Power-Line Frequency Electromagnetic Fields Do Not Induce Changes in Phosphorylation, Localization, or Expression of the 27-Kilodalton Heat Shock Protein in Human Keratinocytes

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The linkage of the exposure to the power-line frequency (50–60 Hz) electromagnetic fields (EMF) with human cancers remains controversial after more than 10 years of study. The *in vitro* studies on the adverse effects of EMF on human cells have not yielded a clear conclusion. In this study, we investigated whether power-line frequency EMF could act as an environmental insult to invoke stress responses in human keratinocytes using the 27-kDa heat shock protein (HSP27) as a stress marker. After exposure to 1 gauss (100 μT) EMF from 20 min to 24 hr, the isoform pattern of HSP27 in keratinocytes remained unchanged, suggesting that EMF did not induce the phosphorylation of this stress protein. EMF exposure also failed to induce the translocation of HSP27 from the cytoplasm to the nucleus. Moreover, EMF exposure did not increase the abundance of HSP27 in keratinocytes. In addition, we found no evidence that EMF exposure enhanced the level of the 70-kDa heat shock protein (HSP70) in breast or leukemia cells as reported previously. Therefore, in this study we did not detect any of a number of stress responses in human keratinocytes exposed to power-line frequency EMF. *Key words:* electromagnetic fields, heat shock proteins, HSP70, HSP27 phosphorylation, HSP27 translocation, keratinocytes, signal transduction, stress. *Environ Health Perspect* 111:281–287 (2003). doi:10.1289/ehp.5395 available via *http://dx.doi.org/* [Online 20 November 2002]

The health risks posed by power-line frequency electromagnetic fields (EMF) are controversial. Almost 50 epidemiologic studies have been published on the topic of residential and occupational exposure to power-frequency fields and cancer risk. The majority of these reports indicate a weak association between exposure to 50–60 Hz fields and the incidence of cancer; however, numerous methodologic flaws reduce their credibility (*1*). Recent studies by the National Cancer Institute indicate that children living near high-voltage power lines do not have an increased risk of lymphoblastic leukemia (*2*). In contrast, other recent reviews of the issue have concluded that it is premature to dismiss concerns about residential EMF and childhood leukemia (*3*). Also controversial are the early epidemiologic data suggesting a connection between EMF and breast cancer (*4,5*); this connection has not been verified by recent studies using animal models (*6*). Although there have been no specific reports regarding the incidence of skin tumors in populations exposed to 60-Hz fields, one study notes that 60 Hz (2 mT) EMF may act as a tumor co-promoter in murine skin previously initiated by the application of chemical carcinogens (*7*). Again, adding to the controversy, using essentially the same protocols, other investigators found no co-promoting activity of EMF (*8*). These uncertainties have led investigators to study the possible effects of power-line frequency EMF on human and mammalian cells in culture where the mechanisms that mediate cellular responses can be dissected. Of note, most of the studies over the past decade have failed to detect any significant cellular responses to EMF exposure (*9,10*).

There are no general models or biomarkers for the assessment of cellular effects of EMF exposure (*11*). It is generally agreed that the low energy EMF, of power-line frequency, is insufficient to cause direct damage to DNA. Therefore, it is unlikely that EMF exposure is able to initiate tumor formation through DNA mutation (*11,12*). An alternative hypothesis is that EMF exposure alters the biochemical signaling pathways that regulate cell proliferation, differentiation, and apoptosis (*12,13*). A number of gene products involved in signal transduction, including tyrosine kinases, phospholipase C, protein kinase C, and protein kinase A, have been linked to effects of EMF exposure (*13–15*), but the results have not been consistent (*12,16,17*). A highly cited study by Lin and Goodman (*18*) reported that EMF exposure induced expression of c-*myc*, an oncogene implicated in a host of cancers including breast cancer. However, attempts to replicate their findings by other investigators have failed (*19–22*). Moreover, Balcer-Kubiczek et al. (*23*) and Loberg et al. (*24*) demonstrated that EMF exposure had no significant effects on expression of a battery of cancer-related genes including c-*myc* in different cell types, including breast cells.

To provide a mechanism for the putative cellular effects imposed by EMF exposure, some investigators have suggested that cells respond to EMF in the same way as they do to other environmental stresses such as heat shock (*25,26*). Numerous cell stressors induce a common protective response: upregulation of a group of proteins called stress or heat shock proteins (HSP). These proteins function as molecular chaperones, binding to other cellular proteins and keeping them in a functional, folding-competent state (*27*). Therefore, the highly conserved heat shock proteins are often used to monitor the impact of various environmental stresses. If low-frequency EMF exposure presents a subtle stress to cells as suggested, it might provoke a response of HSP similar to that exhibited by cells exposed to other insults. For example, if EMF exposure generates or stabilizes free radicals in cells, as recently proposed (*12*), then the downstream activation of the oxidative stress pathway, with phosphorylation of mitogen-activated protein kinases such as p38 or c-*Jun* NH_2-terminal kinase and subsequent upregulation or phosphorylation of heat shock proteins, should be readily measurable (*28,29*). Indeed, Goodman and co-workers have reported that EMF exposure increased expression of the 70-kDa heat shock protein (HSP70) in leukemia and breast cells (*26,30*).

