

Attenuation of constitutive ATM activation and H2AX phosphorylation in human leukemic TK6 cells by their exposure to static magnetic field

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In live cells DNA is being continuously damaged by reactive oxygen species (ROS) generated during aerobic metabolism in mitochondria (reviewed in refs. 1–3). Environmental pollutants, macrophage oxidative burst, and even iatrogenic factors, also contribute to oxidative DNA damage. It has been estimated that ~5,000 DNA single-strand lesions (SSLs) are generated by endogenous oxidants and about 1% of them are converted to DNA double-strand breaks (DSBs) in cell nucleus during a single cell cycle.³ Because some DSBs are repaired by the non-homologous DNA-end joining (NHEJ), the mechanism that is error prone, a progressive accumulation of irreversible DNA damage takes place in live cells with every cell generation. This process of constitutive DNA damage is considered to be the main cause of aging and predisposition to cancer.³

The immunocytochemical detection of histone H2AX phosphorylation on Ser139 (γ H2AX) and activation of ATM through its phosphorylation on Ser1981 (ATM-Ser1981^P) with phospho-specific Abs was shown to provide highly sensitive means to detect and measure the extent of DNA damage in individual cells.^{4,5} The sensitivity of this approach combined with flow- or image assisted-cytometry was high enough to detect and measure the constitutive DNA damage in the untreated normal and tumor cells induced by endogenous metabolically generated oxidants and assess the protection of DNA provided by anti-oxidants and metabolic inhibitors.⁶⁻⁹

There is a large body of evidence implicating static magnetic field (SMF) as the

factor that can affect biological systems through modulation of ROS (reviewed in ref. 10). The way by which SMF affects ROS is by magnetic force interaction on the radicals possessing the unpaired electrons, termed “a radical pair mechanism”.¹⁰ This mechanism provides the means through which the magnetic field can influence the spin of electrons in free radicals thereby affecting their lifetime and the kinetic reactions in which the radicals are involved. Furthermore the electron transport chain in mitochondria represents flow of electrons through the space of mitochondrial intermembrane and potentially may be the target of SMF. Oxidative phosphorylation, the process generating ROS, therefore also can be influenced by SMF. Given the above we were interested whether SMF can affect the constitutive phosphorylation in H2AX and activation of ATM, the biomarkers of constitutive DNA damage.⁶⁻⁹

Exposure of human lymphoblastoid TK6 cells (wt p53) to moderate strength SMF (705 militesla, mT) led to a reduction in expression of both γ H2AX and ATM-Ser1981^P (Fig. 1). The attenuation of expression of the H2AX and ATM phosphorylation upon exposure to SMF was not cell cycle phase specific as the decrease was of comparable degree across all phases of the cycle. The reduction of intensity of γ H2AX in the SMF exposed cells compared to the unexposed ones was 19%, 20% and 24% for the G₁, S and G₂M phases of the cell cycle, respectively. The reduction in intensity of ATM-Ser1981^P IF was 22%, 23% and 21%, respectively. Similar results were reproduced in numerous experiments,

including when the incubators used to culture cells with the magnet and the incubators bearing the control cells were switched. In repeated experiments the differences in the reduction of the mean values of expression of γ H2AX and ATM-Ser1981^P IF in the SMF exposed cultures compared to the respective controls were within the range of the SD as shown in Figure 1B. Exposure of cells to 705 mT intensity SMF for 5 h led also to attenuation of expression of γ H2AX and ATM-Ser1981^P compared to the control. However, the degree of a reduction was lower compared with the 24 h exposure. Thus the reduction of γ H2AX IF was 11%, 11% and 15% for cells in G₁, S and G₂M phases of the cell cycle, respectively. The expression of ATM-Ser1981^P was reduced 12% 13% and 5% for the populations of G₁, S and G₂M cells.

