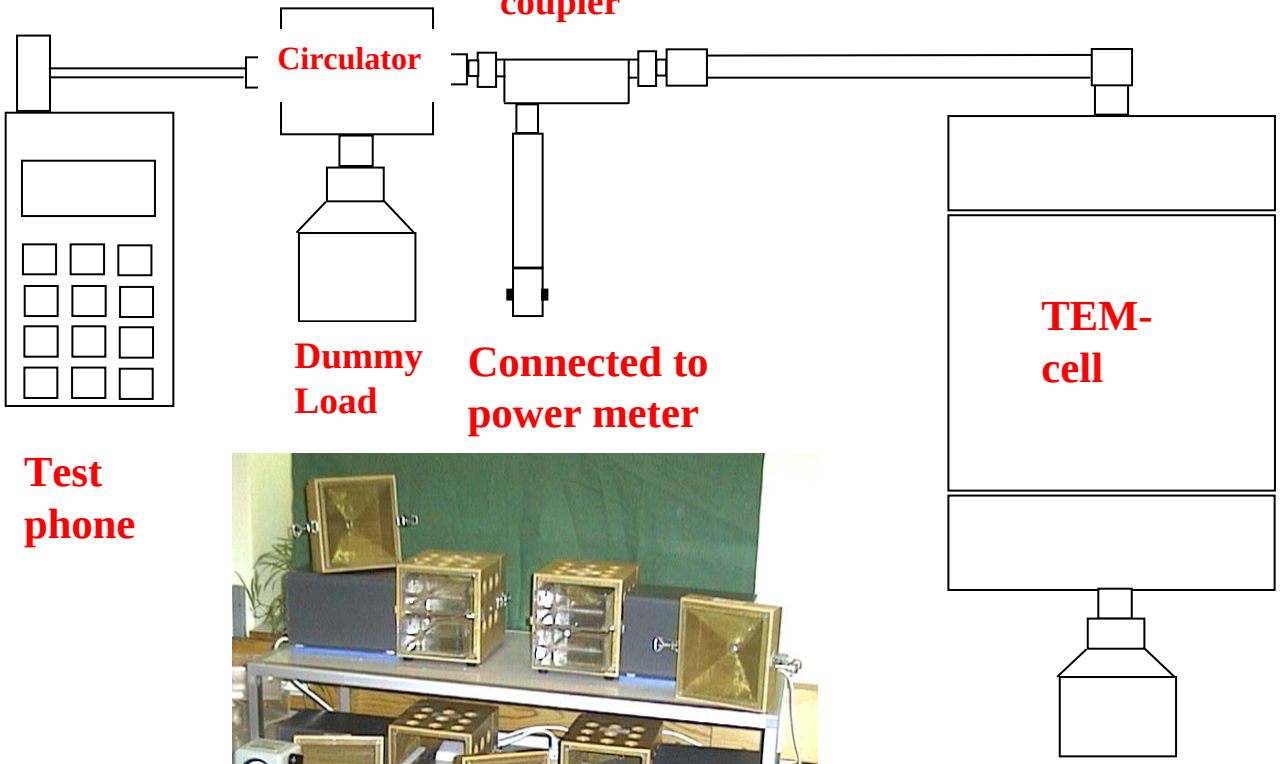
chromatin conformation, such as relaxation of DNA loops (Belyaev et al. 1999).

Several proteins involved in DSB repair, such as phosphorylated histone 2A family member X (γ-H2AX) and tumor suppressor TP53 binding protein 1 (53BP1), have been shown to produce discrete foci that colocalize to DSBs, referred to as DNA repair foci (Kao et al. 2003; Sedelnikova et al. 2002). Analysis of DNA repair foci is currently accepted as the most sensitive and specific technique for measuring DSBs 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 DSBs induced by a very low dose of ionizing radiation, 1 cGy, which results in only 0.4 DSB/cell on average (Markovà et al. 2007). We have also used this technique to analyze 53BP1/γ-H2AX foci in human lymphocytes exposed to MWs from Global

