Transcriptional effects of 50 Hz magnetic fields at 1.2 μT and 100 μT on human breast cancer MCF-7 cells

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Abstract. The International Agency for Research on Cancer (IARC) classified power frequency magnetic fields as a possible human carcinogen. Alteration in transcription programs is a fundamental feature of cancer. Here, using DNA array technology, we examined the transcriptional effects of 50 Hz magnetic fields on human breast cancer MCF-7 cells. It was found that expression of several oncogenes was significantly altered by magnetic-field exposure and that gene expression profilings were similar in MCF-7 cells exposed to magnetic fields at 1.2 μT and 100 μT for 1 week.

1. Introduction

Epidemiological data reported by Wertheimer and Leeper stimulated the research area of biological effects of magnetic fields [1]. They first identified the association between child leukaemia and residential magnetic fields of extremely low frequency. They also reported a nearly threefold increase of breast cancer risk among women younger than 55 who lived near power lines, suggesting that magnetic fields exposure had accelerated development and growth of breast cancer. Subsequent epidemiological studies showed the association between child leukemia and residential magnetic fields [2-6]. The International Agency for Research on Cancer classified power frequency magnetic fields as a possible human carcinogen [7].

Stevens hypothesized that magnetic fields affect pineal gland melatonin secretion in vivo, which, in turn, influence mammary (breast) carcinogenesis [8]. Since then, a number of experimental studies have been conducted in order to test this hypothesis.

Melatonin from the pineal gland is released into the blood stream and cerebrospinal fluid. Melatonin was initially found to function as a mediator of circannual reproductive rhythms as well as of circadian cycles [9]. Subsequently, melatonin was shown to have significantly broader actions...
including oncostatic effects, immune enhancement, and anti-inflammatory functions [10, 11]. Melatonin appears to exert the effects through pharmacologically specific, high affinity receptors. Receptor affinity is sensitive to guanine nucleotides and activation of these receptors results in inhibition of adenylyl cyclase via a pertussis toxin-sensitive mechanism (Fig. 1).

Liburdy originally demonstrated that 60 Hz magnetic fields at 1.2 μT inhibit the antiproliferative effect of melatonin on the MCF-7 cells [12]. In addition, other laboratory independently reported the results that are consistent with this finding concerning the effect of magnetic fields on melatonin [13], supporting the hypothesis. We extended their study by showing the molecular mechanism of the biological effects of magnetic fields. Namely, exposure to magnetic fields causes the uncoupling of the melatonin signal transduction pathway [14, 15]. It is likely that magnetic fields certainly exert its effects on protein levels.

Alteration in transcription programs is a fundamental feature of cancer. Therefore, in this study we employed human DNA arrays of Atlas Glass Human 1.0 Microarray (Clontech Corp), containing 1,081 probe sets for transcriptional analyses of bioeffects of magnetic fields.

2. Materials and Methods

2.1. Cell culture and Magnetic fields exposure. MCF-7 cells were kindly provided by Dr. Liburdy (UCLA, Berkley) and grown in Dulbecco’s modified Eagle’s Medium (Invitrogen, Carlsbad, CA) supplemented with 10% FBS (Gibco BRL), penicillin (100 U/ml), and streptomycin (100 μg/ml) in a humidified atmosphere of 95% air: 5% CO2 at 37°C, as described previously [14-16]. The cells were subcultured (1:4) 1 to 2 times per week. In order to expose the cells to magnetic fields, a 50 Hz sinusoidal magnetic field was generated in a chamber with four Merritt-coil devices. The chamber had four ventilation holes (2.54 cm in diameter) on the top and bottom. A temperature probe was placed inside the chamber to monitor temperature continuously. The anti-parallel mode of operation generated opposing magnetic fields that cancelled and resulted in a true sham exposure. When a current was applied to the parallel configuration, a magnetic field was established. Two identical exposure systems were employed in this study. Each coil system was driven by identical signal generators obtained from NF Electronic Instruments Corp. (Yokohama, Japan). Cell viability was determined by means of the crystal violet staining method.

