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ERK1/2 Phosphorylation, Induced by Electromagnetic Fields, Diminishes During Neoplastic Transformation

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Abstract It has been suggested that electromagnetic (EM) fields can act as co-promoters during neoplastic transformation. To examine this possibility, we studied the effects of 0.8-, 8-, 80-, and 300-μT 60-Hz electromagnetic (EM) fields in INITC3H/10T1/2 mouse fibroblast cells. These cells are transformed carcinogenically by methylcholanthrene, but the neoplastic phenotype can be suppressed indefinitely by the presence of retinyl acetate (RAC) in the culture medium. The effects of EM field exposures were examined at three stages: (1) before initiation of transformation (i.e., RAC in the culture media); (2) early in the transformation process (4 days after withdrawal of RAC); and (3) at full of neoplastic transformation (10 days after withdrawal of RAC). EM field exposures induced significant increases in protein levels for hsp70 and c-Fos and in AP-1 binding activity. EM fields induced phosphorylation of MAPK/ERK1/2 before the onset of transformation, but these increases diminished during the transformation process. No phosphorylation in the other major extracellular stress pathway, SAPK/JNK, was detected in cells exposed to EM fields at any time before, during, or after neoplastic transformation. Human cells HL60, MCF7, and HTB124, exposed to EM fields, also showed MAPK/ERK1/2 phosphorylation. Cells treated with the phorbol ester, TPA, served as positive controls for AP-1 activation, c-Fos protein synthesis, and ERK1/2 phosphorylation. There was no indication that EM fields affected the rate of cell transformation or acted as a co-promoter, under the conditions of this study. J. Cell. Biochem. 78:371–379, 2000.

Key words: co-promotion; stress-activated signal transduction pathways; DNA; electromagnetic fields

Low-frequency electromagnetic (EM) fields have been shown to induce a variety of biosynthetic alterations in cells and tissues [NIEHS Report, 1998; EM Goodman et al., 1995; Hong, 1995]. These effects include changes in enzyme activity [Blank, 1995], increased transcript levels for the immediate early-response genes, *myc* [Lin et al., 1994; 1996; Jin et al., 1997] and *fos* [Phillips et al., 1992; Rao and Henderson, 1996], and for the stress response gene HSP70 [Goodman and Henderson, 1988; Blank et al., 1994; Goodman et al., 1994; Goodman and Blank, 1995; 1998]. These changes are induced by EM fields with minimal perturbation of the cell. The EM field induction of HSP70 gene expression occurs at normal growth temperatures and at an energy density that is 14 orders of magnitude lower than needed to induce HSP70 by heat shock [Goodman and Blank, 1995; 1998]. The EM field-induced stress response has been observed in dipteran, yeast, mouse, and human cells and is used in our experiments as a biomarker for EM field responsiveness.

Despite the extremely large difference in energy required for inducing the expression of HSP70 by EM fields and heat shock, there are some surprising similarities [Goodman and Blank, 1998]. Both EM fields and heat shock induce the classic "heat shock puffs" in the larval salivary gland chromosomes of *Drosophila melanogaster* [Goodman et al., 1992]. Both EM fields and heat shock induce trimerization of heat shock factor 1 (HSF1) and binding to a heat shock element (HSE). However, EM fields induce HSF/HSE binding at an HSE centered

Abbreviations used: EM, electromagnetic; Hz, Hertz (cycle/s); MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated protein kinase; SAPK, stress-activated phospho kinase; RAC, retinyl acetate; HS, heat shock; hsps, heat shock proteins; μT, microTesla; TPA, 12-O-tetradecanoyl-phorbol-13-acetate.

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at −192 (the EM field domain) in the HSP70 promoter [Lin et al., 1997; 1998a,b; 1999], upstream from the HSE at −100 in the heat shock domain [Morimoto, 1993]. When the nGAAn HSE nucleotide sequence at −192 is mutated, the HSE sequence at −100 in the heat shock domain is not sufficient to evoke the EM field response [Lin et al., 1999]. Transactivation of HSP70 by EM fields requires the presence of the nCTCTn nucleotide sequence in the HSP70 promoter and the presence of c-Myc protein.

A recent report from the National Institutes of Environmental Health Sciences (NIEHS) assessed the health effects from exposure to power line frequency electric and magnetic fields and found evidence from epidemiologic, cellular, and animal studies indicating that EM fields are a "possible cause of human cancer." The role of EM fields as a co-promoter was cited in the report as a possible mechanism to enhance neoplastic transformation [NIEHS, 1998].

That EM fields may act as co-promoters was supported by earlier data [Lin et al., 1998a,b] suggesting that EM fields activate at least two signaling pathways: one involving AP-1 binding activation and a second involving trimerization of HSF and binding to HSE that increased hsp70 levels. The requirement of the oncoprotein c-Myc for mediation of the response and the increased synthesis of the oncoprotein c-Fos were additional indications that EM fields might act as co-promoters. Given these observations, and the fact that there is at present no defined mechanism(s) to explain how EM fields interact with cells to induce the variety of cellular alterations reported, we have addressed the question of whether one interaction mechanism may involve EM fields in the rate at which transformed cells become neoplastic.