Epidermal keratinocytes of the skin, by virtue of their location at the outermost surface of the body, are maximally exposed to environmental EMF. Exposure of keratinocytes to other energy source stresses such as UV irradiation or heat induces both the expression and phosphorylation of the 27-kDa heat shock protein (HSP27), a member of the small heat shock protein family, through the reactive oxygen species/p38 mitogen-activated protein kinase-dependent pathway (*31,32*). Thus, we hypothesized that HSP27 induction and/or phosphorylation might likewise serve as an early biomarker for human EMF exposure. We selected 1 gauss (100 μT) exposure based on the range of magnetic field exposures found in industrial and residential environments. These exposures range from 2 mG in typical

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residential settings to 2 G in industrial settings (*33*). Our data demonstrate that EMF exposure has no effects on the phosphorylation, cellular relocalization, or expression of HSP27. In addition, we repeated the experiments reported by the Goodman group (*26,30*), exposing leukemia and breast cells to EMF. In contrast to their reported findings, we did not find an increase in HSP70 induced by EMF exposure.

Materials and Methods

EMF exposure system. The EMF exposure system consists of two identical modules, each housed in identical incubators (Figure 1A–C). A uniform electromagnetic field was applied using a cube-shaped Merritt coil with four square, double-wrapped coils 12.5 inches on a side using 26/11/11/26 turns, respectively (Figure 1A). The wire used was 24-gauge, parallel speaker wire, and the total resistance of a single wire forming four coils was 8.3 ohms. Using speaker wire with two parallel leads allowed us to pass current through the parallel pair in a parallel or antiparallel mode. For EMF exposure, current was passed in a parallel mode through each wire of the pair. For non-EMF–exposed controls, current was passed in an antiparallel manner through the pair of wires so that the magnetic fields generated by each one would cancel each other while generating the same amount of joule heating as in the experimental exposure. This coil was placed inside a mu-metal box (Figure 1B) that was, in turn, placed inside a CO_2 incubator held at 37°C (Figure 1C). The mu-metal box eliminated any external magnetic fields generated by the incubator and the Earth. To compensate for the lack of the Earth's magnetic field, a DC current of 115 mA was driven through each of the parallel coils to simulate the Earth's field of 250 mG. This DC current generated a joule heating of 110 mW in each coil, and we did not detect any temperature increase near the coils due to this joule heating. The AC electromagnetic fields used were at the most 100 µT root mean square, and they were generated by currents of 241 mA root mean square in each of the parallel coils. The distribution of both the DC and AC electromagnetic fields in the incubators was determined using a gaussmeter (Model 9640; F.W. Bell, Orlando, FL). The background field in the incubator where control samples were placed was zero when no current was passed through the coils. We positioned all of the culture dishes in the central area on the shelf where the EMF was very uniform (Figure 1D).

We monitored and adjusted temperature and CO_2 in the control and EMF incubators so that the culture conditions in the two incubators were identical. All experiments were done in a double-blind fashion with randomly labeled samples, so that the identities of EMF-exposed and control samples remained unknown to the experimenters who made measurements until all of the data were analyzed.

Cell culture. Normal human keratinocytes were derived from neonatal foreskin and maintained in Medium 154 (Cascade Biologics, Inc., Portland, OR) as described by Rood et al. (*34*). Cells of passage 5–6 were used in this study. The human skin-derived keratinocytes of HaCaT line (a gift of N. Fusenig) were cultured in Dulbecco's modified Eagle's medium (Life Technologies, Gaithersburg, MD), supplemented with 10% (v/v) bovine calf serum (Hyclone Laboratories Inc., Logan, UT). Both normal and HaCaT keratinocytes were grown at 37°C in a 5% CO_2 incubator and exposed to EMF when cultures had reached confluence.

The HTB124 human mammary epithelial cell line was kindly provided by R. Goodman and cultured following published protocol (*30*). The experiment with HL60 human promyelocytic leukemia cells was performed in the laboratory of R. Goodman, who kindly allowed one of us (B.S.) to participate in the experiment performed there.

Extraction of cellular proteins. The extraction of cellular proteins was carried out at 4°C. The cultures were quickly rinsed with Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS), scraped, and pelleted by centrifugation at 500 × *g*. The cells were resuspended in an extraction buffer (pH 7.4) containing 10 mM Tris, 10 mM NaCl, 2 mM ethylenediamine tetraacetic acid (EDTA), 2 mM phenylmethysulfonyl fluoride (PMSF), and protease inhibitors (2.5 µg/mL aprotinin, 1.0 µg/mL bestatin, 2.5 µg/mL leupeptin, and 1.0 µg/mL pepstatin A), then lysed by ultrasonication on a Sonifer Cell Disrupter Model W140 (Ultrasonics Inc., Plainview, NY). After being rocked for 30 min, cell lysates were centrifuged at 10,000 × *g* for 10 min. The supernatants were aliquoted as cell extracts and stored at –80°C. We determined protein concentrations of the samples using a Bradford protein assay (Bio-Rad Laboratories, Hercules, CA).

