

# Effect of Electromagnetic Field Exposure on Chemically Induced Differentiation of Friend Erythroleukemia Cells

Gang Chen,<sup>1</sup> Brad L. Upham,<sup>1</sup> Wei Sun,<sup>1</sup> Chia-Cheng Chang,<sup>1</sup> Edward J. Rothwell,<sup>2</sup> Kun-Mu Chen,<sup>2</sup> Hiroshi Yamasaki,<sup>3</sup> and James E. Trosko<sup>1</sup>

<sup>1</sup>Department of Pediatrics and Human Development, College of Human Medicine, Michigan State University, East Lansing, Michigan, USA; <sup>2</sup>Department of Electrical Engineering, College of Engineering, Michigan State University, East Lansing, Michigan, USA; <sup>3</sup>Unit of Multistage Carcinogenesis, International Agency for Research on Cancer, Lyon, France

Whether exposure of humans to extremely low frequency electromagnetic fields (ELF-EMF) can cause cancer is controversial and therefore needs further research. We used a Friend erythroleukemia cell line that can be chemically induced to differentiate to determine whether ELF-EMF could alter proliferation and differentiation in these cells in a manner similar to that of a chemical tumor promoter. Exposure of this cell line to 60 Hz ELF-EMF resulted in a dose dependent inhibition of differentiation, with maximal inhibition peaking at 40% and 40 mG (4  $\mu$ T). ELF-EMF at 10 mG (1.0  $\mu$ T) and 25 mG (2.5  $\mu$ T) inhibited differentiation at 0 and 20%, respectively. ELF-EMF at 1.0 (100) and 10.0 G (1,000  $\mu$ T) stimulated cell proliferation 50% above the sham-treated cells. The activity of telomerase, a marker of undifferentiated cells, decreased 100 $\times$  when the cells were induced to differentiate under sham conditions, but when the cells were exposed to 0.5 G (50  $\mu$ T) there was only a 10 $\times$  decrease. In summary, ELF-EMF can partially block the differentiation of Friend erythroleukemia cells, and this results in a larger population of cells remaining in the undifferentiated, proliferative state, which is similar to the published results of Friend erythroleukemia cells treated with chemical-tumor promoters. **Key words:** cancer, cell differentiation, extremely low frequency electromagnetic fields, Friend leukemia cells, telomerase activity. *Environ Health Perspect* 108:967–972 (2000). [Online 7 September 2000] <http://ehpnet1.niehs.nih.gov/docs/2000/108p967-972chen/abstract.html>

The biological effects of extremely low frequency electromagnetic fields (ELF-EMF) have been a concern since Wertheimer and Leeper (1) reported that children living in homes with an excess of electrical wiring configurations suggestive of high current-flow had a higher incidence of cancer. However, subsequent *in vivo*, *in vitro*, and epidemiological studies have produced varying results that often conflict with each other (2–7). Whether ELF-EMF exposure is correlated with human cancer is controversial and therefore needs further research.

Carcinogenesis involves the irreversible genetic alteration of a single cell (initiation), followed by the clonal expansion of that initiated cell (promotion), and the ultimate conversion of these cells to invasive and metastatic neoplastic cells (8). Mutation of DNA plays an important role in carcinogenesis as evidenced by hereditary mutations predisposing humans to cancer, known mutagens being effective initiators of carcinogenesis, and mutations found in the protooncogenes and tumor-suppressor genes in cells of tumors (8). If ELF-EMF contributes to cancer, then it is important to determine the stage or stages of carcinogenesis that it affects. Most studies indicate that ELF-EMF is a nonionizing form of radiation that is too weak to induce thermal effects; therefore, it cannot damage or mutate DNA and lead to mutagenesis (9,10). A few studies have been interpreted as indicating either mutagenic or comutagenic

activity (11,12). However, the weight of the evidence indicates that ELF-EMF exposure would be an extremely weak initiator, if not a noninitiator, of carcinogenesis.

Assuming that ELF-EMF plays a role in the carcinogenic process but that it is not a carcinogenic initiator, it follows that ELF-EMF is likely to be involved in the promotional phase of cancer. The promotional phase of carcinogenesis consists of nonmutagenic and epigenetic mechanisms (13). These mechanisms entail the alteration of gene expression at the transcriptional level (e.g., methylation of DNA or acetylation of DNA-binding proteins), translational level (e.g., alternative splicing or stability of mRNA), or posttranslational level (e.g., protein modification such as phosphorylation). Most tumor promoters are not mutagenic (e.g., phenobarbital, phorbol esters, polybrominated biphenyls, saccharin, peroxisome proliferators, TCDD, DDT) (14), but can alter differentiation (15), inhibit apoptosis (16,17), induce various signal transduction pathways (e.g., protein kinase C, mitogen activated protein kinases) (18), and activate gene expression (19). Similar to tumor-promoting chemicals, ELF-EMF can also alter the transcription and translation of genes such as *hsp70*, *myc*, *jun*, and *fos* (20–23).

To study the possibility that EMF contributes to the promotional stage of cancer, we chose a Friend erythroleukemia cell line to determine the epigenetic effect of ELF-EMF,

*in vitro*, on cell proliferation and differentiation. Friend erythroleukemia cells are primitive erythroid cells that can be induced to differentiate after exposure to either DMSO or hexamethylene bis-acetamide (HMBA). In essence, this cell line mimics an initiated stem cell. After exposure to these chemicals, these cells differentiate as measured by their ability to express globin mRNA and synthesize adult hemoglobin, express a red cell membrane-associated antigen, decrease in volume, change morphologically, and exhibit limited proliferative capacity (24,25). Tumor-promoting effects should therefore inhibit differentiation and stimulate proliferation of DMSO-treated Friend erythroleukemia cells. Such tumor-promoting effects have been observed when Friend erythroleukemia cells were treated with the known model tumor promoter agent 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (26). Our results support the hypothesis that ELF-EMF acts as a tumor-promoting agent in mimicking the tumor promoter TPA by inhibiting DMSO and HMBA-induced Friend erythroleukemia cell differentiation and by stimulating cell proliferation.