For these studies, the well-defined model INITC3H/10T1/2 mouse fibroblast cell line was selected. These cells, originally developed by Bertram [1980], Bertram et al. [1981], and Mordan et al. [1982], have been transformed carcinogenically with methylcholanthrene, but expression of the neoplastic phenotype can be suppressed indefinitely by the presence of retinyl acetate (RAC) in the culture medium. Upon withdrawal of RAC, the neoplastic phenotype is observable by 10 days (manifested as the loss of contact inhibition).

In the experimental protocol used here, INITC3H/10T1/2 cells were exposed to EM fields or the phorbol ester TPA at three stages: (1) before initiation of transformation (i.e., while RAC was in the culture medium); (2) early in the transformation process (on day 4 after withdrawal of RAC from the culture medium); and (3) when full neoplastic transformation had been achieved (10 days after withdrawal of RAC from the culture medium). We sought to determine whether EM fields induced kinase phosphorylation in the two major extracellular stress-activated signal transduction pathways MAPK/ERK or SAPK/JNK during neoplastic transformation. Since earlier experiments showed that EM fields induce increased binding activation of AP-1 [Lin et al., 1998a,b], changes in c-Fos levels and AP-1-DNA binding activation were also examined. Levels of the stress protein, hsp70, were used as biomarkers for the EM field response [Goodman and Blank, 1998]. 12-O-Tetradecanoylphorbol-13-acetate (TPA)-treated cells served as an internal positive control for c-Fos protein synthesis, AP-1 binding, and ERK1/2 phosphorylation [Cano and Mahadevan, 1995].

MATERIALS AND METHODS

Cell Cultures/Growth Conditions

INIT/C3H/10T1/2 cells (a gift from Dr. S.W. Hui, Roswell Park Cancer Institute, Buffalo, NY) have been carcinogenically transformed by methylcholanthrene [Bertram, 1980; Bertram et al., 1981; Mordan et al., 1982]. The expression of the neoplastic phenotype was suppressed indefinitely by the presence of RAC in the culture medium (0.3 μg/ml RAC). When RAC was withdrawn, the neoplastic phenotype was observable in 10 days, manifested phenotypically as the loss of contact inhibition. The cells were maintained in basal medium Eagle (Gibco) (with or without RAC, depending on the experimental protocol) and supplemented with 10% fetal bovine serum (FCS) (Gibco). Cells were split twice weekly with two media changes to ensure that cells were always in exponential growth. All cultures were maintained in a dedicated NuAire model Nu2 700 CO_2 incubator. Cells were discarded after passage ten. Cells were harvested in trypsin-EDTA from the seeding vessel and counted using a hemocytometer. Trypan blue staining

determined the general health of cells at the time of counting.

TPA Induction

12-O-Tetradecanoyl-phorbol-13-acetate(TPA) was used as an internal positive control for AP-1 binding activation, c-Fos synthesis, and ERK1/2 phosphorylation. Cells were treated with 0.1 μg/ml TPA for 1 h before protein extraction [Mordan et al., 1982].

EM Field Exposure Conditions

INIT/C3H/10T1/2 cells in 100-mm Petri dishes (10^6/ml in 15 ml) were exposed (and sham-exposed) to 60-Hz sinusoidal EM fields, using Helmholtz coils (shielded in μ metal containers) in a 37°C incubator (NuAire). To determine the optimum field strength, cells were initially exposed (and sham-exposed) to 0.8-, 8-, 80-, and 300-μT EM fields at 60Hz. Cells were exposed: (1) during suppression of neoplastic phenotype (+RAC); (2) 4 days after withdrawal of RAC (4-RAC); and (3) during full neoplastic transformation (10-RAC).

EM Field Exposure System

Two fully functional exposure units permitted simultaneous sham exposure of control cultures. The Helmholtz coils (Electric Research and Management, Pittsburgh, PA) consisted of 19-gauge wire bundles wound 164 times around a square form 13 cm long and 14 cm wide with 8-cm spacing. The coils were energized by a function generator (11-MHz Wavetek Stabilized Function Generator; model 21). A digital multimeter was used to measure the field intensity and verify the system's operation (Fluke 87 digital multimeter). Field parameters were monitored with a Hitachi V-1065 100-MHz oscilloscope and calibrated inductive search coil (25×; Electro-Biology, Parsippany, NJ). Details of the exposure system, including background magnetic fields in the incubator, harmonic distortion, DC magnetic fields, and mean static magnetic fields in the incubator, both vertical and horizontal components, are described by Jin et al [1997]. The Helmholtz coils are double wound; thus, either an exposure field or a sham field can be created, based on the direction of current flow, without significant field production and as a control for the effects of coil heating.