MICROWAVE EXPOSURE OF PRIMARY HUMAN CELLS

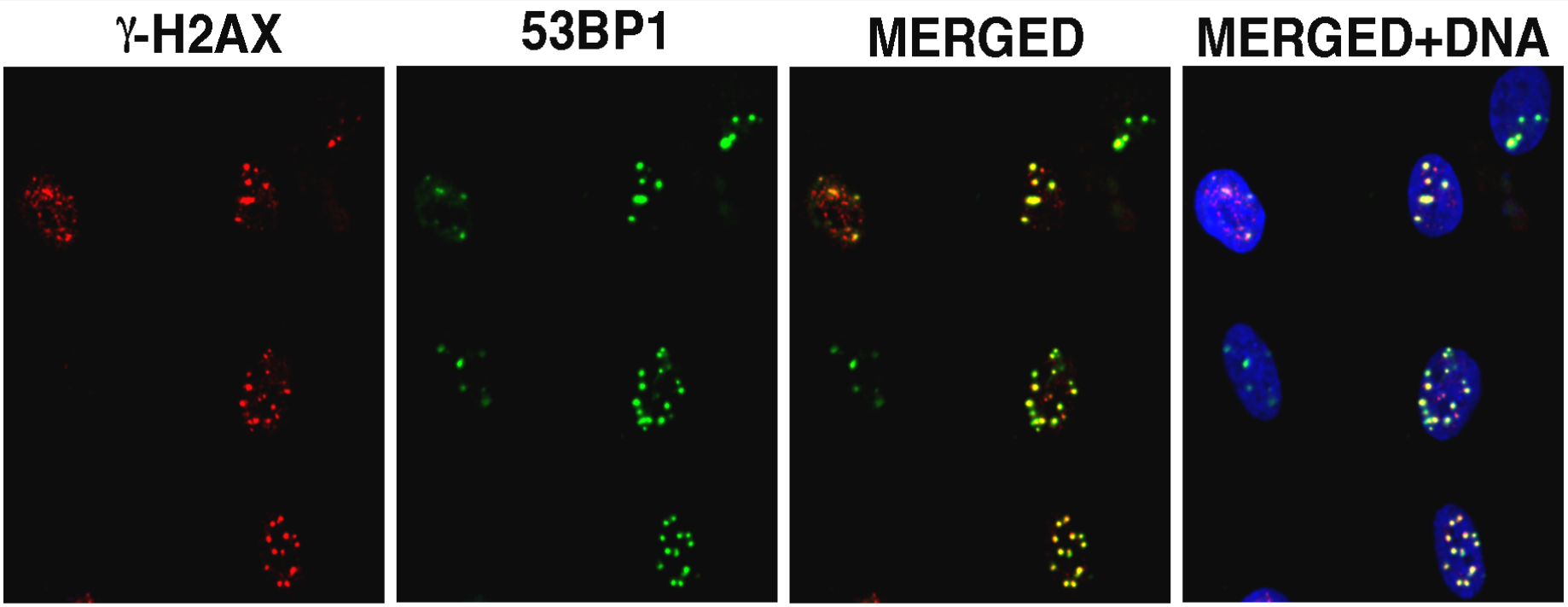
The test-mobile phone is programmed to choose the GSM frequency or UMTS band, and 0.25 W output power.





Distribution of specific absorption rate (FDTD-METHOD)