## Materials and Methods

**Chemicals.** DMSO was obtained from Aldrich Chemical (Milwaukee, WI); acetic acid from EM Science (Gibbstown, NJ); formaldehyde (37%) from J.T. Baker (Phillipsburg, NJ); ethanol from Millennium Petrochemicals (Tuscola, IL); and HMBA and benzidine from Sigma Chemical Co. (St. Louis, MO).

**Electromagnetic field exposure system.** We used a Linear EMF Exposure System model 2 (LES-002-DC; Electric Research and Management Inc., State College, PA). This system consisted of one incubator (Steri-Cult 200; Forma Scientific, Marietta, OH), one

Address correspondence to J.E. Trosko, Michigan State University, 246 Food Safety and Toxicology Bldg., East Lansing, MI 48824 USA. Telephone: (517) 353-6346. Fax: (517) 432-6340. Email: trosko@msu.edu

We thank R. Davenport for her excellent word processing skills in preparing the manuscript and L. King for assisting us with the FACS analyses. We thank the Electrical Power Research Institute (contract UBP950822) for funding this research.

Received 7 March 2000; accepted 1 June 2000.

stock chamber, and two chambers that contained the EMF-generating coils. Eight alternating current magnetic coils per chamber were arranged in groups of four with a Merritt configuration, with the outer coils bucking the inner coils to reduce the fields outside the assembly. The coils were in bifilar arrangement so that they could be energized in parallel fields for sham conditions. The incubator controlled the environment. Three 2.5-horsepower fans distributed air containing 5% CO<sub>2</sub> at 98% relative humidity from the incubator to the chambers through 6.4 cm polyvinyl chloride conduits. The cell culture chambers were at least 0.9 m from the incubator to minimize the ELF-EMF exposure from the incubator to less than the background levels. However, the chambers are constructed from plastic and do not shield from the earth's magnetic field. The sham fields were < 1 mG (0.1 μT). These chambers were 85 × 85 × 90 cm (length × width × height) with the door positioned at the top, the air intake 3 cm from the bottom, and the outtake 25 cm from the top. Cell samples were placed on a grid 10 cm from the bottom and 7 cm above the intake port. ELF-EMF exposure measurements of field strengths using an EFA-1 Field Analyzer probe (Wandel & Goltermann, Eningen, Germany) indicated that the ELF-EMF fields were homogeneous between 10 and 30 cm above the grid; therefore, all samples were placed in this homogeneous exposure zone with the flasks lying down in the horizontal position. Thus, the EMF magnetic fields were perpendicular to the plane of the flask. Also, the exposure system was characterized through field measurements performed by the National Institute of Standards and Technology (NIST; Gaithersburg, MD). Measurements of the magnetic field at the sample plane showed a variation of 2–3% from the specified field, with a spatial uniformity of > 99%. The measured electric field within the chambers at 10 G (1,000 μT) magnetic field strength was found to be < 0.2 V/m. There was no measurable distortion in the 60 Hz magnetic field waveform. The upper limits of field strength and frequency are 10 G (1,000 μT) and 3000 Hz, respectively. Each chamber had a temperature monitor and a three-axis magnetic sensor. The incubator had outputs to allow the computer to monitor temperatures, humidity, and CO<sub>2</sub> levels. The chambers also had ports for monitoring CO<sub>2</sub> levels, which was done daily using a portable fyrite-based indicator system. We also used the computer to set the experiment in either a blind or known mode.

The absolute current induced within the culture medium and within the cell is very small. The maximum induced current in the culture medium was calculated by using

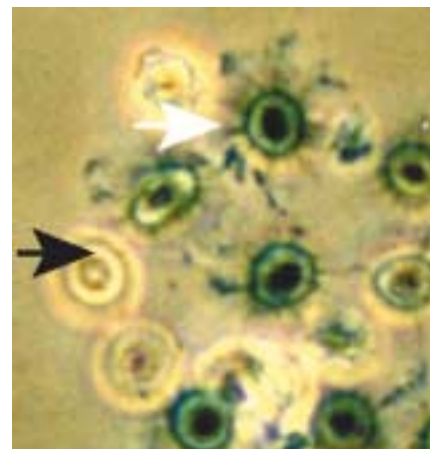
either numerical simulation techniques or the analytical expression for an insulated rectangular box (27). The conductivity of the medium was measured at 9.6 mS/m using a Hanna Instrument conductivity meter (Model HI 8733; Vila do Conde, Portugal). Using flask dimensions of 5.2 × 4.8 cm, the maximum value of the current density induced within the culture medium was calculated to be 6.2  $b_0$  μA/m<sup>2</sup>, where  $b_0$  is the applied magnetic flux density in Gauss. Thus, at 10 G the maximum induced current density is 62 μA/m<sup>2</sup>. The maximum induced current density occurs adjacent to the walls of the flask, while the current density at the center of the flask is zero. The time-average power dissipated by the culture medium is on the order of 2 × 10<sup>-12</sup> W and thus less than a 1 μJ of heat is generated over a period of 3 days. Obviously, the thermal effect from the small induced currents is negligible.