Placement of Samples in the Helmholtz Coils

Cells in Petri dishes were placed on a Plexiglas stand in a horizontal orientation; i.e., the entire area of the dish was exposed to the field. The bottom of the dish was 2 cm below the axis level. The height from dish bottom to top surface of liquid was approximately 1.1 cm; the height of the liquid was 0.6 cm. The calculated electric field was ~11 μV/m for an 8-μT exposure.

μ Metal Shielding

Cells were shielded from stray fields during all exposures (experimental and sham) in μ metal containers [see Jin et al., 1997, for shielding specifications]. Temperature was monitored with a Physitemp (BAT-12) thermocouple probe (PhysiTemp, Hackensack, NJ), sensitive to ±0.1°C.

Protein Lysates

Protein was extracted and lysates prepared from whole cells as previously described [Lin et al., 1997; modification of Mosser et al., 1988]. Protein concentrations were determined with the Bio-Rad protein assay kit (Bio-Rad Laboratories).

Western Blot

Protein lysates were analyzed for hsp70, c-Fos, and phosphorylation of MAPK/ERK1/2 and SAPK/JNK, using the ECL detection system (Amersham). The intensity of the signal was determined with a PhosphorImager 400A (Molecular Dynamics) and quantified using ImageQuant software.

Electrophoretic Mobility Shift Assays

Electrophoretic mobility shift assays (EMSA) were performed for AP-1 binding activity as previously described. In these band-shift assays, oligonucleotides were prepared by GIBCO/BRL[Mosser et al., 1988; Lin et al., 1997] and end-labeled with γ^{32}P-ATP (NEN/DuPont).

Antibodies

Anti-hsp70 was from StresGen, anti-Fos was from Santa Cruz, phosphorylation of ERK1/2 was determined with a MAPK/ERK1/2 antibody kit (New England BioLabs, cat. no. 9100), and phosphorylation of SAPK/JNK was deter-

mined with the SAPK/JNK antibody kit (New England BioLabs, cat. no. 9250).Non-phospho ERK1/2 served as a positive control (New England BioLabs).

Statistical Analyses

A sufficient number of experiments were performed to ensure statistical significance. Samples from each experiment were tested two to three times. A minimum of five separate experiments was performed for each data point. The data were entered into Excel for analysis and the results examined with a two-tailed *t*-test. Statistical significance was determined by a multifactor analysis of variance program (INSTAT). The data are expressed as the ratio of the experimental to the control (E/C) ±standard error of the mean (±SEM).

RESULTS

EM Fields Induced Increased hsp70 in INIT/C3H/10T1/2 Cells

Dose-response thresholds and time course.

To determine baseline effects of EM fields, protein levels for hsp70 were established in initial experiments. In our earlier experiments protein levels for hsp70 proved a consistent and reliable biomarker for EM field effect. Figure 1A shows the hsp70 levels for INIT/C3H/10T1/2 cells exposed to 0.8-μT, 8.0-μT, 80-μT and 300-μT 60-Hz sinusoidal EM fields, during full suppression of the neoplastic phenotype (i.e., cells growing in media containing RAC) and during full neoplastic transformation (i.e., cells growing in media without RAC for 10 days). Cells exposed to an 80-μT EM field induced an average increase in hsp70 of 62% (7 experiments).There was no measurable elevation of hsp70 levels over baseline in protein lysates from cells exposed to field strengths of 0.8 μT and 8.0 μT or to field strengths greater than 80 μT (up to 300 μT). In fully transformed cells (Fig. 1A), no measurable increase in hsp70 levels was induced by EM fields at any field strength tested.

Continuous exposure vs single exposure.

Figure 1B and 1C shows the effect of exposure duration on hsp70 levels. Two modes of exposure were tested, using an 80-μT EM field: (1) continuous exposure (Fig. 1B) up to 180 min with samples taken for protein analysis at 10, 20, 30, 60, 120, and 180 min, followed each time by 20 min out of the field before protein extraction (Fig. 1B); and (2) a single 30-min exposure (Fig. 1C), followed by removal of cells from the field. Samples were then taken for protein analyses at 60, 120, and 180 min after the initial 30-min exposure. Under both continuous and a single 30-min exposure, an 80-μT