For some experiments, cells were lysed using a published protocol (*30*). Cells were harvested in PBS and centrifuged. Cell pellets were frozen overnight at –70°C, lysed in 100 µL of Mosser's buffer (20 mM HEPES, 25% glycerol, 0.42 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM PMSF, and 1.0 mM dithiothreitol, pH 8.0), then centrifuged at 12,000 rpm in an Eppendorf centrifuge for 20 min. We collected the supernatants for gel electrophoresis.

Gel electrophoresis and immunoblotting. We carried out one-dimensional isoelectric focusing (IEF) gel electrophoresis as previously described (*35,36*). A mini IEF gel was prefocused for 30 min at 150 mV. Samples of equal amounts of protein, premixed with a loading buffer, were loaded. IEF was run at 150 mV for 30 min, at 200 mV for 120 min, and at 250 mV for 30 min with 20 mM NaOH as catholyte and 10 mM H_3PO_4 as anolyte. The cellular proteins on the IEF gel were transferred onto Immunobilon-P membranes (Millipore, Bedford, MA) at 100 mV using 0.7% acetic acid solution as the transfer buffer. In SDS-PAGE, samples were loaded into a 10% acrylamide gel, and electrophoresis

Figure 1. EMF exposure system and field distribution. (*A*) Photograph of cube-shaped Merritt coil. (*B*) Photograph of the coil inside the mu-metal box, which was placed inside a CO_2 incubator. (*C*) Photograph of the mu-metal box inside the CO_2 incubator. (*D*) Magnetic field distribution within the closed mu-metal box when a 100 µT (1 gauss) field was applied in the coil. The central region of about 25 square inches was very uniform, and this was where culture plates were positioned. See "Materials and Methods" for details.

was done at 200 mV. We transferred the proteins on the gel onto a membrane at 100 mV for 1 hr.

For immunoblotting, membranes were blocked with 5% non-fat dry milk in PBS, then probed with mouse IgG anti-HSP27 or mouse IgG anti-HSP70 (StressGen Biotechnologies Corp., Victoria, BC, Canada) at 1:2,000–5,000 dilution, or IgG anti-actin (Sigma Chemical Co., St. Louis, MO) at 1:200–400 dilution. The immunoreactive proteins were detected using horseradish peroxidase-linked anti-mouse IgG, then stained with ECL (electrogenerated chemiluminescence) Western blotting detection reagents (Amersham Life Science Inc., St. Louis, MO). We scanned immunoblots on a UMAX S-6E scanner and determined the optical density (OD) of each band using NIH Image 1.61 (National Institutes of Health, Bethesda, MD). We used only images with bands within the linear range of detection for evaluation of protein abundance and densitometery.

To compare the "non-normalized" OD values of HSP (HSP27 or HSP70) bands in control and EMF-exposed groups, the average OD of all HSP bands in each group was taken; then the E/C OD ratio (average OD of HSP bands in EMF-exposed samples over average OD of HSP bands in control samples) was calculated. To normalize OD values of HSP bands based on the amount of proteins loaded in each well, we took the average OD of all actin bands and then calculated the normalizing factor (i.e., loading difference) for each lane by dividing the OD of each individual actin band by the average OD of all actin bands. The normalized OD of each HSP band was obtained by dividing the non-normalized OD of the HSP band by the normalizing factor. Finally, the average of the normalized OD of four HSP bands of each treatment was taken and compared.

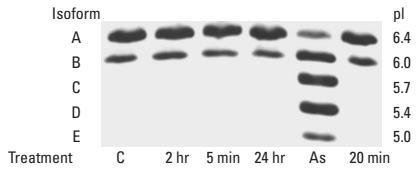

Figure 2. IEF immunoblot of HSP27 isoforms in normal keratinocytes exposed to a 100 µT EMF for 5 min, 20 min, 2 hr, or 24 hr, or treated with 100 µM sodium arsenite (As) for 2 hr. C, control sample. HSP27 isoforms were separated and detected using IEF immunostaining as described in "Materials and Methods." EMF exposure did not induce changes in HSP27 isoforms in these cells. Two independent experiments were done, and similar results were obtained in duplicate samples from each experiment.

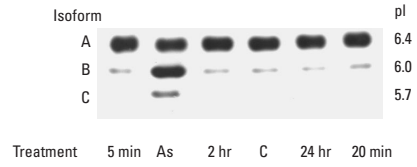

Figure 3. IEF immunoblot of HSP27 isoforms in HaCaT keratinocytes exposed to a 100 µT EMF for 5 min, 20 min, 2 hr, or 24 hr, or treated with 100 µM arsenite (As) for 2 hr. C, control sample. HSP27 isoforms were separated and detected using IEF immunostaining as described in "Materials and Methods." EMF exposure did not induce changes in HSP27 isoforms in the HaCaT cell line. Similar results were obtained in two independent experiments with duplicate samples.