We have also measured the effect of SMF on the capability of cells to oxidize 5'-carboxy-2',7'-dichlorodihydrofluorescein diacetate (H2DCF-DA), the probe of the reactive oxidants. The data distinctly show that this ability was reduced in the cells exposed to SMF for 24 h (Fig. 1C). Considering the exponential scale of the coordinate representing the intensity of H2DCF-DA fluorescence the observed reduction was of approximate range of two orders of magnitude.

The present data thus indicate that exposure of TK6 cells to moderate strength SMF leads to a reduction in the level of constitutive H2AX phosphorylation and ATM activation. Considering the collective evidence obtained from numerous experiments on the effects of a variety of

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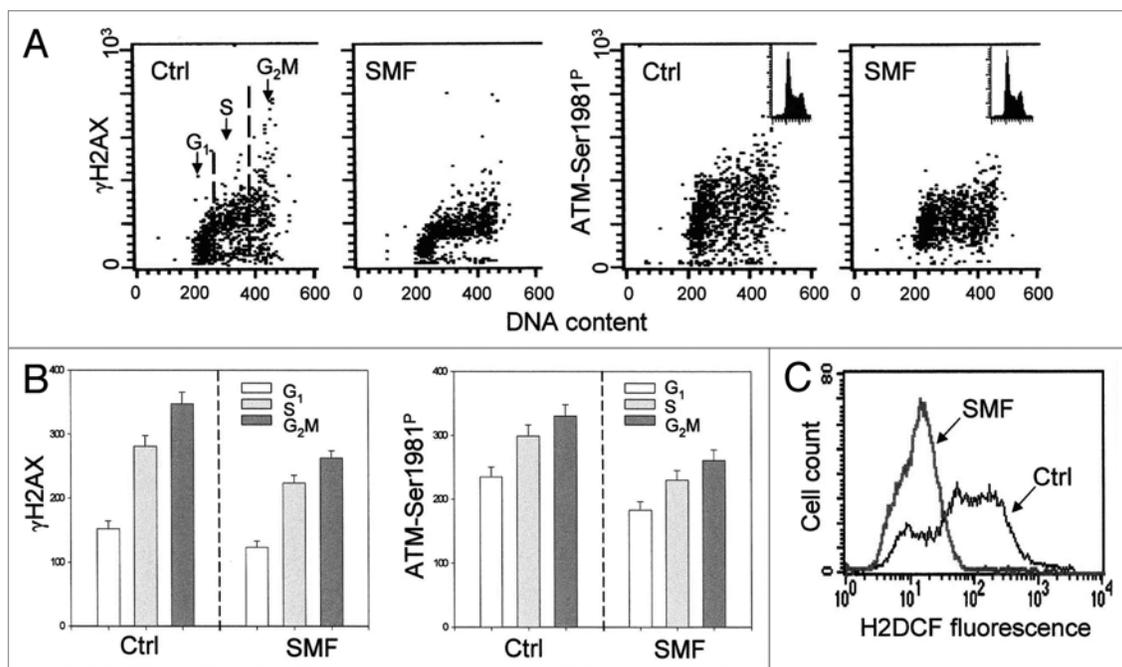


Figure 1. Effect of exposure of TK6 cells to SMF on expression of γ H2AX and ATM-Ser1981P (A and B) and on cells ability to oxidize H2DCF-DA (C). (A) The bivariate distributions of γ H2AX IF and ATM-Ser1981P IF vs. cellular DNA content of the untreated cells (Ctrl) and the cells exposed to 705 mT for 24 h (SMF). The DNA content frequency histograms of the cells from the respective cultures are shown in right panels. (B) Attenuation of H2AX and ATM phosphorylation by SMF in relation to the cell cycle phase. The mean values of γ H2AX and ATM-Ser1981P immunofluorescence (\pm SD) were estimated for populations of cells in G₁, S and G₂M phases of the cell cycle by gating analysis based on differences in DNA content as shown in (A) and plotted as bar graphs. (C) Effect of SMF on the ability of TK6 cells to oxidize H2DCF-DA. The cells in culture were exposed for 24 h to SMF. During the final 30 minutes DCF-DA was added into the culture to final concentration 10 μ g/ml, the cells were then rinsed and their green fluorescence was measured by flow cytometry. Note a decrease in intensity of H2DCF-DA fluorescence in cells exposed to SMF.