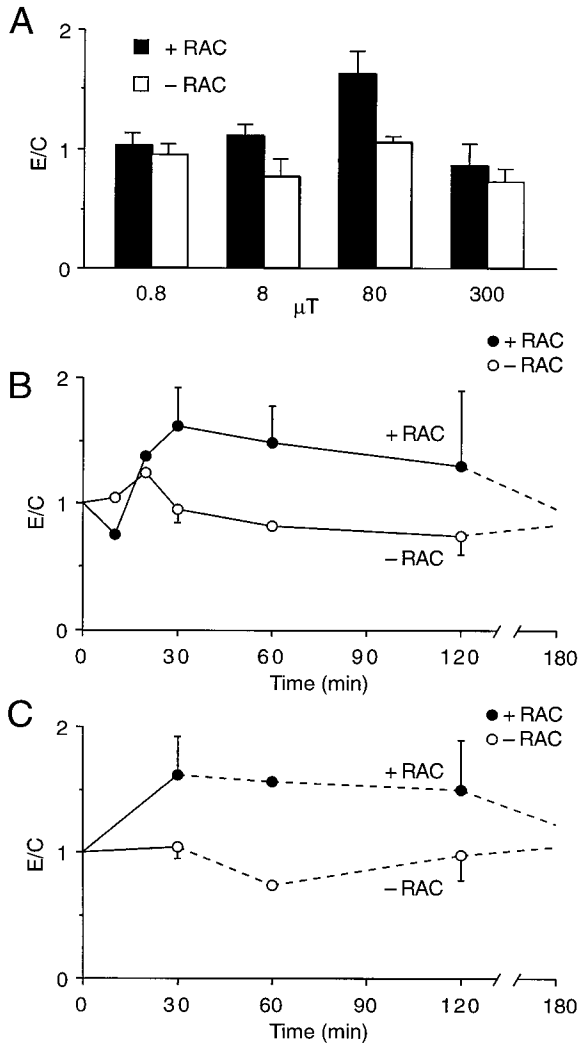

Fig. 1. Dose-response thresholds and time course of hsp70 in INIT/C3H/10T1/2 cells exposed to 60-Hz EM fields before and after neoplastic transformation. Western blot analysis. **A:** Dose-response thresholds of hsp70 in INIT/C3H/10T1/2 cells exposed and sham-exposed. cells were exposed for 30 min, followed by 20 min out of the EM field before protein extraction (7 experiments). **B:** Effect of time of EM field exposures on hsp70 levels using continuous (80 μT 60Hz) exposure up to 180 min with samples taken at 20, 30, 60, 120, and 180 min, followed by 20 min out of the field before protein extraction (6 experiments). **C:** Single 30-min exposure, followed by removal of cells from the field for protein analyses at 60, 120, and 180 min after the initial 30-min exposure (6 experiments).

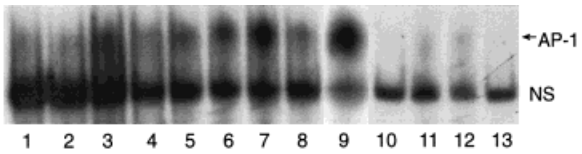

Fig. 2. Electromobility shift assay of AP-1 binding activation by an 80 μT 60-Hz EM field before, during and at full neoplastic transformation TPA incubations = 1 h, EM exposures = 30 min, sham-exposed controls = 30 min. **Lanes 1–4**, samples from cells growing in medium containing RAC. **Lane 1**, sham-exposed control; lane 2, EM 20 min; **lane 3**, EM 40 min; **lane 4**, EM 60 min. **Lanes 5–9**, samples from cells 4 days after withdrawal of RAC. **Lane 5**, sham = exposed control; **lane 6**, EM 20 min; **lane 7**, EM 40 min; **lane 8**, EM 60 min; **lane 9**, TPA 60 min. **Lanes 10–13**, samples from fully transformed cells. **Lane 10**, sham-exposed control; **lane 11**, EM 20 min; **lane 12**, EM 40 min; **lane 13**, EM 60 min.

EM field increased hsp70 levels more than 60% over baseline within 30 min in medium containing RAC. The EM field induced increases remained elevated for more than 2 h before decreasing very gradually. EM fields did not induce a measurable increase in hsp70 levels over baseline in cells that had been fully transformed with any time point, field strength, or mode of exposure tested.

EM fields induced increased AP-1 binding activation in INIT/C3H/10T1/2 cells.

An EMSA illustrates the time course of AP-1 binding activation in cells exposed to an 80 μT EM field (Fig. 2). The effect of EM fields on AP-1 binding activation in cells growing in medium containing RAC (transformation suppressed) is shown in lanes 1–4. At 40 min, the effect of EM field exposure on AP-1 activation (lane 3) was almost threefold, as compared with the sham-exposed control (lane 1); 4 days after withdrawal of RAC from the culture medium, the effect of EM fields on AP-1 activation at 40 min was reduced (lane 6). The effect of EM fields on fully transformed cells is shown in lanes 11–13 (lane 10 is a sham-exposed sample). No measurable effect of EM fields on AP-1 activation was detected in fully transformed cells (RAC withdrawn from culture medium at least 10 days). The effect of TPA on AP-1 binding activity was used throughout as a positive control; effects of 1 h of TPA treatment in cells at 4 days after withdrawal of RAC can be seen in lane 9.