Immunofluoresence staining. Keratinocytes were plated on 12-mm diameter round coverslips and cultured until nearly confluent before use. After treatments, cells were fixed with 4.0% paraformaldehyde in PBS, permeabilized with 0.1% Triton X-100 in PBS, then blocked in a blocking buffer of 2% bovine serum albumin/PBS. The blocked samples were incubated with mouse IgG anti-HSP27 (1:250 in the blocking buffer) at 4°C overnight and detected with biotinylated anti-mouse antibody (1:500 in the blocking buffer) for 1 hr, then stained with fluorescein isothiocyanate (Sigma; 1:1,000 in the blocking buffer) for 30 min. We examined and photographed coverslips with a Dage-MTI camera (model CCD-72T; Dage-MTI, Michigan City, IN). Digital images were captured and stored in a computer using NIH Image 1.61.

Results

Phosphorylation of HSP27 in keratinocytes. In unstressed normal keratinocytes, the non-phosphorylated HSP27 (HSP27A, pI 6.4) isoform is predominantly expressed along with a small amount of the monophosphorylated isoform, HSP27B (pI 6.0). Exposure of cells to a 1 gauss (100 µT), 60 Hz electromagnetic field did not induce the phosphorylation of HSP27

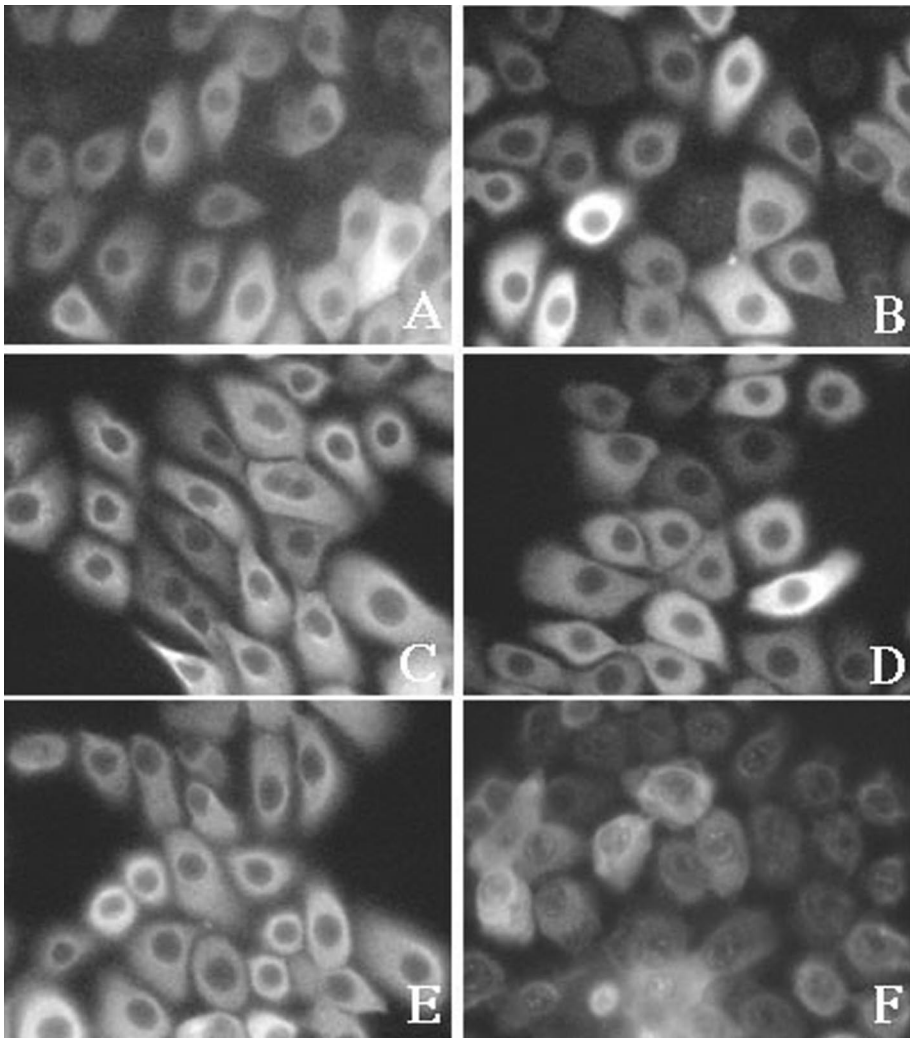

Figure 4. Photomicrographs of control cells not exposed to EMF (*A*), cells exposed to 100 µT EMF for 5 min (*B*), 20 min (*C*), 2 hr (*D*), or 24 hr (*E*), or treated with 100 µM arsenite for 2 hr (*F*). HSP27 was visualized by immunofluorescence as described in "Materials and Methods." EMF exposure did not induce the translocation of HSP27 from the cytoplasm to the nucleus in normal keratinocytes. Similar results were obtained in two independent experiments with triplicate samples.