**Cell culture and induction of differentiation.** Friend erythroleukemia cell line 19-9 was maintained in Eagle's basal medium (catalog no. M-7278, lot 17H2380; Sigma Chemical Co.) supplemented with 5% fetal calf serum (catalog no. 16000-044, lot no. 1021367; Gibco BRL, Grand Island, NY), and subcultured twice a week. We transferred cells into fresh medium 1 day before the induction of differentiation. For the induction of differentiation, we transferred the cells to fresh medium containing 2% DMSO or 4 mM HMBA at a cell density of 1 × 10<sup>5</sup> cells/mL, distributed to 25-cm<sup>2</sup> cell-culture flasks with screw-on caps, and immediately placed into the exposure and sham chambers. The average time between the dilution of cells in the DMSO or HMBA-containing medium and placement of the flasks into the chambers was < 1 min. This was done to minimize the time between the induction of signal transduction pathways and the exposure of these induced signaling pathways to the ELF-EMF. We used flasks with screw-top caps to minimize evaporation that could induce osmotic stress, which is known to induce cell-signaling pathways. We found that the use of culture plates resulted in significant evaporation, which resulted in high levels of differentiation in the absence of an inducer. We did not see this effect when we used the flasks with screw-top caps that were loosely screwed on.

**Hemoglobin determination. Benzidine staining technique.** Benzidine was dissolved in 12% acetic acid to a final concentration of 0.4% (w/v). Just before the cells were stained for hemoglobin, 40 μL of 30% H<sub>2</sub>O<sub>2</sub> was added to 1 mL 0.4% benzidine solution, which was then added to a 5-mL cell suspension (25). The cells that stained dark blue-green (Figure 1) were scored positive for

hemoglobin. We counted the stained versus unstained cells using a hemocytometer, and the results were expressed as a percentage of cells that were stained. The undiluted cell suspension was added to the hemocytometer, and the cells were counted in five of the nine grids (4 corners and the center grid), with the total number of cells ranging between 200 and 500 cells. The first three experiments at 1.0 G (100 μT) were counted by two different individuals and the final numbers agreed to within 1% of each other.

**Immunostaining and flow cytometry.** Cells were collected and centrifuged at 200g for 10 min and washed with phosphate-buffered saline (PBS). This washing was repeated 2 times, and then the cells were fixed in 4% formalin for 15 min. The membranes of the fixed cells were permeabilized for the antibody step with 75% ethanol. Then the cells were washed 1 time with 50% ethanol, 1 time with 25% ethanol, and 1 time with PBS. These fixed cells were resuspended for 1 hr in a blocking agent containing 10% normal goat serum that was dissolved in PBS. The blocking step was continued for an additional 1 hr using a 1% solution of normal goat serum and 0.1% bovine serum albumin in PBS. A 1:200 dilution of the primary antibody was added to this cell suspension and incubated overnight with constant shaking at 4°C. Then the cells were washed 3 times with PBS, and the secondary antibody was added at a 1:200 dilution in PBS containing 1% normal goat serum and 0.1% bovine serum albumin for 1 hr at room temperature. After incubation with the secondary antibody, the cells were washed 3 times with PBS and resuspended in PBS at 1 × 10<sup>5</sup> cells/mL and then analyzed using a Vantage fluorescence activated cell sorter (FACS) (Becton; Dickinson, San Jose, CA).



**Figure 1.** A microscopic photograph at 200× illustrating benzidine-stained cells containing hemoglobin versus unstained cells lacking hemoglobin. The white and black arrows indicate benzidine-positive and negative stained cells, respectively.

**Cell proliferation.** Cells were seeded into 25-cm<sup>2</sup> flasks at  $1 \times 10^4$  cells per flask and exposed to ELF-EMF or sham conditions. The medium was changed every 2 days by pelleting the cells at 200g and resuspending in fresh medium. We determined cell density by measuring DNA concentration. DNA was quantified from cells that were collected from flasks and then centrifuged at 200g for 10 min, washed with PBS 2 times, and then lysed in 3 mL 0.1 N NaOH. We determined the DNA concentration (28) by measuring the absorbance at 260 nm using a DU 7400 Beckman Diode Array Detector (Beckman Coulter, Fullerton, CA).

**Polymerase chain reaction-based telomerase assay.** The cell suspension was centrifuged and washed with PBS. This step was repeated once and then cells were resuspended in PBS at a concentration of  $1 \times 10^6$  cells/mL and aliquoted to three microcentrifuge tubes. After the cells were centrifuged at 12,000g for 20 min and 4°C, the PBS was carefully removed, and the cell pellets were stored at -85°C. For the telomerase assay, the cell pellet containing  $1 \times 10^6$  cells was thawed and diluted in 200  $\mu$ L of 1 $\times$  CHAPS {3-[(3-cholamidopropyl) dimethyl-ammonio]-1-propanesulfonate} buffer giving a final cell concentration of 5,000 cells/ $\mu$ L lysis buffer for 30 min at 4°C. Then the samples were centrifuged at 12,000g for 20 min at 4°C. The cell lysate for each sample was aliquoted to several new tubes and stored at -85°C. We examined telomerase activity by the telomeric repeat amplification protocol (TRAP) assay (29) using the TRAPeze Telomerase Detection Kit (Oncor, Gaithersburg, MD). This protocol includes primers of a 36 base pairs (bp) internal positive standard for amplification, thus providing a positive control for accurate quantitation of telomerase activity within a linear range close to 2.5 logarithmic units. Each analysis included a negative control (CHAPS-lysis buffer instead of cell lysate), a heat-inactivated control (sample incubated at 85°C for 10 min before the assay), and a positive control (breast carcinoma cell line MCF-7). For RNase treatment, 10  $\mu$ L of extract was incubated with 1  $\mu$ g of RNase for 20 min at 37°C. The products of TRAP assay were resolved by electrophoresis in a non-denaturing 12% polyacrylamide gel electrophoresis in a buffer containing 54 mM Tris-HCl (pH 8.0), 54 mM boric acid, and 1.2 mM EDTA. The gel was stained with Syber Green (Molecular Probes, Inc., Eugene, OR) and visualized at either 302 nm or 254 nm using an ultraviolet transilluminator. We captured and analyzed images using a NucleoVision760 ccd cooled camera image analyzer (NucleoTech Corporation, San Mateo, CA). The products generated

from TRAP assay were quantified using the following formula:

$$\text{TPG (total product generated units)} = \frac{[(x - x_0)/d]/[(r - r_0)/c_R] \times 100,$$

where  $x$  and  $x_0$  represent signals corresponding to the TRAP product ladder bands of non-heat-treated and heat-treated sample lanes, respectively, and  $r$  and  $r_0$  represent signals from 1 $\times$  CHAPS lysis buffer control (i.e., primer-dimer/polymerase chain reaction contamination control) and TSR8 (DNA quantitation control), respectively. The signal from the internal standard (TSK1) in non-heat-treated samples and TSR8 quantitative control are  $c$  and  $c_R$ , respectively.