anti-oxidants and metabolic inhibitors^{6-9,11} such a reduction in all probability reflects the SMF-induced attenuation of oxidative DNA damage caused by endogenous ROS originating from aerobic metabolism. The observed reduction in cells ability to oxidize H2DCF, which is a recognized bioprobe of ROS,¹² provides supporting evidence that the degree of constitutive oxidative DNA damage is reduced by SMF.

There is very extensive and often conflicting literature on biological effects of SMF. Perusal of the literature indicates that the character of the SMF-induced effects depends on intensity of the magnetic force and duration of the treatment. The recent article by Okano¹⁰ provides the most comprehensive and elucidating review of the literature devoted to biochemical and biological effects of SMF. In the present study the cells were exposed to the moderate-intensity SMF, which generally is defined as within the millitesla (1 to 10³ mT) range.¹⁰ Therefore to present our results within the context of the published data we limit the discussion to the effects of SMF of that

intensity range on ROS and DNA integrity in live cells.

Exposure of pregnant rats to 128 mT SMF was reported neither to affect normal pregnancy nor to induce a detectable oxidative DNA damage.¹³ The authors observed however the increase in the level of the ROS scavenger glutathione (GSH) but no changes in the level of ROS were seen. The same group of authors reported the induction of another ROS scavenger, metallothionein, in the liver and kidney of male rats exposed to 128 mT SMF.¹⁴ The elevation of the level of ROS scavengers was also observed in rat male testes.¹⁵ However the level of the DNA oxidation product 8-oxo-dG was increased concurrently with a decrease in testosterone level, although there was no evidence of altered spermatogenesis in rats exposed to SMF.¹⁵

Exposure of lymphocytes to 7 mT SMF alone for 3 h was reported to have no effect on integrity of their DNA but when the exposure was combined with the FeCl₂ treatment significant degree of DNA damage was seen, likely, reflecting necrotic or

apoptotic cell death.¹⁶ In fact, the accelerated progression of apoptosis to its later, the necrotic-like phase, was observed in the cells exposed to SMF in which apoptosis was induced by the antitumor drug camptothecin.¹⁷ Previous studies demonstrated that SMF modulate Ca²⁺ fluxes in human glioblastoma primary cultures and interfere with the apoptotic pathway of these cells under apoptogenic chemical or physical stress.¹⁸ It appears however that the effects of SMF in modulation of the apoptotic process depend on the cell type but not on the inducer of apoptosis.¹⁹

How our present findings that SMF attenuates the level of ROS and reduces extent of CHP and CAA can be accounted in the context of the presented above observations of the effects of SMF in other cell systems? The observed decrease in the level of ROS is consistent with many reports on induction of the radical scavengers in different cell types. Induction of scavengers thus could contribute to the observed decline in ROS. It should be noted however that SMF through the "radical pair

mechanism” facilitating the interconversion of the singlet to triplet excitation state (intersystem crossing) prolongs lifetime of reactive oxidants.²⁰ In the absence of the radicals scavenger mechanism one would expect an increase rather decrease in the level of ROS. However, still another mechanism may play a role. As mentioned, SMF may interfere with the electron transport chain in mitochondria reducing cell respiration, the process that generates reactive oxidants. Surprisingly little information on the effect SMF on this process is available in the literature although some reports suggest this mechanism.²¹ If the process of electron transport and in consequence the oxidative phosphorylation are perturbed by SMF, the effect would be analogous to that of reduction of cell respiration by the diet restriction or the “diet restriction mimetics”, known to decrease oxidative DNA damage and increase longevity. However, we were unable to find any report on the effect of SMF on animals longevity.

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