(Figure 2). There was neither an immediate (5 or 20 min) nor late (24 hr) change in the isoform pattern of HSP27 in response to EMF exposure. In a positive control, we treated the cells with 100 μM sodium arsenite ($NaAsO_2$) for 2 hr, and the phosphorylation of HSP27 was noted, as has been previously reported (*32*). The arsenite-treated cells exhibited a marked reduction of the nonphosphorylated HSP27A and significant increase in monophosphorylated HSP27B. The further phosphorylation of HSP27 resulted in an increased level of biphosphorylated HSP27C (pI 5.7) and triphosphorylated HSP27D isoforms (pI 5.4) and a small amount of a fifth isoform.

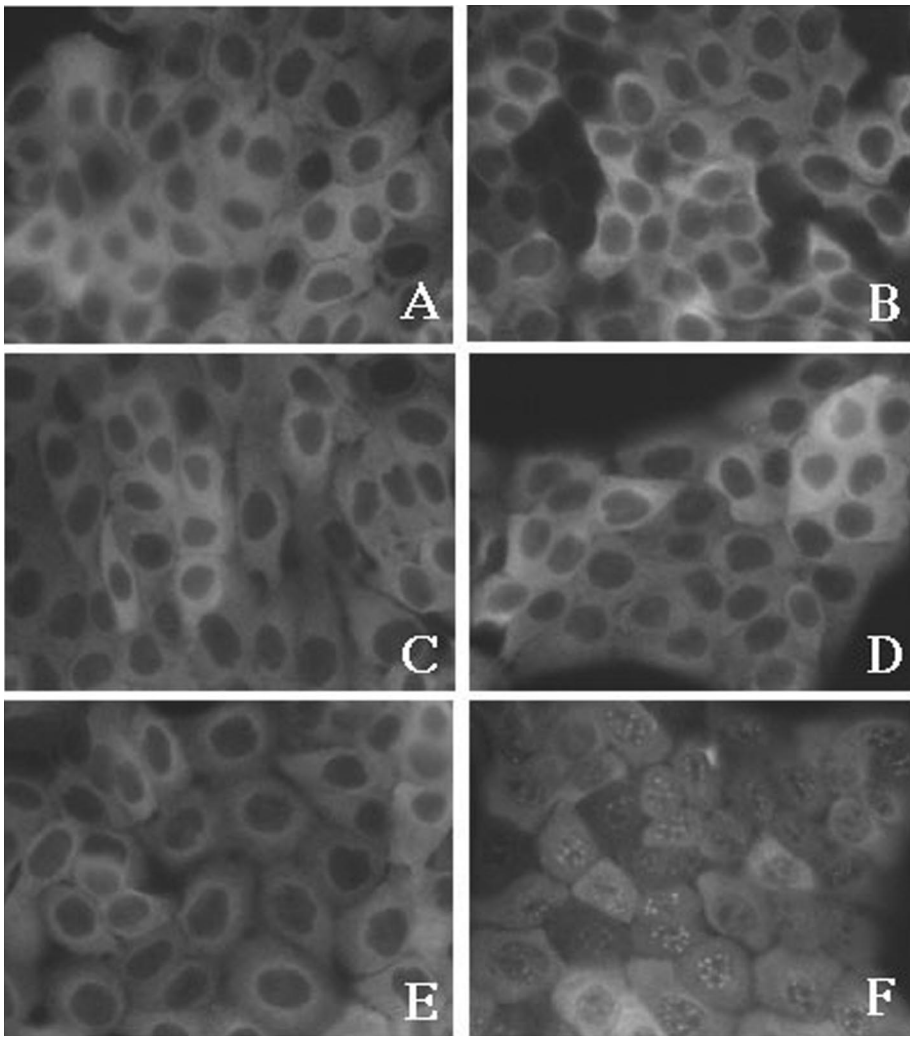

Figure 5. Photomicrographs of control HaCaT cells (*A*), HaCaT cells exposed to 100 μT EMF for 5 min (*B*), 20 min (*C*), 2 hr (*D*), or 24 hr (*E*), or treated with 100 μM arsenite for 2 hr (F). HSP27 was visualized by immunofluorescence as described in "Materials and Methods." EMF exposure did not induce the translocation of HSP27 from the cytoplasm to the nucleus. Similar results were obtained in two independent experiments with triplicate samples.

Table 1. Quantitative analysis (OD) of HSP27 isoforms shown in Figure 2.

Sample	A	B	C	D	E	Total	S/C
Control	1,683	763				2,446	1.000
EMF							
5 min	1,630	687				2,317	0.947
20 min	1,493	724				2,217	0.906
2 hr	1,663	850				2,513	1.027
24 hr	1,706	635				2,341	0.957
Arsenite	516	1,417	1,616	1,654	558	5,761	2.355

The optical density of each band on the blot in Figure 2 was measured with NIH Image 1.61 using arbitrary units. Total OD is the sum of OD values of all HSP27 isoform bands in a sample. The S/C (sample/control) OD ratio was calculated by dividing the total OD of a sample by the total OD of the control.