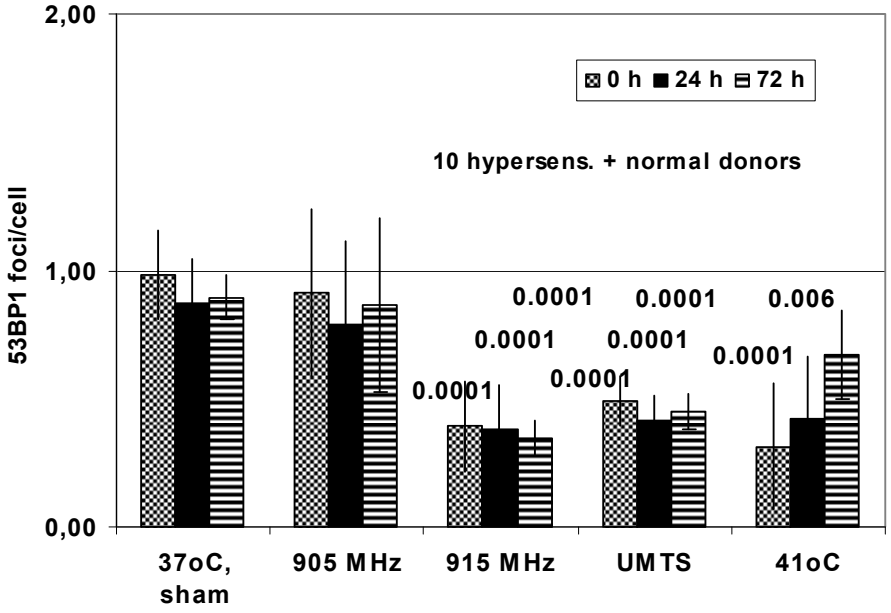
H2AX is phosphorylated (γ-H2AX) through decondensed DNA-domain, 2 Mb, containing DSB. Many other proteins including tumor suppressor p53 binding protein 1 (53BP1) re-localize at the DSB-containing domain forming discrete foci that are referred to as DNA repair foci and can be visualized by immunofluorescence