## Results

Both DMSO- and HMBA-induced Friend erythroleukemia cells can be used to express hemoglobin, a differentiation marker (Table 1). However, DMSO consistently caused a higher percentage of cells to express hemoglobin than HMBA-treated cells and was chosen as the inducer of differentiation in subsequent experiments. EMF-ELF at a field-strength of 1.0 G (100 mT) inhibited differentiation 35% and 25% in DMSO and HMBA-treated cells, respectively (Table 1). A FACS technique was used as an alternative

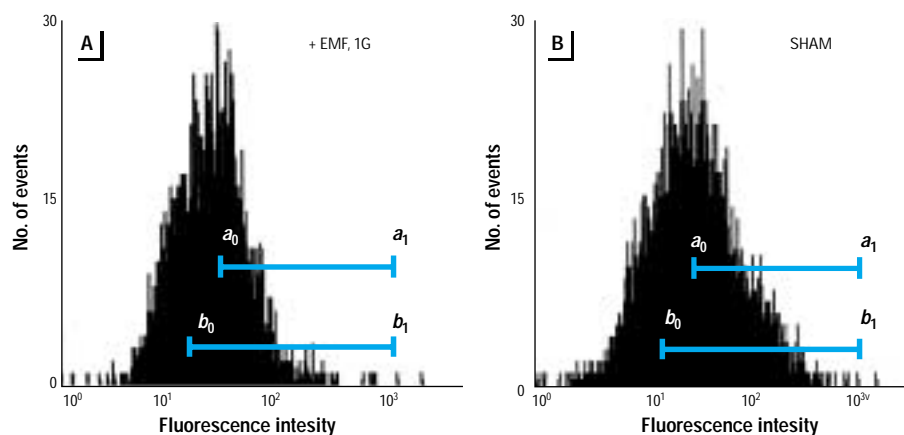
method of quantifying the percentage of cells containing hemoglobin. Figure 2 illustrates how the data from the FACS analysis were compiled. The channels were set according to the results of several experiments that determined the fluorescence profiles of Friend erythroleukemia cells fixed without antibodies and with secondary antibodies alone (data not shown). Also, primary and secondary antibodies were incubated with Friend erythroleukemia cells that were not treated with a differentiating inducer. From these experiments, the fluorescence intensity below  $b_0$  was attributed to the nonspecific binding of secondary antibodies, and the fluorescence intensities between  $a_0$  and  $b_0$  were from background levels of fluorescence in undifferentiated cells (Figure 2). The number of events in channel  $a_0$  and  $a_1$  was attributed to the specific binding of the primary antibodies to hemoglobin and the results were reported as a ratio of ELF-EMF:sham (Figure 2). Friend erythroleukemia cells treated with DMSO and exposed to 1.0 G (100  $\mu$ T) ELF-EMF resulted in a shift of the peaks to lower fluorescence intensities, thereby decreasing the ELF-EMF:sham ratio to < 1.00 (Figure 2). An average ratio of  $0.69 \pm 0.22$  was calculated from seven FACS experiments in which the sham and ELF-EMF chamber each had one flask per experiment.

**Table 1.** Inhibition of DMSO- or HMBA-induced differentiation of Friend erythroleukemia cells exposed to 1.0 G (100  $\mu$ T), 60-Hz ELF-EMF.

Differentiation inducer	Percent differentiated cells		Ratio of +EMF:sham
	Sham	(+) EMF	
DMSO	28.9 $\pm$ 9.2	18.9 $\pm$ 8.1*	0.64 $\pm$ 0.14
HMBA	16.7 $\pm$ 4.7	12.2 $\pm$ 3.8*	0.74 $\pm$ 0.30

Hematocrit counting of benzidine-stained cells was used to determine the percentage of cells containing hemoglobin. The data represent the percent average  $\pm$  standard deviation at the 95% confidence interval. There were three replicates (flasks) per experimental trial. There were 15 and 4 experimental trials for DMSO and HMBA treatments, respectively. Ten of the 15 DMSO trials and none of the 4 HMBA trials were blind studies.

\*Significant at the  $p < 0.005$  level as determined by a paired  $t$ -test.



**Figure 2.** An example of a FACS analysis showing the shift in fluorescence intensity after exposure to 1.0 G (100  $\mu$ T) ELF-EMF. The channels were set according to the results of previous experiments. Fluorescence <  $b_0$  were seen in DMSO-treated cells incubated with only the secondary antibodies. Fluorescence <  $a_0$  were seen in Friend cells incubated with primary and secondary antibodies but not treated with DMSO. In channel  $a_0 - a_1$ , there were 3,152 events for sham and 1,832 events for EMF treatment, with a ratio of EMF:Sham of 0.58.

