Static magnetic fields modulate X-ray-induced DNA damage in human glioblastoma primary cells

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Although static magnetic fields (SMFs) are used extensively in the occupational and medical fields, few comprehensive studies have investigated their possible genotoxic effect and the findings are controversial. With the advent of magnetic resonance imaging-guided radiation therapy, the potential effects of SMFs on ionizing radiation (IR) have become increasingly important. In this study we focused on the genotoxic effect of 80 mT SMFs, both alone and in combination with (i.e. preceding or following) X-ray (XR) irradiation, on primary glioblastoma cells in culture. The cells were exposed to: (i) SMFs alone; (ii) XRIs alone; (iii) XR, with SMFs applied during recovery; (iv) SMFs both before and after XR irradiation. XR-induced DNA damage was analyzed by Single Cell Gel Electrophoresis assay (comet assay) using statistical tools designed to assess the tail DNA (TD) and tail length (TL) as indicators of DNA fragmentation. Mitochondrial membrane potential, known to be affected by IR, was assessed using the JC-1 mitochondrial probe. Our results showed that exposure of cells to 5 Gy of XR irradiation alone led to extensive DNA damage, which was significantly reduced by post-irradiation exposure to SMFs. The XR-induced loss of mitochondrial membrane potential was to a large extent averted by exposure to SMFs. These data suggest that SMFs modulate DNA damage and/or damage repair, possibly through a mechanism that affects mitochondria.

Keywords: static magnetic field; ionizing radiation; comet assay; DNA fragmentation; mitochondrial membrane potential; glioblastoma

INTRODUCTION

Whether exposure to Static Magnetic Fields (SMFs) affects DNA integrity in human cells remains a matter of debate. Many studies suggest that SMFs have no effect on cell growth rate, with results showing that cell cycle distribution is not influenced even by extremely strong SMFs (up to a maximum of 7.05 T) [1]. By contrast, we (and others) show that SMFs do have structural and functional effects according to the cell types and to the intensity, duration and orientation of the field used [2–4]. The majority of evidence suggests that SMFs do not induce genotoxic effects [5, 6], however, some studies have reported a mutagenic effect for SMFs [7, 8]. SMFs have been shown to reduce constitutive DNA damage signaling, which is considered to indicate DNA damage induced by endogenous oxidants, as has been demonstrated by analyzing oxidative phosphorylation [9]. It is of paramount importance to establish whether SMFs do cause DNA damage and, if so, to determine what doses, duration of exposure and type of SMFs (homogeneous or inhomogeneous) induce such damage, the underlying mechanisms of action, and which end-points need to be evaluated.

It has been suggested that free radicals play a key role in the mechanisms underlying DNA damage [10]. Indeed, one
of the potential mechanisms through which SMFs may interact with the living organism is via electronic interactions, in which magnetic fields influence the kinetics of the reactions with radical pair intermediates [11]. It has also been postulated that the magnetic field can increase the concentration of free radicals in living cells [11, 12]. Free radicals have, in turn, been shown to alter the mitochondrial membrane potential (ΔΨm) and to induce genetic damage [13, 14]. At the cellular level, lipids, proteins, carbohydrates and nucleic acids may be damaged by reactions with free radicals [15, 16]. These damaging reactions give rise to functional and morphological disturbances in the cell, due to oxidative/nitrosative/carboxilative stress, which lead to reversible or irreversible DNA damage [11]. The mechanisms underlying ionizing radiation (IR)-induced DNA damage, unlike those associated with exposure to SMFs, have been thoroughly investigated in recent decades. The effects induced by IR, such as XRs, on biological tissues, have been shown to be mediated by free radicals. In recent years, IR has been used as a primary tool for studying oxidative stress responses in various organisms ranging from prokaryotes to higher eukaryotes. Molecular insights into how reactive oxygen species (ROS) generated by radiation can damage DNA have served as a basis for investigating the nature of mutagenesis, disease, aging, and innumerable biological processes ending in cell death [17, 18]. Mitochondria are assumed to be responsible for ROS production [19]. Indeed, IR has been shown to upregulate the mitochondrial electron transport chain function [20]. ROS attack DNA readily, generating a variety of DNA lesions, such as oxidized bases and strand breaks [20–22]. It has also been reported that magnetic fields interfere with the genotoxic activity of physical and chemical agents [23, 24]. However, there is a general lack of data regarding the effect of co-exposure to SMFs and XRs. The aim of this study was to investigate the genotoxicity of exposure to SMFs alone, as well as the genotoxicity of exposure to SMFs prior to and/or following XRs in a very sensitive primary line of human glioblastoma cells obtained by surgical biopsy, which in a previous study have been demonstrated to be highly responsive to SMFs [2, 24].