To avoid possible variability of experimental results resulting from the use of different strains of normal keratinocytes, the study was also performed with keratinocytes of an immortalized line HaCaT, which is reported to differentiate in a nearly normal fashion (*37*). In these cells, the HSP27B isoform was less abundant in unstressed cells, and arsenite treatment induced fewer phosphorylated isoforms as compared to normal keratinocytes. However, as in normal keratinocytes, HSP27 was not phosphorylated, and its isoform pattern remained unchanged in HaCaT cells after EMF exposure (Figure 3).

Cellular relocalization of HSP27 in keratinocytes. We examined the subcellular distribution of HSP27 using indirect immunofluorescence staining. In the unstressed, normal, or HaCaT keratinocytes, HSP27 was primarily located throughout the cytoplasm and was absent from the nucleus. After treatment with arsenite, HSP27 molecules were translocated into the nuclei and formed bright granular aggregates within the nucleus in both normal (Figure 4F) and HaCaT (Figure 5F) keratinocytes. However, this relocalization of HSP27 from the cytoplasm to the nucleus was not observed in either normal (Figure 4) or HaCaT (Figure 5) keratinocytes that had been subjected to short- or long-term EMF exposure.

Synthesis of HSP27 in keratinocytes. Some environmental insults may also increase the abundance of HSP27 in addition to inducing its phosphorylation. However, the total amount of all HSP27 isoforms in EMF-exposed samples in Figures 2 and 3 appeared approximately equal to that of the unexposed sample. To determine if total HSP27 was increased, we totaled the optical densities (OD) of each isoform band in the IEF blot

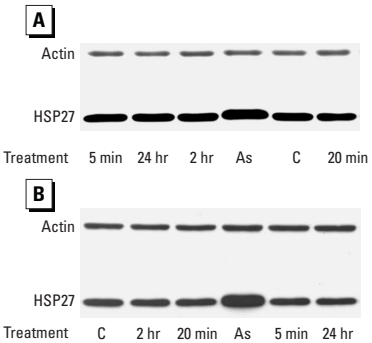

Figure 6. Immunoblots of HSP27 in (*A*) HaCaT keratinocytes and (*B*) normal keratinocytes exposed to 100 μT EMF for 5 min, 20 min, 2 hr, or 24 hr, or treated with 100 μM arsenite for 2 hr. C, control. HSP27 and actin in the samples were separated and detected using Western blotting as described in "Materials and Methods." EMF exposure did not induce an increase in HSP27 in either HaCaT or normal keratinocytes.

(Figure 2) for each experimental condition (each lane). The OD measurement data (Table 1) confirm that during EMF exposure, the total level of HSP27 in normal keratinocytes remained unchanged. In the arsenite-treated positive control cells, the total amount of HSP27 increased by 1.3-fold.

We further investigated the effect of EMF exposure on HSP27 abundance in keratinocytes using SDS-PAGE/Western blotting. To reduce possible variations due to unequal loading of the samples, actin was used as an internal loading control. The amount of HSP27 in HaCaT cells exposed to EMF from 5 min to 24 hr was virtually unchanged from unexposed controls (Figure 6A). Similar results were obtained in normal keratinocytes (Figure 6B). As positive controls, increases in HSP27 abundance in cells treated with arsenite (100 µM, 2 hr) were detectable.

To reduce experimental error, we analyzed multiple replicate samples for HSP27 levels. In the experiment reported in Figure 7, each of the control and 24 hr EMF-exposed treatment contained four samples of HaCaT keratinocytes. The OD of each HSP27 band on the Western blot was measured, and normalized against the OD of the actin band in the same lane. Data analysis with either the non-normalized OD or normalized OD suggests that EMF exposure did not induce significant increases in HSP27 level. Without the normalization, the HSP27 level was 962 ± 184 (arbitrary units; n = 4) in EMF-exposed cells, compared to 951 ± 102 (n = 4) in controls, yielding an EMF/control OD ratio of 1.012. With the normalization, the HSP27 level was 980 ± 136 in EMF-exposed samples, versus 930 ± 114 in controls, with an EMF/control OD ratio of 1.054 (Figure 7B).

Taken together, IEF and SDS-PAGE data reveal that the HSP27 abundance in keratinocytes is not affected by EMF exposure. Thus, electromagnetic fields, as applied in this study, do not induce *de novo* synthesis of HSP27.

Synthesis of HSP70 or HSP27 in breast or leukemia cells. To determine if the effects of EMF on heat shock proteins are specific for certain types of HSP or restricted to certain types of cells, the study was expanded to include HSP70 along with HSP27 and to include HTB124 human mammary epithelial cells. This cell line was chosen because EMF exposure has been reported to increase the expression of HSP70 in HTB124 cells (*30*). The amount of HSP70 or HSP27 in HTB124 culture was not increased in response to short- or long-term EMF (8 µT) exposure, and neither of the stress proteins were induced in HaCaT cells (Figure 8A). In another experiment, HTB124 cells were exposed to 8 µT EMF for 20 min, a condition reported to induce HSP70 expression (*26*). To maximize comparability of results, the cells were lysed using either our protocol or Goodman's protocol (*26*). The results indicated that there was no difference between the levels of HSP70 in the samples extracted using either lysis protocol. With either protocol, the HSP70 kept not affected by EMF exposure (Figure 8B). As a positive control, arsenite elicited a measurable increase in HSP70 expression in these cells.