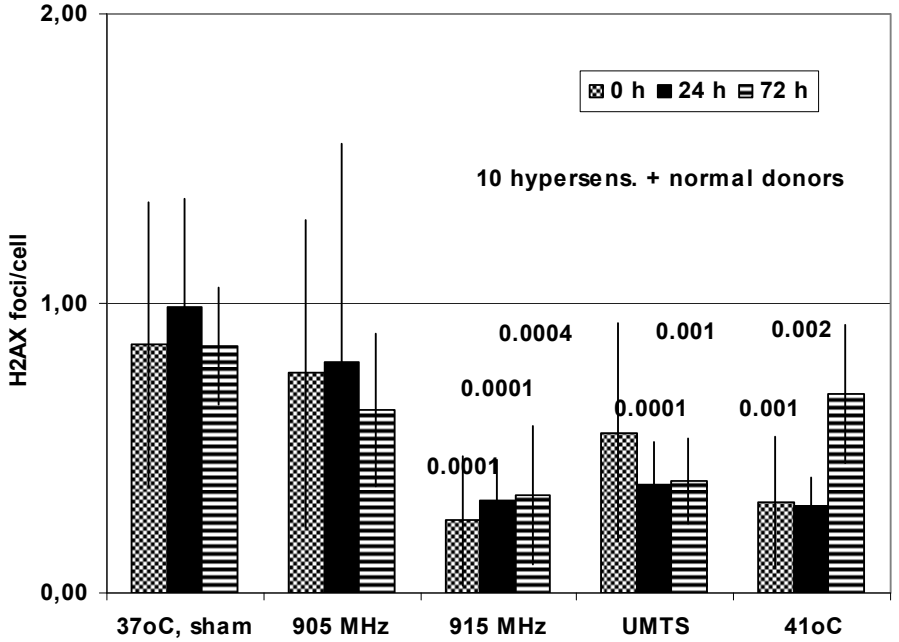


VH-10 cells, 12 h following irradiation with 3 Gy

E. Markova, N. Schultz, and I. Y. Belyaev, *Int J Radiat Biol,* vol. 83, pp. 319-329, May 2007.

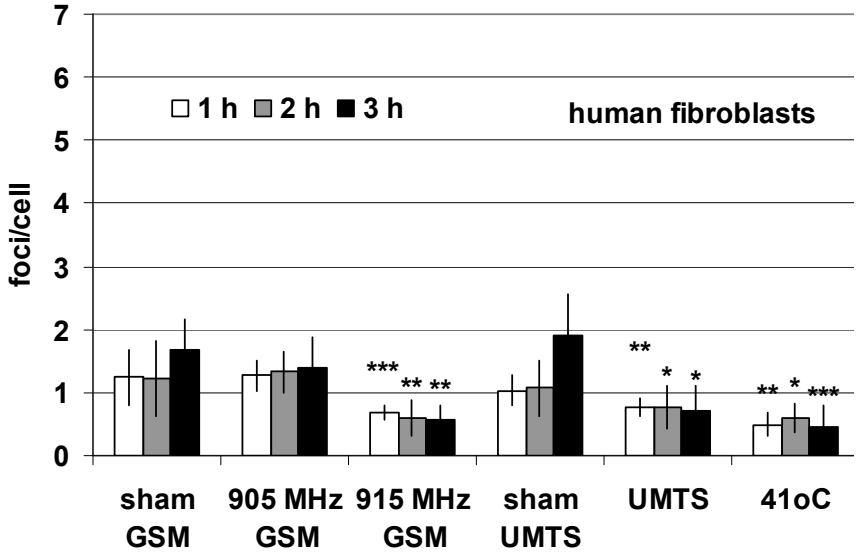
Role of frequency: Inhibitory effects of 915 MHz GSM and 1947 MHz UMTS on DNA repair foci remain 72 h after exposure of human lymphocytes to MW. No effects at 905 MHz