The inhibitory effect of ELF-EMF on the differentiation of Friend erythroleukemia cells was dose dependent (Table 2 and Figure 3). At 0.01 G (1  $\mu$ T), there was no significant inhibition of differentiation. Maximal inhibition occurred at an approximate value of 0.05 G (5  $\mu$ T). Although the extent of differentiation varied with each experiment at the various field strengths, maximum inhibition was approximately 40–50% (Table 2 and Figure 3). The percentage of differentiating cells appeared to be a function of passages. The number of passages after the cells were thawed from liquid nitrogen was < 5 for the 0–0.05 G (5  $\mu$ T) experiments, 5–10 for the experiments at 1.00 G (100  $\mu$ T), and 10.0 G (1,000  $\mu$ T), and 10–15 for the 0.10 and 0.50 G experiments. Apparently the cells subjected to higher numbers of passages differentiated less than cells that underwent low numbers of passages. An ANOVA showed no statistical differences among 0.05 G (5  $\mu$ T), 0.1 G (10  $\mu$ T), 0.5 G (50  $\mu$ T), 1.0 G (100  $\mu$ T), and 10 G (1,000  $\mu$ T) at the  $p = 0.001$  level, indicating that the level of inhibition by ELF-EMF was not a function of differentiation levels. The variation between the different dose treatments appeared to be a function of the number of trials. The highest variance was observed at 1.00 G (100  $\mu$ T), where there were 15 trial experiments. The variation dropped almost 50% for the 0.05 G (5  $\mu$ T) experiment that had only two trials and dropped almost 75% for the remaining experiments that only had one trial run.

Telomerase activity, a marker of undifferentiated stemlike cells, was high in Friend erythroleukemia cells grown in DMSO-free media (Figure 4). Induction of differentiation with DMSO caused a 100 $\times$  decrease in telomerase activity in the sham-treated cells but only a 10 $\times$  decrease in the ELF-EMF (1.0 G, 100  $\mu$ T) exposed cells. Cell proliferation was also affected by ELF-EMF (Figure 5). The DNA content, a marker of cell number, increased 1.5 times compared to

the sham-treated cells after a 4-day exposure to 1.0 G (100  $\mu$ T) and 10.0 G (1,000  $\mu$ T) ELF-EMF.

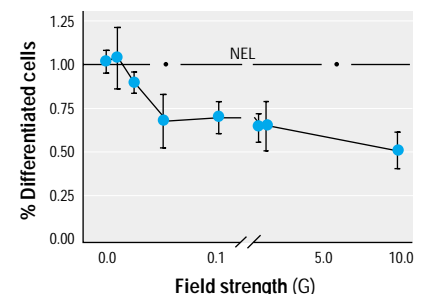
## Discussion

Driven by concerns raised by epidemiologic studies suggesting a potential cancer risk after exposures to ELF-EMF field strengths above the average exposure of 2 mG (0.2  $\mu$ T) (6,7,30), this study was designed to determine whether there was a biological effect relevant to the carcinogenic process. We used an *in vitro* cell differentiation system to determine if ELF-EMF could alter chemically induced differentiation and proliferation of Friend erythroleukemia cells.

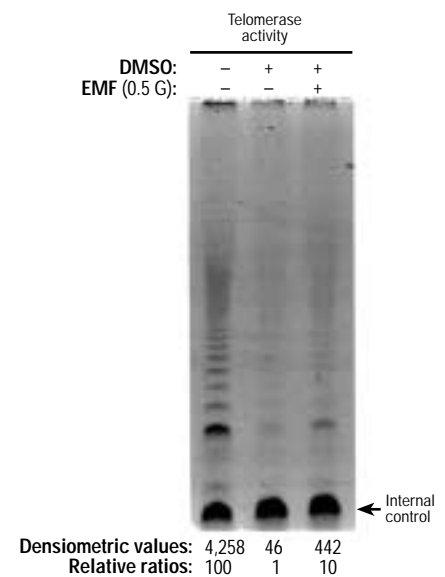
Our results, using two independent assays to measure hemoglobin content, demonstrated that inhibition of chemically induced hemoglobin-containing cells by ELF-EMF was dose dependent. In addition, we showed that exposure to ELF-EMF after chemical induction of differentiation in the Friend cells blocked the reduction in telomerase activity. The reduction of telomerase activity is a normal consequence of cells terminally differentiating or senescing (31). The corollary experiment to the induction of differentiation is a measurement of the exposed population of the Friend cells to proliferate as measured by DNA synthesis. The result was an increase in cell proliferation, which was probably the consequence of a larger subpopulation of undifferentiated cells that did not lose their capacity to proliferate.

Our results contrast to those reported by Revoltella et al. (32). They saw no effect of ELF-EMF on the proliferation and differentiation of a Friend erythroleukemia cell line. We can only speculate on an explanation of the differences between their system and ours. One could be differences in the strain of Friend cells used. After induction of cell differentiation by DMSO, Revoltella et al.'s cell line continued to proliferate to the same level as the cells not treated with DMSO. Our cell line had decreased proliferation when treated with either DMSO or HMBA.

Also, we minimized the time between the exposure to DMSO and ELF-EMF so that the inhibitory effect of ELF-EMF on the cell signaling pathways induced by DMSO was almost immediate. The difference of time between DMSO and ELF-EMF exposure in Revoltella et al.'s experiments were not reported (32), so we are unable to make the comparisons. This may be important because the DMSO-induced differentiation process has two distinct phases (i.e., pre- and post-commitment), and TPA is known to inhibit the precommitment stage (33). Also, their ELF-EMF coils were placed at the top and bottom, whereas ours were placed on each side of the growth chambers. This could also be a potential explanation of the differences because Blackman et al. (34–36) demonstrated that ELF-EMF perpendicular to the static fields of the earth had the greatest effect on



**Figure 3.** Dose response of DMSO-induced differentiation of Friend erythroleukemia cells exposed to various field strengths. The data are ELF-EMF:sham ratios calculated from the data presented in Table 2. NEL, no effect level.



**Figure 4.** The effect of 1.0 G (100  $\mu$ T) ELF-EMF on telomerase activity. Telomerase activity was measured using the TRAP assay. The internal control was used to quantify the signal amplification efficiency among the samples. The densitometric values were measured using our Nucleotech gel doc scanning system.

**Table 2.** Dose response of various field strengths at 60-Hz ELF-EMF on the differentiation of Friend erythroleukemia cells.