EM Fields Induced Increased c-Fos Protein Levels in INIT/C3H/10T1/2 Cells

Figure 3A presents as actual counts, the effect of 80-μT EM fields (lane 2) or TPA (lane 3)

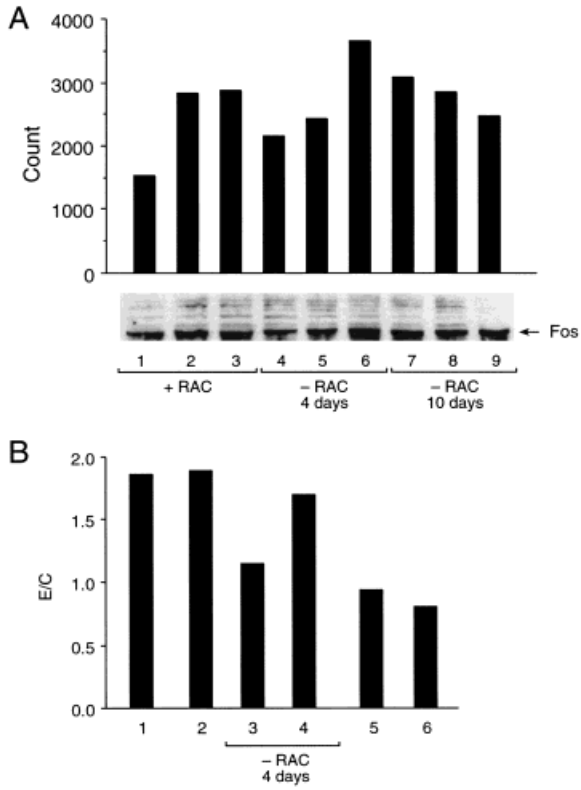

Fig. 3. Effect of 80 μT 60-Hz EM fields on c-Fos levels before, during, and at full neoplastic transformation.(11 experiments). TPA incubations = 1 h, EM exposures = 30 min, sham-exposed controls = 30 min. **A:** PhosphorImager counts. **Lanes 1–3**, samples from cells growing in medium containing RAC. **Lane 1**, sham-exposed control; **lane 2**, EM field; **lane 3**, TPA. **Lanes 4–6**, samples from cells 4 days after withdrawal of RAC. **Lane 4**, sham-exposed control; **lane 5**, EM; **lane 6**, TPA. **Lanes 7–9**, samples from fully transformed cells. **Lane 7**, sham-exposed control; **lane 8**, EM; **lane 9**, TPA. **B:** E/C = experimental value/control value. **Lane 1**, EM +RAC (transformation suppressed); **lane 2**, TPA +RAC; **lane 3**, EM 4-RAC (4 days after withdrawal of RAC); **lane 4**, TPA 4-RAC; **lane 5**, EM fully transformed; **lane 6**, TPA fully transformed.

on c-Fos protein levels in cells in which transformation was suppressed (medium containing RAC). Lane 5 shows the effect of EM fields on c-Fos levels at the beginning of transformation (4 days after withdrawal of RAC from culture medium), as compared with the sham-exposed sample (lane 4) and TPA-treated sample (lane 6). In cells displaying the full neoplastic phenotype, c-Fos is still elevated (lane 8).

The counts shown in Figure 3A are presented in Figure 3B as the ratio of the experimental counts/control counts (E/C). A twofold increase in c-Fos protein levels was measured in cells growing in RAC and exposed to either EM fields (lane 1) or TPA (lane 2). The effect of EM fields on

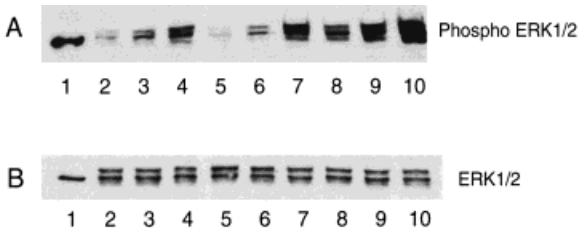

Fig. 4. Magnetic field-induced MAPK/ERK1/2 phosphorylation (12 experiments). TPA incubations = 1 h, EM exposures = 30 min, sham-exposed controls = 30 min. **A:** Phospho ERK1/2. **Lane 1**, positive phospho control; **lane 2**, sham-exposed control +RAC (transformation suppressed); **lane 3**, EM +RAC; **lane 4**, TPA +RAC; **lane 5**, sham-exposed 4-RAC (4 days after withdrawal of RAC); **lane 6**, EM field 4-RAC; **lane 7**, TPA 4-RAC; **lane 8**, sham-exposed 10-RAC (full neoplastic transformation); **lane 9**, EM fields 10-RAC; **lane 10**, TPA 10-RAC. **B:** ERK1/2. **Lane 1**, positive nonphospho control; **lane 2**, sham-exposed control +RAC (full neoplastic suppression); **lane 3**, EM +RAC; **lane 4**, TPA +RAC; **lane 5**, sham-exposed 4-RAC (4 days after withdrawal of RAC); **lane 6**, EM field 4-RAC; **lane 7**, TPA 4-RAC; **lane 8**, sham-exposed 10-RAC (full neoplastic transformation); **lane 9**, EM fields 10-RAC; **lane 10**, TPA 10-RAC.

c-Fos levels was diminished to baseline levels 4 days after the withdrawal of RAC from the medium for 4 days (lane 3), and below baseline 10 days after the withdrawal of RAC (lane 5). A similar pattern in c-Fos levels was observed in cells treated with TPA (lanes 4 and 6).