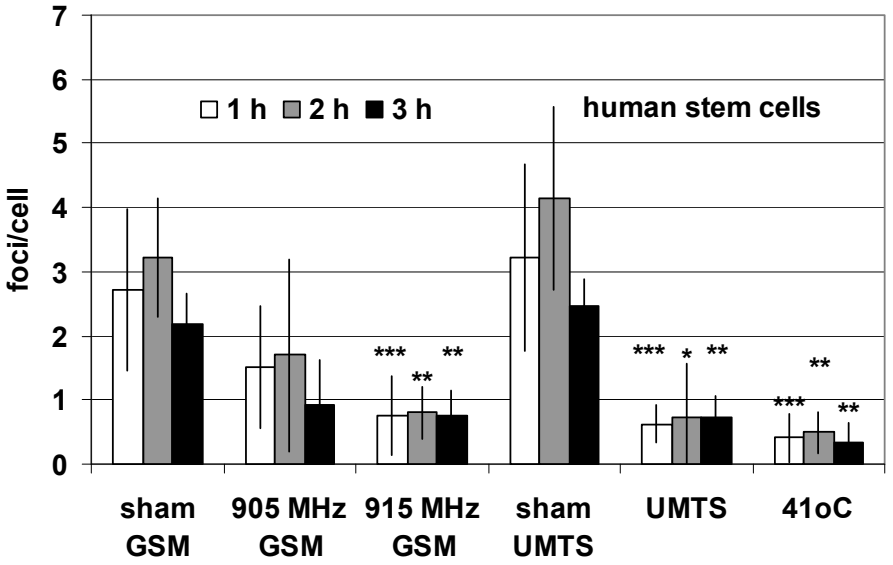




Both molecular markers, γ-H2AX и 53BP1, show the same results

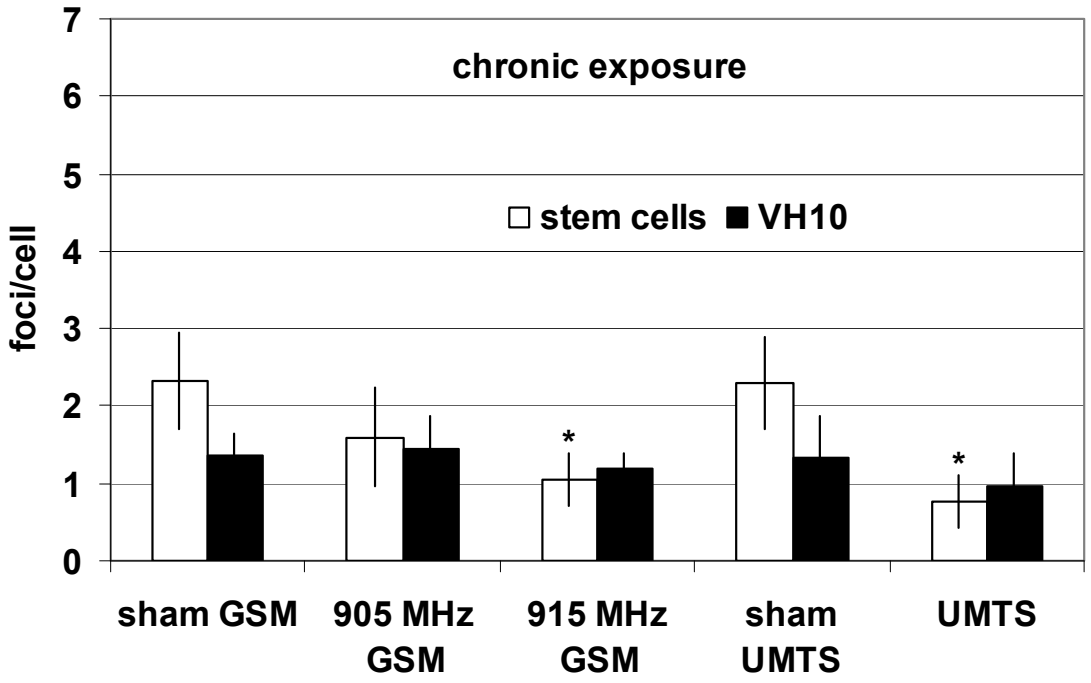
Role of cell type: MW exposure inhibited DNA repair foci in human fibroblasts and mesenchymal stem cells from adults. Stem cells were most sensitive to exposure.



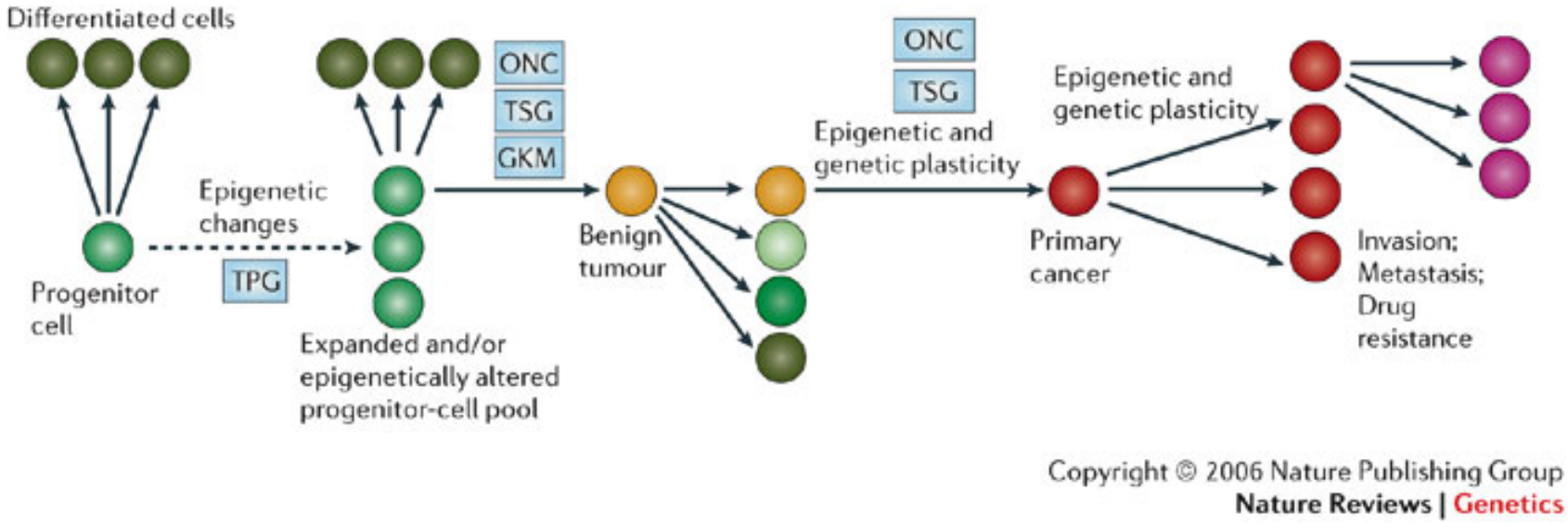


Role of cell type: Contrary to fibroblasts, human mesenchymal stem cells from adults did not adapt to effects of MW