**MATERIALS AND METHODS**

**Cell culture**

Cells were taken from a patient with Stage III multiform glioblastoma undergoing a brain tumor resection for therapeutic purposes. These cells were selected from one particular patient out of four under study, since they showed the highest sensitivity to chemical and physical agents [24]. The secondary use of the human specimens was approved by the hospital ethical board, and the patients’ data were adequately anonymized. Primary cultures were grown in RPMI medium supplemented with 5% fetal calf serum and used in their early passages. Cells in exponential growth, in triplicate Petri dishes for each time-point under investigation, were exposed to SMFs and/or XRs, as reported below and illustrated in the scheme in Table 1. In order to submit all the samples to the same environmental conditions (i.e. light and temperature), the control cells underwent sham procedures (e.g. transportation to the XR generator, with a delay of return to the incubator equal to XR irradiation time) and are referred to as ‘sham’ throughout the paper. In the case of simple exposure to SMF, sham-treated cells were submitted to the same environmental conditions as SMF-treated cells, except for the lack of exposure to SMFs.

**SMF exposure**

The exposure system has been previously described in detail [3] and is briefly described here. SMFs of known flux densities were produced by custom-made 4 cm × 4 cm Neodymium magnetic plaques. These magnetic plaques were placed underneath the Petri dish at a 1-mm distance from the cell monolayer in a custom-made device, which kept them suspended in the incubator at least 10 cm from any metallic surface (to avoid distortion of the field). Samples were also placed > 10 cm from each other (at which distance the magnetic field of the plaque magnets is undetectable). The position of the samples inside the incubator was randomly chosen. The fields were axial, with the magnetic North (the pole of the magnet which would be repulsed by the Earth’s North magnetic pole) vector crossing the cells.

The magnitude of the magnetic field of each plaque was 80 ± 5 mT (1 mT = 10 G) and was checked by means of a gaussmeter (Hall-effect Gaussmeter, GM04 Hirst Magnetic Instruments, UK). The gaussmeter was also used to measure the magnetic field inside the incubator (0.5 ± 0.02 mT), which must be considered as background for all the cell cultures used in this study, including sham cells, and added to the 80 mT magnetic field for the exposed cells. Magnets, in contrast with other exposure devices, do not produce temperature variation. For schematic protocols of treatments and sampling times see Table 1.

For the comet assay, experiments were repeated twice on each of three independent culture dishes for each experimental

| Treatment         | Sampling time (h) |
|-------------------|-------------------|
| SHAM              | 3                 |
| XR                | 6                 |
| SMF               | 12                |
| SMF + XR          | 20                |
| SMF + XR + SMF    | 24                |

° = comet assay, # = JC-1.
point. For JC-1 analysis, experiments were repeated twice with a single measure within each experiment for each time-point.

XR irradiation
Cells in triplicate Petri dishes for each time-point, were irradiated with 5 Gy XR generated by a CHF 320 G generator (Giraldoni, Mandello del Lario, LC, Italy) equipped with a Cu filter of 0.5 mm, operating at 250 keV, 15 mA, delivering a dose rate of 1.1 Gy/min. Cells underwent 6 or 20 h of recovery in the incubator before the comet assay. For ΔΨm investigation, cells were analyzed after 3, 6 and 20 h of recovery (Table 1).

XR irradiation and SMF
Cell cultures were exposed to 5 Gy XRs, and during their recovery continuously exposed to SMF for 6 or 20 h. In addition, an experimental group was exposed to 6 h of SMF before XR irradiation, followed by recovery under SMF for 6 and 20 h (Table 1).

Comet assay
The alkaline comet assay was performed on viable cells as previously described [25]. Dead and apoptotic cells were removed during rinsing. Trypan blue staining demonstrated that adherent cells contained no more than 2% and 4% of dead cells in sham cells and 5-Gy-irradiated cells, respectively. It has been reported that detaching cells with trypsin may increase the cells’ ROS production [26]; however, scraping is considered worse; thus we used trypsin, so as to minimize cellular stress. Cells were thoroughly rinsed three times with 37°C, Ca- and Mg-free, sterile PBS, then incubated at 37°C with 1 ml 0.25% trypsin/EDTA solution for ~4 min, checking during this period the numbers of detached cells. Trypsinization was stopped by adding complete medium, incubation continuously exposed to SMF for either 6 or 12 h. By contrast, a significant difference emerged between the two groups of cells in both the SMF (for either 6 or 12 h). In all cases, P-values < 0.05 were considered statistically significant. The statistical tests were performed using MATLAB software.