EM Fields Induce Increased Phosphorylation of MAPK/ERK1/2

Kinase phosphorylation was examined to determine whether either or both MAPK/ERK1/2 or SAPK/JNK, the two major stress-activated pathways, were involved in EM field-induced stress (Fig. 4A). There was a twofold increase in ERK1/2 phosphorylation in fully suppressed cells exposed to EM fields (i.e., in +RAC culture medium) (lane 3), as compared with the sham-exposed sample (lane 2). The sample from the TPA-treated cells is in lane 4. A reduced effect of EM fields on MAPK/ERK1/2 phosphorylation was noted in 4 days after RAC withdrawal (lane 6). The effect of EM fields on ERK1/2 phosphorylation in fully neoplastic cells (lane 9) was only slightly elevated over the level in the sham-exposed cells (lane 8). TPA induction of MAPK/ERK1/2 phosphorylation (lanes 4, 7, and 10) is more pronounced than EM field effect throughout. The positive control for phosphorylation, phospho-ERK1/2, is in lane 1. Neither 80-μT EM fields nor TPA induced kinase phosphorylation in SAPK/JNK (data not shown).

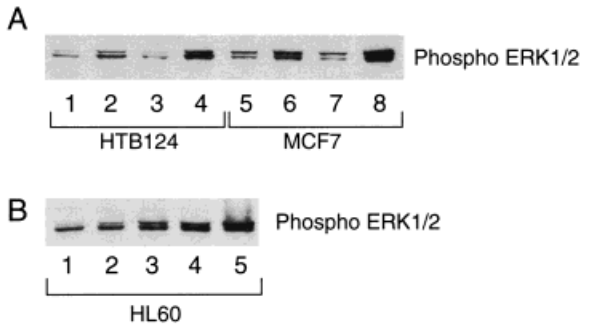

Fig. 5. Induction of MAPK/ERK1/2 phosphorylation in human cells by 8 μT and 80 μT 60-Hz EM fields (5 experiments). **A:** HTB 124 and MCF7 cells. **Lanes 1,5**, sham-exposed; **lanes 2,6**, EM at 80 μT for 30 min; **lanes 3,7**, EM at 8 μT for 30 min; **lanes 4,8**; TPA 1 h. **B:** HL60 cells. **Lane 1**, sham-exposed; **lane 2**, EM at 8 μT for 30 min; **lane 3**, EM at 80 μT for 30 min; **lane 4**, EM at 80 μT for 30 min; **lane 5**, TPA for 1 h.

Figure 4B shows background nonphospho ERK1/2 in cells exposed to 80-μT EM fields or treated with TPA before transformation (lanes 3 and 4), early in transformation (lanes 6 and 7), and at full transformation (lanes 9 and 10). At full transformation, EM fields and TPA had little or no effect. The positive control (lane 1) is nonphospho ERK1/2 and lanes 2, 5, and 8 are the unexposed sham controls.

EM Fields Induce MAPK/ERK1/2 Phosphorylation in Three Human Cell Lines

Three human cell lines known to respond to EM fields with a 2-3 fold induction of HSP70 expression [Goodman and Blank, 1998] were tested to determine whether 8.0-μT and/or 80-μT 60-Hz EM fields induce MAPK/ERK1/2 phosphorylation in human cells. Figure 5A shows the induction of MAPK/ERK1/2 phosphorylation by EM fields in HTB124 (lanes 2 and 3) (normal breast cells from a patient with a genetic history of breast cancer) and in MCF7 (lanes 6 and 7)(human breast carcinoma) cells after 30-min EM field exposures. Figure 5B shows the induction of MAPK/ERK1/2 phosphorylation by EM fields in HL60 cells (human leukemic cells) after 30 min EM field exposures (lanes 2–4). All three cell lines showed greater induction of ERK1/2 phosphorylation with an 80-μT EM field than with an 8.0-μT EM field. There was a pronounced induction of ERK1/2 phosphorylation by TPA in all three cell lines. SAPK/JNK was not phosphorylated by EM fields in these human cell lines.

DISCUSSION

Signaling Pathways During EM Field Interaction With Cells

It is currently believed that activation of the stress response occurs when extracellular signals affect receptors in the plasma membrane and that this subsequently initiates the specific signal transduction cascades involved in regulating cell proliferation, differentiation, and metabolism. Each cascade is thought to pass its message to the cell nucleus via protein kinases that propagate and amplify the signal, with different molecules activating different pathways. The final step in activation of gene expression requires the DNA-binding of specific transcription factors (e.g., HSF, AP-1) to specific nucleotide sequences in the promoter of the gene. Extracellular signals that affect transcription factor binding activity also affect steps in this process [reviewed in Waskiewicz and Cooper, 1995; Karin, 1995].