Exposure during 2 weeks, 1 hour/daily

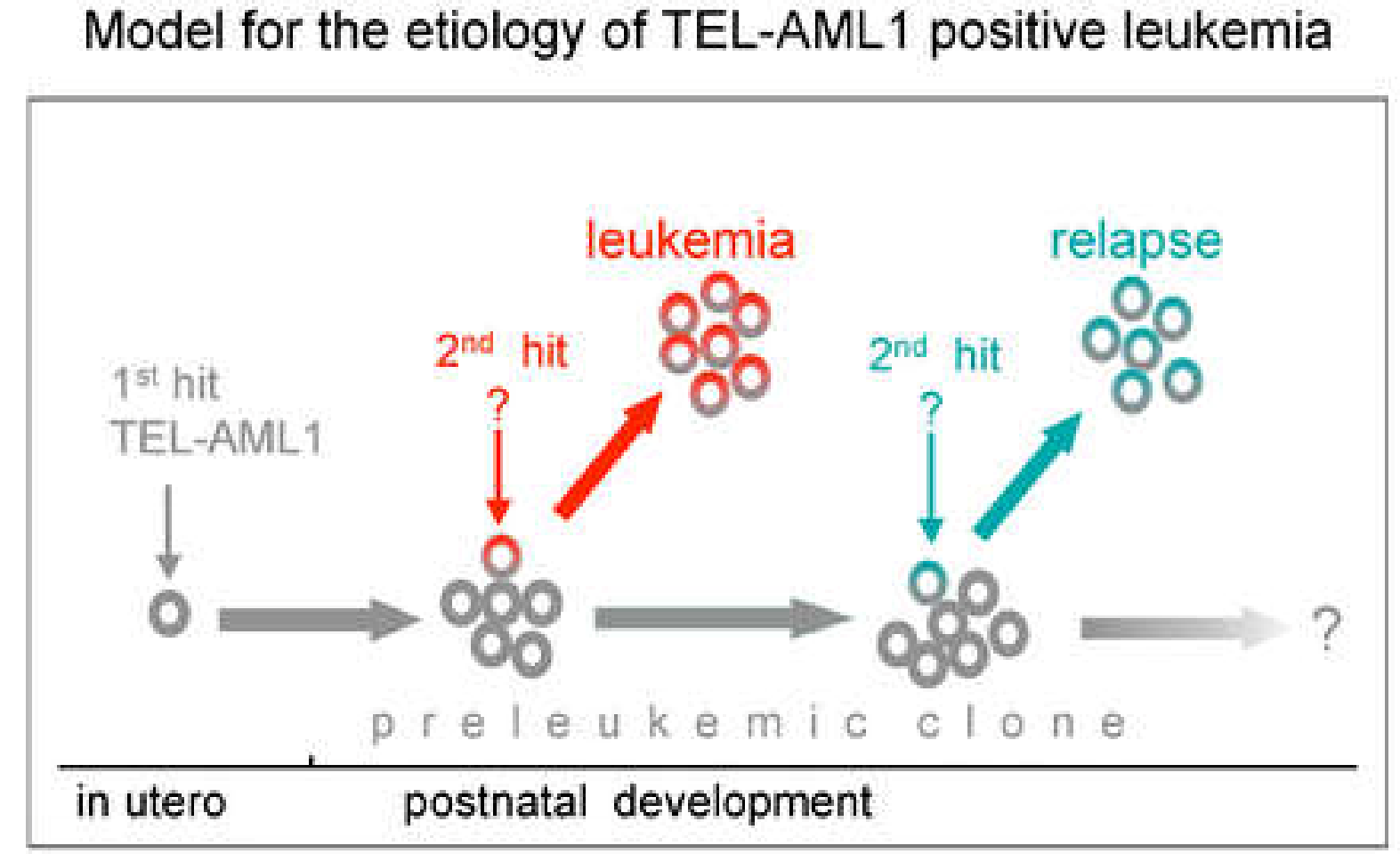


Results with stem cells may be especially important because different cancer types (tumors and leukemia) often originate from stem cells by well-known genetic and recently suggested epigenetic changes