To control for geographical alteration in EMF, or other unknown experimental conditions that might have explained the difference in the observation in our laboratory in Davis, California, compared to those obtained by Goodman and co-workers in their New York laboratory, we replicated experimental conditions reported by Lin et al. (*26*) in that laboratory. HL60 human promyelocytic leukemia cells were exposed to 8 µT EMF for 20 min in the New York laboratory, then lysed using the procedure as described by Lin et al. (*38*). An aliquot of each sample was analyzed by Western blotting in the New York laboratory, and we were unable to confirm the EMF-associated increase in HSP70 synthesis (data not shown). Another aliquot of each sample was transported on dry ice to our laboratory for Western blotting analysis (Figure 9). A similar negative result was obtained: The non-normalized ratio of HSP27 level in EMF-exposed cells versus HSP27 level in control (E/C ratio) was 0.971. After normalization against actin, the E/C ratio was 0.997.

Discussion

In this study we investigated the effects of power-line frequency EMF on the heat shock protein HSP27 in human epidermal keratinocytes. To establish plausible causality of EMF-dependent effects with multiple parameters, we examined the phosphorylation, cellular redistribution, and total amount of HSP27 after EMF exposure. We took several precautions to eliminate experimenter's bias and reduce experimental errors, including using a double-blind protocol, assay and data analysis, use of positive controls and loading references, assessment of multiple samples, and testing a range of EMF exposure duration. Many of these measures have been suggested and used by other investigators (*19,22*).

The responses of heat shock proteins in living cells to environmental insults may occur at the transcriptional and posttranslational levels. As a posttranslational modification, the phosphorylation of heat shock proteins represents the more immediate stress response. A

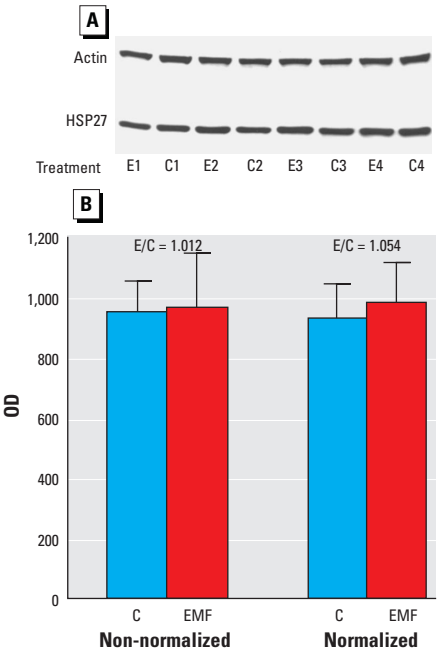

Figure 7. Effects of 24-hr EMF exposure on HSP27 level in HaCaT keratinocytes. Abbreviations: C, control sample; E, 24-hr EMR-treated sample. (*A*) Immunoblot of HSP27 and actin. Similar results were obtained from three independent experiments. (*B*) OD measurements of HSP27 bands in 7A (average of non-normalized or normalized OD values of HSP27 bands in the control or the EMF-exposed group). The E/C ratio is the ratio of the average OD of HSP27 bands in the EMF-exposed group over the average OD of HSP27 bands in the control group. See "Materials and Methods" for details.

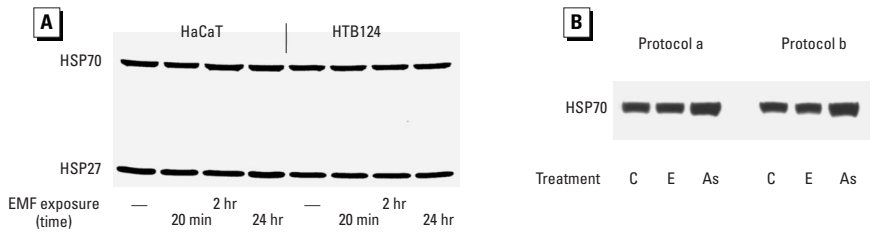

Figure 8. Immunoblots showing that EMF exposure does not increase HSP70 or HSP27 in either HTB124 breast cells or HaCaT keratinocytes. (*A*) HaCaT and HTB124 cells exposed to 8 µT EMF for various times; cells of the two cultures were lysed, and HSP70 and HSP27 were separated and detected by Western blotting as described in "Materials and Methods." Similar results were obtained in duplicate samples. (*B*) HTB124 cells exposed to 8 µT EMF (E) for 20 min or treated with 100 µM arsenite (As) for 2 hr. C, control cells. Cells were lysed using the protocol reported by Lin et al. (*38*) (protocol a) or using our protocol (protocol b). HSP70 in the samples was detected by Western blotting. See "Materials and Methods" for details.

variety of environmental stressors are able to induce serine phosphorylation of HSP27 in keratinocytes (*32,39*), resulting in multiple easily separable isoforms. However, as our data demonstrate, this is not the case for EMF exposure. Neither short- (5–20 min) nor long-term (24 hr) exposure had any detectable effect on HSP27 phosphorylation in either normal keratinocytes or the immortalized HaCaT keratinocyte line. Because we were able to detect characteristic changes in HS27 phosphorylation induced by arsenite (*32,40*), it is clear that our experimental system has the sensitivity to detect a change in HSP27 isoform pattern if one were elicited by EMF exposure.