Field strength, G ( $\mu$ T)	Percent differentiated cells		EMF:sham
	Sham	(+) EMF	
0.00 (0)	40.5 $\pm$ 0.9	41.5 $\pm$ 2.1	1.02
0.01 (1)	32.8 $\pm$ 2.7	34.1 $\pm$ 4.1	1.04
0.025 (2.5)	52.6 $\pm$ 2.7	46.4 $\pm$ 2.0	0.88
0.05 (5)	40.0 $\pm$ 5.2	26.4 $\pm$ 2.7*	0.66
0.10 (10)	15.2 $\pm$ 1.4	10.7 $\pm$ 1.7*	0.70
0.50 (50)	16.1 $\pm$ 0.4	9.8 $\pm$ 1.1*	0.61
1.00 (100)	28.9 $\pm$ 9.2	18.9 $\pm$ 8.1*	0.65
10.0 (1,000)	24.8 $\pm$ 2.0	12.7 $\pm$ 3.5*	0.51

Hematocrit counting of benzidine-stained cells was used to determine the percentage of cells containing hemoglobin. Each experiment had three to four replicates (flasks). There was one experiment trial per EMF dose, except for at 0.05 and 1.00 G (100  $\mu$ T), where there were 2 and 15 experiments done, respectively.

\*The averages between the sham and ELF-EMF-exposed cells were significant at the  $p < 0.001$  level as determined by a paired *t*-test for DMSO-induced differentiated cells.

neurite outgrowth of PC-12 cells, whereas fields applied parallel to the earth's static fields resulted in no difference.

Because the potential risk of cancer has been a primary concern of ELF-EMF exposures, understanding both the mechanisms of carcinogenesis and the potential relevance of our *in vitro* system must be delineated. Carcinogenesis is a multistage, multimechanism process (37,38), consisting of initiation, promotion, and progression phases. The weight of the evidence appears to have negated the possibility of a mutagenic or initiating potential of ELF-EMF fields (39). Consequently, if ELF-EMF exposure contributes to the carcinogenic process, it must act as a tumor promoter. Therefore, by identifying characteristics of tumor promoters, and assuming ELF-EMF as a physical agent has properties identical to chemical promoters, such as phorbol esters, phenobarbital, and dioxin, it should have the ability to alter differentiation (40), to stimulate the clonal expansion of initiated cells by either or both stimulation of proliferation (13), and to block apoptosis (41). In addition, many tumor promoters seem to have threshold levels of no effect (42,43).

It might seem that the use of an established erythroleukemia cell line and the results obtained from these cells would have no relevance to the potential mechanistic role that ELF-EMF might have in carcinogenesis. The carcinogenic initiation process is one that blocks the ability of a clonally expandable cell (possibly a stemlike cell) to terminally differentiate and become immortalized (13). In the promotion phase this initiated cell clonally expands so that additional genotypic and phenotypic changes occur to bring about an invasive, metastatic neoplastic cell (13). Therefore, our results indicate that the process of inducing a kind of terminal differentiation, as measured by the expression of the globin gene and the production of hemoglobin in an erythroleukemia line, can be inhibited by ELF-EMF. In other

words, these ELF-EMF fields mimic some of the properties of known chemical tumor promoters such as TPA.

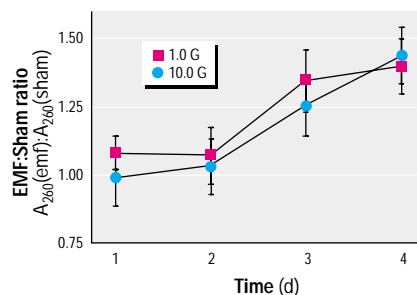
In the absence of precise knowledge of the mechanisms by which DMSO induces Friend erythroleukemia cell differentiation, it is difficult to discuss the mechanisms of its inhibition by ELF-EMF. However, the most obvious physical mechanism produced by exposing cell cultures to a 60-Hz magnetic field is the creation of an induced electric current both within the electrically conducting culture medium and within the cells themselves. Because the size of the sample is small compared to the wavelength of the field, the induced current may be estimated using Faraday's law. Modeling of the container, medium, and free-floating cells reveals that a circulating current is produced within the culture medium roughly proportional to the distance from the center of the flask. This current passes tangentially around the insulating cell membrane. A much smaller circulating current is induced within the cell itself, proportional to the distance from the center of the cell. Thus, a significant tangential shear current exists at the cell membrane. The ratio of the external to internal current at the membrane is roughly proportional to the ratio of the product of the medium conductivity and distance of the cell from the center of the flask to the product of the conductivity of the cell and the cell radius. Assuming that the conductivities of the medium and the cell are roughly the same, the ratio of external to internal shear current for a 5- $\mu\text{m}$  diameter cell located near the edge of the  $5.2 \times 4.8$  cm flask is on the order of  $5 \text{ cm}/0.00025 \text{ cm} = 20,000$ . This result has been verified through simulations using the finite element technique. We therefore speculate that the EMFs probably interfere with the DMSO or HMBA membrane-trigger signal transduction system needed to alter, epigenetically, the gene expression found in the hemoglobin-containing cells. This may be due to the currents induced within individual cell's plasma membranes or the external to internal shear current difference of 20,000 as calculated above. However, the EMF effect on a plasma membrane signaling protein is probably not from the external current alone. At 0.05 G (5  $\mu\text{T}$ ) and 10 G (1,000  $\mu\text{T}$ ), we calculated an induced current density of  $62 \mu\text{A}/\text{m}^2$  and  $0.31 \mu\text{A}/\text{m}^2$  at the walls of the flask, respectively. Although this was a 200 $\times$  difference between 0.05 G (5  $\mu\text{T}$ ) and 10 G (1,000  $\mu\text{T}$ ), the final result of EMF-induced inhibition was similar between these field strengths, indicating that external currents alone may not be the critical factors in controlling the differentiation potential of these cells.