Feinberg AP *et al.* (2005) The epigenetic progenitor origin of human cancer
Nat Rev gene. **7:** 21–33 doi:10.1038/nri1748

Leukemia is arising from the transformation of a single cell, which is usually a pluripotent hematopoietic stem/progenitor cells (HSC) or a more mature progenitor cell



(Greaves et al.,2003)

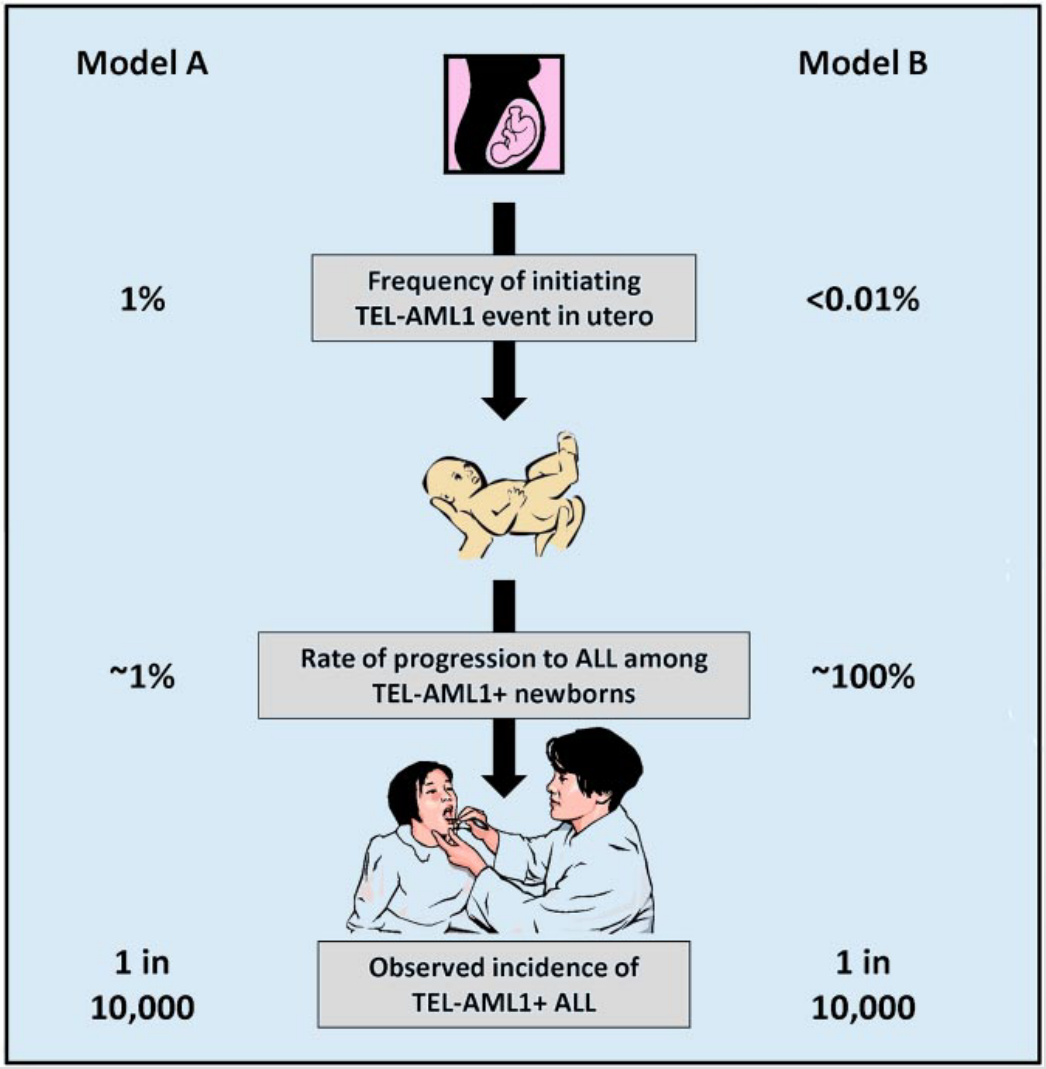
1st hit: Most frequent leukemic gene fusions (characteristic leukemic chromosomal aberrations) which are hallmark of many leukemias