Fig. 1. The schematic drawing of melatonin signalling pathway. Melatonin acts through its receptor, G protein, and adenylyl cyclase to elicit the physiological responses such as circadian rhythms, seasonal reproduction, and oncostatic effects. This pathway was specifically disrupted by exposure to magnetic field. The primary structure of human melatonin receptor is represented with deduced amino acids (a single letter).
2.2. DNA array analyses. Total cellular RNA was isolated from control and magnetic fields-treated cells (1.2 µT or 100 µT; one week) by single-step guanidinium thiocyanate-phenol-chloroform extraction, as described previously [17]. The quality and quantity of RNA was measured by spectrophotometry and electrophoresis on denaturing agarose gel. The first strand DNA was synthesized using Moloney murine leukemia virus reverse transcriptase (Invitrogen) with random heximers as primers. Fluorescent-labeled cDNA probes were synthesized from 10 µg total RNA in the presence of dATP, dCTP, dTTP, dGTP, aminoally-dUTP, Cy3 and Cy5 mono-reactive dyes (Amersham Pharmacia Biotech, NJ), and PowerScript reverse transcriptase (Clontech). The labeled cDNA was purified using Atlas NucleoSpin Extraction Spin Columns (Clontech). Atlas Glass Human 1.0 Microarray (Clontech) were prehybridized in GlassHyb hybridization buffer, containing the sheared salmon testes DNA for 30 min at 50 °C in a hybridization chamber (Clontech). The heat-denatured probe was hybridized overnight at 50 °C. The arrays were washed with 2 x SSC (1 x SSC = 150 mM NaCl/ 15 mM sodium citrate, pH 7.0) twice at room temperature for 5 min, twice with 2 x SSC plus 1% SDS at room temperature for 30 min, and twice with 0.1 x SSC plus 0.5% SDS at room temperature for 30 min. The arrays were then scanned. Image acquisition and quantification were performed using an AtlasImage (Clontech).

3. Results and Discussion

The DNA array was performed using Atlas Glass Human 1.0 Microarray. There were 1,081 probe sets. The probe sets were categorized into oncogenes and tumor suppressor genes, DNA damage repair-related genes, cell cycle-related genes, cell signalling-related genes, ligand genes, and channel and transporter genes.

Gene expression levels were calculated as the ratio of expression level in the exposed cells to that in the control cells. Only estimated levels of gene expression are shown in Fig. 2. Table 1 summarizes the changes of levels in gene expression of molecules that are oncogenes or tumor suppressor genes.
suppressor genes. Genes listed were functionally categorized according to the information provided by BD Biosciences Clontech. The levels of gene expression of 95% genes were unchanged. The expression levels of 39 genes were increased by more than 2-fold, whereas that of 12 genes were decreased by 2-fold or less as a result of magnetic fields exposure (1.2 \( \mu \)T for 1 week). The similar changes in patterns and the extent of gene expression were obtained by exposure to 100 \( \mu \)T magnetic fields for 1 week. The highest change was about 6-fold increase.

It has been shown that among these proto-oncogenes, the expression of Ets oncogenes was induced by magnetic fields in several kinds of cultured cells [18]. Ets is a family of transcription factors that play important roles in cell development, cell differentiation, apoptosis, and tissue remodelling [19, 20]. The aberrant expression of Ets genes leads to malignant transformation and tumor progression. The level of Ets oncogene has been associated with the grade of malignancy and prognosis in several types of tumors including breast cancer [19, 21]. The mechanism through which magnetic fields modulate gene expression is largely unknown because in single photon interaction picture, it has been considered difficult to explain the mechanism, due to its low energy. However, it has been proposed that weak magnetic fields can cause charge movement, resulting in conformation change of the macromolecules due to redistribution of charges [22]. Thus, it might be possible that magnetic fields may alter biological processes such as gene expression through their effects on charge distribution.

Since Atlas Glass Human 1.0 Microarray used in this study has limited in number of probe sets (only 1,081), it is necessary to establish the transcriptome of magnetic fields-exposed MCF-7 cells, using whole human genome DNA array. This is under way in our laboratory.

4. Conclusion
Based on ‘Melatonin Hypothesis’[7], we previously demonstrated that magnetic fields exerted its effects on the protein levels in cultured MCF-7 cell, seen as a disruption of protein-protein interactions in a melatonin-signaling pathway. Furthermore, in this study we demonstrated the effects of magnetic fields on transcriptional levels by showing that the magnetic field increased expression of some oncogenes and was decreased some of others. The patterns of the change were similar at 1.2 \( \mu \)T and 100 \( \mu \)T, eliminating the possibility of artifacts during exposure to magnetic fields. The pathological consequences of these alterations remain to be determined.

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