ΔΨm
Depolarization of ΔΨm was evaluated by the incorporation of 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethyl-benzimidazol-carbocyanin iodide (JC-1) (Molecular Probe, Invitrogen, Milan Italy). Cells, harvested as previously described, were stained at 3, 6 and 20 h of recovery after XR irradiation. Cells at the concentration of 106/ml in PBS were incubated with 3 µg/ml (final concentration) JC-1 for 15 min in the dark at 37°C, from a stock solution of JC-1 at 1mg/ml in DMSO. Then, cells were washed once in PBS. Green vs red fluorescence was determined with a PASIII PARTEC flow cytometer (Partec, Muenster, Germany). At least 20 000 cells were counted for each cytogram. Estimation of emitted fluorescence was performed on forward/side scatter-gated cytograms.

RESULTS
SMF effects on glioblastoma cells
No significant difference was observed in either the TD or TL between sham-treated cells and cells exposed to 80 mT SMF (for either 6 or 12 h). By contrast, a significant difference emerged between the two groups of cells in both the TD and TL at 24 h exposure, as shown in Fig. 1. It is noteworthy that as the culture times grew longer, the sham cells also exhibited a significant increase in the comet parameters,
indicating that a certain amount of DNA fragmentation may have occurred as a result of active metabolic endogenous activity in these cells.

**XR and SMF interactions on glioblastoma cells**

As expected, the exposure of cells to 5 Gy XRs induced a high degree of DNA fragmentation, as demonstrated by the consistent increase in the TD and TL, at both 6 h and 20 h following exposure (Figs 2 and 3), though it should be borne in mind that the standard deviation was quite high (see Discussion). The results for SMFs applied after XR irradiation are also shown in Figs 2 and 3. They reveal a dramatic reduction in DNA fragmentation when recovery was performed under SMF exposure ($P < 0.01$). No major change was observed when 6 h SMF pretreatment was performed before XR irradiation.

**ΔΨm**

In order to assess the ΔΨm, known to be affected by XR irradiation, we investigated the green vs red fluorescence JC-1
staining using flow cytometry. The results demonstrated a consistent shift of the red fluorescence according to treatment. A representative histogram with the cumulative results of the four groups analyzed after 6 h of recovery is shown in Fig. 4. As expected, and as emerges in the figure, a dramatic decrease in 590 nm fluorescence, indicating $\Delta \Psi_m$, is observed in the XR samples (green line) when compared with the sham samples (blue line). A less marked shift in 590 nm fluorescence was observed in the SMF-treated cells.

What is very interesting is that the samples exposed to SMFs after XR irradiation unexpectedly displayed a significant recovery in the amount of 590 nm fluorescence, thereby pointing to rescue from $\Delta \Psi_m$, which is likely due to the presence of SMF during recovery.

The time-course of $\Delta \Psi_m$ during recovery is illustrated in Fig. 5. These results highlight the ratios between the 590 nm fluorescence-positive cells and the 590 nm fluorescence-negative cells (i.e. RN2 vs RN1 of Fig. 5) analyzed after 3, 6

![Graph showing % tail DNA and 90% tail length](image-url)

Fig. 2. Comet assay results of cells exposed to XR irradiation that underwent 6 h of recovery in the absence and in the presence of SMFs. TD and TL are shown in the upper and lower panels, respectively. The results of triplicate samples of two independent experiments are reported together with the SD. Five groups are reported in the figure. These groups are (i) sham: cells not exposed to any treatment but submitted to the same environmental conditions; (ii) XR: cells exposed to XR irradiation only; (iii) SMF: cells exposed to SMFs only; (iv) XR+SMF: cells exposed to SMFs during 6 h of recovery following XR irradiation; (v) SMF+XR+SMF: cells exposed to SMFs prior to XR irradiation and during 6 h of recovery. Recovery under SMFs significantly (asterisk) reduced the TD and TL ($P < 0.001$) compared with XR irradiation alone.
and 20 h of recovery following XR irradiation. The two-way Anova test demonstrated an effect of both time \((P < 0.001)\) and treatment \((P = 0.004)\), as well as an interaction effect (of time and treatment) \((P = 0.002)\). Using Scheffé’s multiple comparisons test, a significantly lower \(\Delta \Psi_m\) was observed at 3 h in the XR and XR + SMF groups, than in the sham cells. However, the presence of SMFs for 3 h during recovery yielded a significantly higher \(\Delta \Psi_m\) compared with XR irradiation alone, thereby demonstrating the ability of SMFs to modulate the \(\Delta \Psi_m\). A significant difference was observed in all the groups at 6 h, whereas at 20 h the \(\Delta \Psi_m\) was restored and no statistical significance was observed between the treatments. It is noteworthy, however, that both XR and XR + SMF groups displayed a trend toward an increased \(\Delta \Psi_m\) when compared with sham-treated cells.