- Characteristic leukemic chromosomal aberrations result in leukemic gene fusions
- characteristic leukemic translocations (TEL-AML1) have been found in umbilical cord blood of newborns

Chromosomal aberration	Gene fusion	[bp]	incidence of childhood leukemia
t(12;21)	TEL – AML1	298 (259)	25% ALL
t(1;19)	EA2 – PBX	373 (400)	3-5% ALL
t(9;22)	BCR – ABL p190	521 (347)	2-5% ALL
t(4;11)	MLL – AF4	184-673	5% ALL
t(8;21)	AML1 – ETO	395	8% AML
t(15;17)	PML – RARA	381 (345)	3-9% AML
inv(16)	CBFβ – MYH11	242-545	8-9% AML

Competing models of TEL-AML1 leukemogenesis
(Brown 2011)

Two studies provide direct evidence in support of Model A, Mori et al in which 567 umbilical cord blood samples from babies in the United Kingdom and Italy were screened and 6 were found to contain TEL-AML1 transcripts, with estimated TEL-AML1 cell frequencies of 10^{-3}. These data were confirmed by the group from Czech Republic (Zuna et al, 2011), that of the cord blood tested, 5 of 253 (2%) bore the *TEL/AML1* fusion. *Data from identical twin studies provide indirect evidence in support of Model A. Recent data by Lausten-Thomsen et al , 2011, suggests an alternative Model B. In this model, the initiating event (TEL-AML1 fusion) is as rare as the disease itself, implying that a high proportion (perhaps 100%) of babies born with a detectable TEL-AML1 fusion are destined to develop TEL-AML1+ ALL.*



Our data, validated by the NIC certified laboratory, show that incidence of infants with gene fusions depend on sensitivity of assay

# UCB	TEL-AML1 positivity CRI	NCI	MLL-AF4 positivity CRI	NCI	BCR-ABL p190 posit. CRI	NCI	Nested PCR
$P41_p$	**1/6**	**0/3**	**0/2**	**0/3**	**1/4**	**1/3**	**$1/3_{p190}$**
$P52_p$	**0/3**	**0/3**	**0/2**	**1/3**	**1/5**	**1/3**	**0/1**
$P68_T$		**1/3**	**1/2**	**0/3**	**0/2**	**0/3**	**0/1**
$P139_p$	**1/3**	**0/3**	**0/2**	**1/3**	**1/2**	**0/3**	**0/1**
$P140_p$	**3/4**	**0/3**	**0/2**	**0/3**	**5/5**	**0/3**	**0/1**
$P141_p$	**0/3**	**0/3**	**0/2**	**0/3**	**2/2**	**1/3**	**0/1**
$P144_p$	**3/3**		**0/2**		**2/2**		**$1/1_{p190}$**
$P145_p$	**0/3**	**1/3**	**1/2**	**0/3**	**2/5**	**1/3**	**0/1**
$P206_p$		**0/3**	**0/2**	**0/3**	**0/2**	**1/3**	**0/1**
Σ	**17/100**		**4/173**		**33/173**		

According to multiplex PCR, all samples were negative for the examined gene fusions at the sensitivity level of 20-100 copies/10^5 cells. Using RT-QPCR and nested PCR with sensitivity of 1-3 copies/10^5 cells we found 4 probands from 200 with BCR-ABL p190 which were confirmed in the certified laboratory at NCI. Based on the obtained data, we conclude that the incidence of infant cases with amount of leukemic gene fusions 1-3 copies/10^5 cells may be about 2%.

Does NT MW induce leukemic gene fusions in infant hematopoietic cells?

GSM exposure did not induce DSB under most tested conditions.
Significant increase in RNA expression was observed in infant mononuclear cells after GSM exposures at 4 mW/kg and 40 mW/kg.
EA2-PBX and AML-ETO leukemic gene fusions were found by RQ-PCR after exposure at 40 mW/kg in preliminary experiments.
Please, see poster 5 presented by Alexandra Somsedikova
Work in progress

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