Exposure of cells to environmental stresses may also induce intracellular redistribution of HSP27. In unstressed cells, HSP27 is found exclusively in the cytosol. Following stress, some of HSP27 molecules bind to cytoskeletal elements and act as molecular chaperones to stabilize actin filaments (*41,42*). Concomitantly, some HSP27 molecules are translocated into the nucleus, where they are thought to be associated with and protect ribonucleoproteins (*39*). Such translocation from the cytoplasm to the nucleus is seen in keratinocytes that have been stressed with heat, UVB irradiation, or oxidants (*32,43*). In this study, although HSP27 was relocalized to the nucleus after arsenite treatment in normal and HaCaT keratinocytes, there was no nuclear HSP27 translocation observed in the cells exposed to EMF.

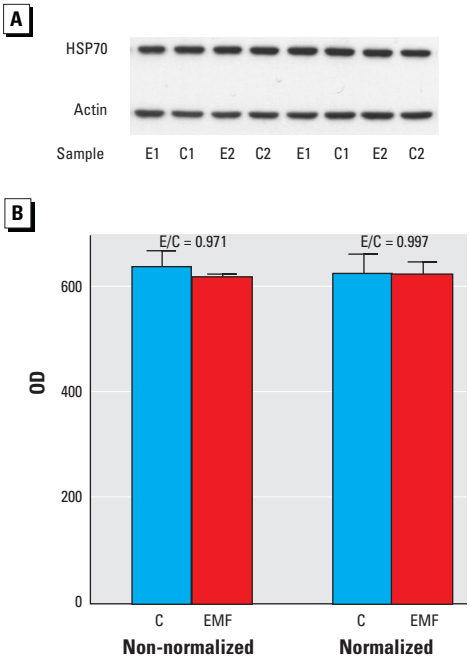

Figure 9. Effect of EMF exposure on HSP70 level in HL60 leukemia cells. Cells were grown and then exposed to 8 μT EMF for 20 min as described by Lin et al. (*26*). Abbreviations: C, control sample; E, EMF-treated sample. (*A*) Immunoblot of HSP70 in HL 60 cells; each sample was loaded into two duplicate wells for Western blotting. (*B*) OD measurement of HSP70 bands in 9A (average of non-normalized or normalized OD values of HSP70 bands in the control or the EMF-exposed group). The E/C ratio is the ratio of the average OD of HSP70 bands in the EMF-exposed group over the average OD of HSP70 bands in the control group. See "Materials and Methods" for details.

Stress-initiated signals also upregulate the expression of heat shock protein genes, presumably by activating heat shock transcription factors that are targeted to heat-shock elements within the promoter regions of these genes (*39*). Lin et al. (*26*) reported that a brief exposure of 8 μT EMF provided a stress sufficient to increase level of HSP70 transcripts by 0.7-fold and level of HSP70 proteins by 1.2-fold in HL60 leukemia cells, presumably promoted by the increase in c-*myc* expression. In human breast cells, they found that a 1-hr EMF exposure or a repeated 20-min EMF exposure led to 30–40% increases in HSP70 protein (*30*). More recently, Pipkin et al. (*44*) reported that both HSP70 and HSP27 were induced in HL-60 cells after a 1 mT EMF exposure for 2 hr.

In contrast, we found that synthesis of HSP27 in human keratinocytes was not sensitive to EMF exposure. When cells were exposed to 100 μT EMF for 5 min, 30 min, 2 hr, and 24 hr, the total levels of HSP27 demonstrated no significant change at any time point compared to the controls exposed to ambient EMF background. We further explored the possibility that the effects of EMF on heat shock proteins are specific for certain heat shock proteins or restricted to certain cell types by attempting to reproduce the results that Goodman and co-workers have reported. Our data demonstrate that the lack of HSP27 response to EMF exposure is not restricted to keratinocytes. Breast cells (HTB124) previously tested by Goodman and colleagues showed no significant change in abundance of HSP27 after exposure to EMF (Figure 8A). Similarly, we were not able to observe any EMF-associated increase in HSP70 level using either breast cells (HTB124) or leukemia cells (HL60). Other investigators have also failed to demonstrate that EMF exposure induces an increase in the synthesis of HSP70 in epithelial carcinoma-derived cells and HL60 cells (*45,46*).

In summary, in this study we failed to detect any of a number of stress responses in keratinocytes exposed to power-line frequency EMF. Not only synthesis of heat shock proteins but also two other parameters of phosphorylation and translocation were not affected by power-line frequency EMF. Evaluation of these three parameters consistently demonstrated that EMF does not elicit the stress responses that are induced by heat shock or other environmental insults. Our study joins a growing body of evidence that suggests that power-line frequency EMF exposure does not elicit detectable cellular responses.

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