**DISCUSSION**

Over the past decade, the possible biological effects (including genotoxicity) of SMFs have been investigated in a wide
Very surprisingly, SMFs modulated the XR response, reducing the degree of DNA fragmentation. It should be borne in mind that our findings were characterized by a high standard deviation, which may be ascribable to several factors: the intrinsic heterogeneity of the primary culture cells, reflecting the in vivo situation, might not contain a homogeneous cell genotype; differences in cell cycle phase sensitivity may also account for a range in the response. The difference that emerged from our experiments was, however, highly significant, despite dispersion of the data.

A recent study has also revealed a ‘beneficial’ effect of an homogeneous SMF on the repair of radiation-damaged DNA in leukocytes, though only after 4 h of recovery [38]. What explanation may be provided for our results showing that SMFs attenuate the level of DNA fragmentation and reduce the two comet parameters? It should be noted that SMFs prolongs the lifespan of reactive oxidants through the ‘radical pair mechanism’, which facilitates the interconversion of the singlet to the triplet excitation state (intersystem crossing) and would be expected to enhance rather than reduce DNA damage [10]. Yet another mechanism that might explain our findings is the interference by SMFs in the electron transport chain in mitochondria; cell respiration, which is the process that generates reactive oxidants, may be reduced by this interference. Surprisingly little information is available in the literature on the effect of SMFs on this process. If the process of electron transport, and consequently of oxidative phosphorylation, is perturbed by the SMF, oxidative DNA damage would be reduced. Our results demonstrate that a decreased mitochondrial depolarization occurs after XR irradiation in the presence of an SMF during recovery. Our previous observations on the same cells [24] demonstrated that the SMF rescues cells from apoptosis after they have been exposed to chemical agents. This result is in accordance with the attenuation of the loss of the ΔΨm induced by SMF exposure during XR irradiation recovery reported here. The present data shed light on the mechanism of interaction during combined treatment with IR and non-IR by pointing to a possible key role for mitochondria. The attenuation of the loss of the ΔΨm following exposure to an SMF as observed by us may impair the release of ROS from the mitochondrial interior, their direct transfer to nuclei, and also the induction of secondary radicals, thereby preventing oxidative DNA damage.

The fact that XR-induced DNA damage is still marked after 20 h of recovery, while the ΔΨm is completely restored is, despite not being the primary aim of this study, of considerable interest. A possible explanation for the persistent DNA damage might be the occurrence of the second wave of ROS, which occurs after XR irradiation [20, 39, 40]. Indeed, whereas IR instantaneously induces the formation of water...
radiolysis products that contain some ROS, the latter are also believed to be released secondarily by biological sources in irradiated cells. Mitochondria are assumed to be responsible for this secondarily IR-induced ROS production [20], though they may not be the only source. The type of ROS produced following radiation might vary depending on the time of recovery. In this regard, following XR irradiation, the results we obtained with 2′-7′ dichlorofluorescin, which labels hydrogen peroxide, differed from those we obtained with dihydroethidium, which labels superoxide anions (data not shown), thus pointing to the existence of a highly complex network involving ROS, DNA damage-triggering, and the recovery time-scale. The uncoupled results we observed between DNA repair and ΔΨm 20 h after XR irradiation may also be due to the fact that DNA repair and recovery of ΔΨm follow different kinetic patterns.

The role of SMFs in protecting mitochondria, and consequently of modulating the genotoxic effects of IR, is clearly of considerable importance and warrants further investigation. The results of the present study represent a proof of principle that SMFs can interfere with IR and raise the very important question of whether the reduced XR-induced DNA damage caused by SMFs results in a reduced effectiveness of radiotherapy when guided by MRI. The magnetic field used in this study is much weaker than that used in MRI (which are in the order of magnitude of milliTesla and Tesla, respectively). However, remarkable biological effects have been shown for both weak and strong SMFs [24, 41, 42]. Notwithstanding, there is lack of exhaustive studies showing the intensity-dependence of these phenomena in the very same experimental model, thus making it hard to extrapolate conclusions from weak to strong SMFs and vice versa. Since our study was limited to low intensity SMFs, the issue of comparing weak SMFs with strong SMFs, such as that used in MRI, warrants further investigation. Nonetheless, since the combined use of SMFs and IR is usually related to diagnostic applications, based on animal studies the antagonistic effects of this combination on biological structures might benefit patient health [43].