Mitogen-activated protein kinases (MAPKs) modulate many cellular events and play an important role in signal transduction in eukaryotic cells. The MAPKinase family includes the ERK, JNK, and p38 kinases; they are found in three interwoven signal transduction cascades. These kinases phosphorylate and thus activate transcription factors in response to mitogens and growth factors, as well as a variety of stresses. Both threonine and tyrosine phosphorylation are necessary for activation of MAP/ERKinases. Eukaryotic cells use either MAPK or SAPK cascades, in combination with other signaling pathways in response to extracellular stresses. This leads to alterations in the phosphorylation status of the transcriptional machinery to reflect a specific stimulus [Waskiewicz and Cooper, 1995; Hafen, 1998].

MAPKinases (44 kDa and 42 kDa), when activated, translocate to the nucleus and activate transcription of immediate early response genes. EM fields are also known to increase transcript levels of two immediate early response genes, c-*fos* and c-*myc* [Rao and Henderson, 1997; Phillips et al., 1992; Jin et al., 1997]. When ERKs become strongly activated, as for example by growth factors, phorbol esters (TPA) or, as we have shown here, by EM fields, activation of JNK is minimal. Because EM fields are a gentle extracellular stress (as compared with such disruptive agents as cyclohexamide, UV, growth factors, or phorbol esters), they provide a much less disruptive model for defining these pathways.

EM Field Interactions During Neoplastic Transformation

In this report we show that:

1. The ability of EM fields to induce elevated levels of hsp70 and c-Fos occurs primarily before the onset of neoplastic transformation.
2. The ability of EM fields to induce AP-1 binding-activation and c-Fos and hsp70 levels was diminished when RAC was withdrawn from the culture medium.
3. The ability of EM fields to induce ERK1/2 phosphorylation was most pronounced when neoplastic transformation was suppressed.
4. EM fields did not activate the SAPK/JNK pathway before, during, or after neoplastic transformation.
5. EM fields induced ERK1/2 phosphorylation in human leukemic cells (HL60), human breast carcinoma cells (MCF7) and "normal" human breast cells (HTB124), in addition to the rodent INIT/C3H/10T1/2 cells.

It has been suggested that EM fields can act as co-promoters, i.e., that they can accelerate the process whereby cells become neoplastic. The experiments described here show no such effect of EM fields. The gradual loss of the stress response, which is a consistent and reliable biomarker for these cells, as well as the response to TPA, the positive control, are both indications of a change in properties of the transformed cell that now make it unresponsive to the extracellular stress from EM fields. Thus, co-promotion by EM fields is not a mechanism in the model system used in this report.

Can Extremely Low-Frequency EM fields Act Directly on Nuclear DNA?

Although it is generally believed that information arriving at the surface of the cell is transmitted as a signal through serine/threonine kinases in the cytoplasm to transcription factors in the nucleus, other interaction mechanisms should also be considered. Based on significant differences between EM field-induced stress and other forms of stress (e.g., heavy metals, amino acid analogues, heat), the conventional stress-activated signal transduction pathways may not necessarily be

the only mechanism for extracellular signaling to the nucleus. There are several lines of evidence that support a direct effect of EM fields on DNA through interaction with conducting electrons in the DNA [Blank and Goodman, 1997; 1999].

Magnetic fields penetrate the nucleus without attenuation, and it has been shown that DNA conducts electrons through its π-stacked arrays of heterocyclic base pairs [McClellan et al., 1990]. Vulnerable sites on the DNA (e.g., guanine) can suffer damage due to electron flow even when an oxidizing agent is attached to the DNA at a distance [Dandliker et al., 1997]. Because EM fields interact with moving charges, their interaction with the conducting electrons in the DNA could occur. Different DNA sequences have different conductivities [Meggers et al., 1998] and specific nucleotide sequences may function as "receptors" for these low-frequency EM fields. In response to fields acting on the moving charges in DNA, conformational changes could occur. The identification of an EM field-sensitive enhancer sequence [Lin et al., 1999] provides evidence of EM field interaction at the regulatory level of the gene and could eventually provide structural information about a EM field "receptor" on the DNA as well.

Both direct interaction of magnetic fields with DNA as well as signal transduction cascades initiated at the cell membrane lead to signal amplification. In the membrane model, signal transduction is via cascades using phosphokinases. In the direct interaction with DNA model, signal transduction could be effected by interaction with large electron flows. However, both models leave unanswered the key question: How can DNA be destabilized to initiate transcription? The membrane model relies on transcription factor binding to specific nucleotide sequences in the promoter DNA. By contrast, the direct interaction with DNA model suggests that disturbance of electron flows within the DNA result in chain bending or kinking that destabilizes the quiescent state.

Since EM field-induced stress is stimulated at very low energy levels, with minimal perturbation of the cell, it offers a unique experimental tool for examining the steps involved in the cellular response to extracellular signals.