Finally, we can ask the question, what might be the relevance of these data on

mouse erythroleukemia cells to the potential induction of human cancers by ELF-EMF? Any biological effect of one *in vitro* experiment cannot be automatically equated to the entire and complex carcinogenic process in human beings. However, if the biological effect is real and relevant to one of the mechanisms of human carcinogenesis (in this case, the blocked differentiation in the tumor promotion phase), ELF-EMF exposure must be further examined in more direct and relevant experimental systems. In a human population, if ELF-EMF fields can act as a potential human tumor promoter, then the individual being exposed needs to have initiated cells on which ELF-EMF can act (to stimulate the clonal expansion), and be at a field strength above a threshold level, and the individual must also be exposed regularly for a chronic length of time. Consequently, any epidemiological study must be designed to test ELF-EMF as a tumor promoter, not simply as a potential carcinogen. The design must be a mechanistically based, biologically relevant model of carcinogenesis.

## REFERENCES AND NOTES

- Wertheimer N, Leeper E. Electrical wiring configurations and childhood cancer. *Am J Epidemiol* 109:273-284 (1979).
- Bracken MB, Belanger K, Hellenbrand K, Addresso K, Patel S, Triche E, Leaderer BP. Correlates of residential wiring code used in studies of health effects of residential electromagnetic fields. *Am J Epidemiol* 148:467-474 (1998).
- Dockerty JD, Elwood JM, Skegg DC, Herbison GP. Electromagnetic field exposures and childhood cancers in New Zealand. *Cancer Causes Control* 9:299-309 (1998).
- Stevens RG, Davis S. The melatonin hypothesis: electric power and breast cancer. *Environ Health Perspect* 104 (suppl 1):135-140 (1996).
- McBride ML, Gallagher RP, Theriault G, Armstrong BG, Tamaro S, Spinelli JJ, Deadman JE, Finchan S, Robson D, Choi W. Power-frequency electric and magnetic fields and risk of childhood leukemia in Canada. *Am J Epidemiol* 149:831-842 (1999).
- National Research Council. Possible Health Effects of Exposure to Residential Electric and Magnetic Fields. Washington, DC:National Academy Press, 1997.
- NIEHS EMF-RAPID Program. Health Effects from Exposure to Power-line Frequency Electric and Magnetic Fields. NIH Publication No. 98-4400. Research Triangle Park, NC:National Institute of Environmental Health Sciences, 1999.
- Trosko JE, Chang CC, Madhukar BV, Dupont E. Oncogenes, tumor suppressor genes and inter cellular communication in the oncogeny as partially blocked ontogeny hypothesis. In: *New Frontiers in Cancer Causation* (Iversen OL, ed). Washington, DC:Taylor and Francis Press, 1993:181-197.
- McCann J, Dietrich F, Rafferty C, Martin AO. A critical review of the genotoxic potential of electric and magnetic fields. *Mutat Res* 297:61-95 (1993).
- McCann J, Dietrich F, Rafferty C. The genotoxic potential of electric and magnetic fields: an update. *Mutat Res* 411:45-86 (1998).
- Miyakoshi J, Koji Y, Wakasa T, Takebe H. Long-term exposure to a magnetic field (5 mT at 60 Hz) increases X-ray-induced mutations. *J Radiat Res (Tokyo)* 40:13-21 (1999).
- Walleczek J, Shiu EC, Hahn GM. Increase in radiation-induced HPRT gene mutation frequency after nonthermal exposure to nonionizing 60 Hz electromagnetic fields. *Radiat Res* 151:489-497 (1999).
- Trosko JE, Ruch RJ. Cell-cell communication in carcinogenesis. *Front Biosci* 3:208-236 (1998).
- Trosko JE, Chang CC. Nongenotoxic mechanisms in




**Figure 5.** The effect of ELF-EMF on the proliferation of Friend erythroleukemia cells as a function of DNA content. The cells were removed from the EMF chamber at the end of each day, and DNA was extracted from the cells with 0.1 N NaOH and quantified by measuring the absorbance at 260 nm.