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**REFERENCES**

1. Schiffer IB, Schreiber WG, Graf R et al. No influence of magnetic fields on cell cycle progression using conditions relevant for patients during MRI. *Bioelectromagnetics* 2003;24:241–50.
2. Teodori L, Albertini MC, Ugazzoni F et al. Static magnetic fields affect cell size, shape, orientation, and membrane surface of human glioblastoma cells, as demonstrated by electron, optic, and atomic force microscopy. Cytometry A 2006;69:75–85.

3. Coletti D, Teodori L, Albertini MC et al. Static magnetic fields enhance skeletal muscle differentiation in vitro by improving myoblast alignment. Cytometry A 2007;71:846–56.

4. Tenuzzo B, Chionna A, Panzarini E et al. Biological effects of 6 mT static magnetic fields: a comparative study in different cell types. Bioelectromagnetics 2006;27:560–77.

5. Chekhun VF, Lozovskaya YV, Lukyanova NY et al. Assessment of the cyto- and genotoxic effects of a nanoferrimagnetic and a static magnetic field in vivo. Cytol Genet 2013;47:179–87.

6. McCann J, Dietrich F, Rafferty C et al. A critical review of the genotoxic potential of electric and magnetic fields. Mutat Res 1993;297:61–95.

7. Potenza L, Cucchiarini L, Piatti E et al. Effects of high static magnetic field exposure on different DNAs. Bioelectromagnetics 2004;25:352–5.

8. Takao K, Ikehata M, Nakagawa M. Estimation of genetic effects of a static magnetic field by a somatic cell test using mutagen-sensitive mutants of Drosophila melanogaster. Bioelectrochemistry and Bioenergetics 1995;36:95–100.

9. Halicka HD, Darzynkiewicz Z, Teodori L. Attenuation of constitutive ATM activation and H2AX phosphorylation in human leukemic TK6 cells by their exposure to static magnetic field. Cell Cycle 2009;8:3238–40.

10. Okano H. Effects of static magnetic fields in biology: role of free radicals. Front Biosci 2008;13:6106–25.

11. Jaje J, Grzegorczyk J, Zmyslony M et al. Effect of 7 mT static magnetic field and iron ions on rat lymphocytes: apoptosis, necrosis and free radical processes. Bioelectrochemistry 2002;57:107–11.

12. Jouini FJ, Abdolmaleki P, Ghanati F. Oxidative stress in broad bean (Vicia faba L.) induced by static magnetic field under natural radioactivity. Mutat Res 2012;741:116–21.

13. Wang JQ, Chen Q, Wang X et al. Dysregulation of mitochondrial calcium signaling and superoxide flashes cause mitochondrial genomic DNA damage in Huntington disease. J Biol Chem 2013;288:3070–84.

14. Baker K, Staecker H. Low dose oxidative stress induces mitochondrial damage in hair cells. Anat Rec (Hoboken) 2012;295:1868–76.

15. Duan J, Kasper DL. Oxidative depolymerization of polysaccharides by reactive oxygen/nitrogen species. Glycobiochemistry 2011;21:401–9.

16. Brieger K, Schiavone S, Miller FJ, Jr et al. Reactive oxygen species: from health to disease. Swiss Med Wkly 2012;142: w13659.

17. Maynard S, Schurman SH, Harboe C et al. Base excision repair of oxidative DNA damage and association with cancer and aging. Carcinogenesis 2009;30:2–10.

18. Azzam EI, Jay-Gerin JP, Pain D. Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury. Cancer Lett 2012;327:48–60.

19. Kikusato M, Toyomizu M. Crucial role of membrane potential in heat stress-induced overproduction of reactive oxygen species in avian skeletal muscle mitochondria. PLOS ONE 2013;8:e64412.

20. Yamamori T, Yasui H, Yamazumi M et al. Ionizing radiation induces mitochondrial reactive oxygen species production accompanied by upregulation of mitochondrial electron transport chain function and mitochondrial content under control of the cell cycle checkpoint. Free Radic Biol Med 2012;53:260–70.