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REFERENCES

Bertram JS. 1980. Structure-activity relationships among various retinoids and their ability to inhibit neoplastic transformation and to increase cell adhesion in the C3H/10T1/2clone8 cell line. Cancer Res 40:3141–3146.

Bertram JS, Mordan LJ, Blair SJ, Hui S. 1981. Effects of retinoids on neoplastic transformation, cell adhesion and membrane topography of cultured 10T1/2 cells. Ann NY Acad Sci 358:218–236.

Blank M, Goodman R. 1997. Do electromagnetic fields interact directly with DNA? Bioelectromagnetics 18:111–115.

Blank M, Goodman R. 1999. Electromagnetic fields may act directly on DNA. J Cell Biochem 75:369–374.

Blank M, Khorkova O, Goodman R. 1994. Changes in polypeptide distribution stimulated by different levels of electromagnetic and thermal stress. Bioelectrochem Bioenerg 33:109–114.

Cano E, Mahadevan LC. 1995. Parallel signal processing among mammalian MAPKs. TIBS 20:117–122.

Dandliker PJ, Holmlin RE, Barton JK. 1997. Oxidative thymine dimer repair in the DNA helix. Science 275: 146–167.

Goodman EM, Greenebaum B, Marron MT. 1993. Altered protein synthesis in a cell-free system exposed to a sinusoidal magnetic field. Biochim Biophys Acta 1202:107–112.

Goodman R, Blank M. 1995. Biosynthetic stress response in cells exposed to electromagnetic fields. Adv Chem 250:423–436.

Goodman R, Blank M. 1998. Magnetic field stress induces expression of *hsp70.* Cell Stress Chaperones 3:79–88.

Goodman R, Henderson A. 1988. Exposure of salivary gland cells to low-frequency electromagnetic fields alters polypeptide synthesis. Proc Natl Acad Sci USA 85:3928–3932.

Goodman R, Blank M, Lin H, Khorkova O, Soo L, Weisbrot D, Henderson A. 1994. Increased levels of HSP70 transcripts are induced when cells are exposed to low frequency electromagnetic fields. Bioelectrochem Bioenerg 33:115–120.

Hafen E. 1998. Kinases and phosphatases—a marriage is consummated. Science 280:1212–1213.

Hong FT. 1995. Magnetic field effects on biomolecules, cells and living organisms. BioSystems 36:187–229.

Jin M, Lin H, Han L, Opler M, Maurer S, Blank M, Goodman R. 1997. Biological and technical variables in *myc* expression in HL60 cells exposed to 60Hz electromagnetic fields. Bioelectrochem Bioenerg 44:210–217.

Karin M. 1995. The regulation of AP-1 activity by mitogen-activated protein kinases. J Biol Chem 270:16483–16486.

Lin H, Opler M, Head M, Blank M, Goodman R. 1997. Electromagnetic field exposure induces rapid, transitory heat shock factor activation in human cells. J Cell Biochem 66:482–488.

Lin H, Head M, Blank M, Han L, Jin M, Goodman R. 1998a. *Myc*-mediated transactivation of HSP70 expression following exposure to magnetic fields. J Cell Biochem 69:181–188.

Lin H, Head M, Blank M, Han L, Goodman R. 1998b. Magnetic field activation of protein-DNA binding. J Cell Biochem 70:297–303.

Lin H, Blank M, Goodman R. 1999. A magnetic field-responsive domain in the human HSP70 promoter. J Cell Biochem 75:170–176.

McClellan JA, Boublikova P, Palcek E, Lilley DMJ. 1990. Superhelical torsion in cellular DNA responds directly to environmental and genetic factors. Proc Natl Acad Sci USA 87:8373–8377.

Meggers E, Michel-Beyerle ME, Giese B. 1998. Sequence dependent long range hole transport in DNA. J Am Chem Soc 120:12950–12955.

Mordan LJ, Bergin LM, Budnick L, Meegan RR, Bertram JS. 1982. Isolation of methylcholanthrene-"initiated" C3H/10T1/2 cells by inhibiting neoplastic progression with retinyl acetate. Carcinogenesis 3:279–285.

Morimoto RI. 1993. Cells in stress: transcriptional activation of heat shock genes. Science 259:1409–1410.

Mosser DD, Theodrakis NG, Morimoto RI. 1988. Coordinate changes in heat shock element-binding activity and HSP70 gene transcription rates in human cells. Mol Cell Biol 8:4736–4744.

NIEHS. 1998. Assessment of health effects from exposure to power line frequency electric and magnetic fields.

Phillips JL, Haggren W, Thomas WJ, Ishida-Jones T, Adey R. 1992. Magnetic field induced changes in specific gene transcription. Biochim Biophys Acta 1132:140–144.

Rao S, Henderson AS. 1996. Regulation of c-*fos* is affected by electromagnetic fields. J Cell Biochem 63:356–358.

Waskiewicz AJ, Cooper JA. 1995. Mitogen and stress response pathways: MAP kinase cascades and phosphatase regulation in mammals and yeast. Curr Opin Cell Biol 7:798–805.