- carcinogenesis: role of inhibited intercellular communication. In: *Carcinogen Risk Assessment: New Directions in the Qualitative and Quantitative Aspects*. Banbury Report 31. (Hart RW, Hoerger FD, eds). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1988:139–170.
15. Yamasaki H. Modulation of cell differentiation by tumor promoters. In: *Mechanisms of Tumor Promotion, Vol IV*, (Slaga TJ, ed). Boca Raton, FL: CRC Press, 1984:1–26.
  16. Bursch W, Lauer B, Timmermann-Trosiener I, Barthel G, Schuppler J, Schulte HR. Controlled death (apoptosis) of normal and putative preneoplastic cells in rat liver following withdrawal of tumor promoters. *Carcinogenesis* 5:453–458 (1984).
  17. Trosko JE, Goodman JI. Intercellular communication may facilitate apoptosis: implications for tumor promotion. *Mol Carcinog* 11:8–12 (1994).
  18. Schenk PW, Snaar JB. Signal perception and transduction: the role of protein kinases. *Biochim Biophys Acta* 1449:1–24 (1999).
  19. Imbra RJ, Karin M. Phorbol ester induces the transcriptional stimulatory activity of the SV40 enhancer. *Nature* 323:555–558 (1986).
  20. Phillips JL, Haggren W, Thomas WJ, Ishida JT, Adey WR. Magnetic field-induced changes in specific gene transcription. *Biochim Biophys Acta* 1132:140–144 (1992).
  21. Lin H, Blank M, Jin M, Goodman R. Electromagnetic field stimulation of biosynthesis: Changes in c-myc transcript levels during continuous and intermittent exposures. *Bioelectrochem Bioenerg* 39:215–220 (1996).
  22. Lin H, Head M, Blank M, Han L, Jin M, Goodman R. Myc-mediated transactivation of HSP70 expression following exposure to magnetic fields. *J Cell Biochem* 69:181–188 (1998).
  23. Lin H, Opler M, Head M, Blank M, Goodman R. Electromagnetic field exposure induces rapid, transitory heat shock factor activation in human cells. *J Cell Biochem* 66:482–488 (1998).
  24. Giroldi L, Hollstein M, Yamasaki H. Cellular-oncogene expression in Friend erythroleukemia cells: relationship to differentiation, commitment and TPA effects. *Carcinogenesis* 9:817–821 (1988).
  25. Yamasaki H, Martel N, Fusco A, Ostertag W. Continuous suppression of globin gene expression and differentiation of Friend erythroleukemia cells by phorbol 12-myristate 13-acetate (PMA) despite the loss of PMA binding sites by down regulation. *Proc Natl Acad Sci USA* 81:2075–2079 (1984).
  26. Yamasaki H, Saint VL, Martel N. Long-term effect of a tumor promoter, 12-O-tetradecanoylphorbol-13-acetate, on induced differentiation of Friend leukemia cells. *Cancer Res* 40:3780–3785 (1980).
  27. Wang W, Eisenberg SR. A three-dimensional finite element method for computing magnetically induced currents in tissues. *IEEE Trans Magn* 30:5015–5023 (1994).
  28. Li IC, Chang CC, Trosko JE. Thymidylate synthetase gene as a quantitative mutation marker in Chinese hamster cells. *Mutat Res* 243:233–239 (1990).
  29. Kim NW, Piatyzek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL, Shay JW. Specific association of human telomerase activity with immortal cells and cancer. *Science* 266:2011–2015 (1994).
  30. Boorman GA, Anderson LE, Morris JE, Sasser LB, Mann PC, Grumbein SL, Hailley JR, McNally A, Sills RC, Haseman JK. Effect of 26 week magnetic field exposures in a DMBA initiation-promotion mammary gland model in Sprague-Dawley rats. *Carcinogenesis* 20:899–904 (1999).
  31. Fu W, Begley JG, Killen MW, Mattson MP. Anti-apoptotic role of telomerase in pheochromocytoma cells. *J Biol Chem* 274:7264–7271 (1999).
  32. Revoltella RP, Trombi L, Petrini M, Grassi B, Manara G, Mese ED. Low-frequency electromagnetic fields do not affect cell growth, erythroid differentiation, and virus production in variant lines of untreated and dimethyl sulfoxide-treated Friend erythroleukemia cells. *Electro- and Magnetobiol* 12:135–146 (1993).
  33. Yamasaki H, Fibach E, Nudel U, Weinstein IB, Rifkind RA, Marks PA. Tumor promoters inhibit spontaneous and induced differentiation of murine erythroleukemia cells in culture. *Proc Natl Acad Sci USA* 74:3451–3455 (1977).
  34. Blackman CF, Benane SG, House DE. Frequency-dependent interference by magnetic fields of nerve growth factor-induced neurite outgrowth in PC-12 cells. *Bioelectromagnetics* 16:387–395 (1995).
  35. Blackman CF, Blanchard JP, Benane SG, House DE. Empirical test of an ion parametric resonance model for magnetic field interactions with PC-12 cells. *Bioelectromagnetics* 15:239–260 (1994).
  36. Blackman CF, Blanchard JP, Benane SG, House DE. The ion parametric resonance model predicts magnetic field parameters that affect nerve cells. *FASEB J* 9:547–551 (1995).
  37. Weinstein IB, Gattoni CS, Kirschmeier P, Lambert M, Hsiao W, Backer J, Jeffrey A. Multistage carcinogenesis involves multiple genes and multiple mechanisms. *J Cell Physiol(suppl)* 3:127–137 (1984).
  38. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 61:759–767 (1990).
  39. Trosko JE. Human health consequences of environmentally-induced modulated gene expression: Potential roles of ELF-EMF induced epigenetic versus mutagenic mechanisms of disease. *Bioelectromagnetics* 21:402–406 (2000).
  40. Yamasaki H, Enomoto T, Hamel E, Kanno Y. Membrane interaction and modulation of gene expression by tumor promoters. In: *Cellular Interactions by Environmental Tumor Promoters* (Fujiki H, Hecker E, Moore R, Sugimura T, Weinstein IB, eds). Tokyo: Japan Scientific Societies Press, 1984:221–233.
  41. Bursch W, Oberhammer F, Schulte HR. Cell death by apoptosis and its protective role against disease. *Trends Pharmacol Sci* 13:245–251 (1992).
  42. Trosko JE, Chang CC, Medcalf A. Mechanisms of tumor promotion: potential role of intercellular communication. *Cancer Invest* 1:511–526 (1983).
  43. Teeguarden JG, Dragan YP, Singh J, Vaughan, Xu YH, Goldsworthy T, Pitot HC. Quantitative analysis of dose- and time-dependent promotion of four phenotypes of altered hepatic foci by 2,3,7,8-tetrachlorodibenzo-p-dioxin in female Sprague-Dawley rats. *Toxicol Sci* 51:211–223 (1999).

# Challeng

1.800.38.BE.

## SMART





EDUCATION  
EXCELLENCE  
PARTNERSHIP

When it comes to the environment ...



## YOU CAN'T BELIEVE EVERYTHING YOU READ

So you'd better know who you can trust. *Environmental Health Perspectives* draws on more than 25 years of scientific publishing, backed by the authority of the National Institutes of Health—National Institute of Environmental Health Sciences, to provide you with the most current and credible information available to help you make decisions about your health and the environment.

Each month we delve into the issues you care about such as:

- water, air, and soil pollution
- urban sprawl
- population and world health
- toxic pesticides
- environmental products and services



Call 1-800-315-3010 today  
to subscribe and visit us online at

<http://ehp.niehs.nih.gov/>