21. Sedelnikova OA, Redon CE, Dickey JS et al. Role of oxidatively induced DNA lesions in human pathogenesis. Mutat Res 2010;704:152–9.

22. Wang Y. Bulky DNA lesions induced by reactive oxygen species. Chem Res Toxicol 2008;21:276–81.

23. Amara S, Abdelmalek H, Garrel C et al. Influence of static magnetic field on cadmium toxicity: study of oxidative stress and DNA damage in rat tissues. J Trace Elem Med Biol 2006;20:263–9.

24. Teodori L, Goehde W, Valente MG et al. Static magnetic fields affect calcium fluxes and inhibit stress-induced apoptosis in human glioblastoma cells. Cytometry 2002;49:143–9.

25. Giovanetti A, Deshpande T, Basso E. Persistence of genetic damage in mice exposed to low dose of X rays. Int J Radiat Biol 2008;84:227–35.

26. Dinnen RD, Mao Y, Fine RL. The use of fluorescent probes in the study of reactive oxygen species in pancreatic cancer cells. Methods Mol Biol 2013;980:321–9.

27. Collins AR. The comet assay for DNA damage and repair: principles, applications, and limitations. Mol Biotechnol 2004;26:249–61.

28. Leszczynski D. Rapporteur report: cellular, animal and epidemiological studies of the effects of static magnetic fields relevant to human health. Prog Biophys Mol Biol 2005;87:247–53.

29. Repacholi MH, Grandolfo M, Jammet H et al. Guidelines on limits of exposure to static magnetic fields. International Commission on Non-Ionizing Radiation Protection. Health Phys 1994;66:100–6.

30. Vecchia P, Matthes R, Feychting M et al. Amendment to the ICNIRP “Statement on medical magnetic resonance (MR) procedures; protection of patients”. Health Phys 2009;97:259–61.

31. Nakahara T, Yaguchi H, Yoshida M et al. Effects of exposure of CHO-K1 cells to a 10-T static magnetic field. Radiology 2002;224:817–22.

32. Miyakoshi J. Effects of static magnetic fields at the cellular level. Prog Biophys Mol Biol 2005;87:213–23.

33. Sarvestani AS, Abdolmaleki P, Mowla SJ et al. Static magnetic fields aggravate the effects of ionizing radiation on cell cycle progression in bone marrow stem cells. Micron 2010;41:101–4.

34. Nguyen PL, Chen MH, D’Amico AV et al. Magnetic resonance image-guided salvage brachytherapy after radiation in select men who initially presented with favorable-risk prostate cancer: a prospective phase 2 study. Cancer 2007;110:1485–92.

35. Suzuki Y, Ikehata M, Nakamura K et al. Induction of micronuclei in mice exposed to static magnetic fields. Mutagenesis 2001;16:499–501.

36. Potenza L, U baldi L, De Sanctis R et al. Effects of a static magnetic field on cell growth and gene expression in Escherichia coli. Mutat Res 2004;561:53–62.
37. Lee CH, Chen HM, Yeh LK et al. Dosage-dependent induction of behavioral decline in *Caenorhabditis elegans* by long-term treatment of static magnetic fields. *J Radiat Res* 2012;53:24–32.

38. Kubinyi G, Zeitler Z, Thuroczy G et al. Effects of homogeneous and inhomogeneous static magnetic fields combined with gamma radiation on DNA and DNA repair. *Bioelectromagnetics* 2010;31:488–94.

39. Kaneyuki Y, Yoshino H, Kashiwakura I. Involvement of intracellular reactive oxygen species and mitochondria in the radiosensitivity of human hematopoietic stem cells. *J Radiat Res* 2012;53:145–50.

40. Yoshino H, Kiminarita T, Matsushita Y et al. Response of the Nrf2 protection system in human monocyctic cells after ionising irradiation. *Radiat Prot Dosimetry* 2012;152:104–8.

41. Teodori L, Grabarek J, Smolewski P et al. Exposure of cells to static magnetic field accelerates loss of integrity of plasma membrane during apoptosis. *Cytometry* 2002;49:113–8.

42. Sakurai T, Terashima S, Miyakoshi J. Effects of strong static magnetic fields used in magnetic resonance imaging on insulin-secreting cells. *Bioelectromagnetics* 2009;30:1–8.

43. Gu Y, Hasegawa T, Yamamoto Y et al. The combined effects of MRI and X-rays on ICR mouse embryos during organogenesis. *J Radiat Res* 2001;42:265–72.