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Research Article

Effect of 60 Hz electromagnetic fields on the activity of hsp70 promoter: an *in vivo* study

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Abstract

Exposure to EMFs (electromagnetic fields) results in a number of important biological changes, including modification of genetic expression. We have investigated the effect of 60 Hz sinusoidal EMFs at a magnetic flux density of 80 μT on the expression of the luciferase gene contained in a plasmid labelled as pEMF (EMF plasmid). This gene construct contains the specific sequences for the induction of hsp70 (heat-shock protein 70) expression by EMFs, as well as the reporter for the luciferase gene. The pEMF vector was electrotransferred into quadriceps muscles of BALB/c mice that were later exposed to EMFs. Increased luciferase expression was observed in mice exposed to EMFs 2 h daily for 7 days compared with controls ($P<0.05$). These data along with other reports in the literature suggest that EMFs can have far-reaching effects on the genome.

Keywords: 60 Hz electromagnetic fields; Hsp70 promoter; gene expression; luciferase; *in vivo*; muscle

1. Introduction

Human 70 kDa hsp70 (heat-shock protein 70) gene is regulated by a number of stimuli, including heat shock (for a review, see Craig, 1985; Said et al., 2010), heavy metals (Wu et al., 1986; Planelló et al., 2010), serum replenishment after serum starvation (Wu and Morimoto, 1985), infection by adenovirus (Yang et al., 2008; Li et al., 2009) and magnetic fields with flux densities ranging from microtesla to millitesla (Blank and Goodman, 2009; Goodman et al., 2009).

Biophysical input, including electric and EMFs (electromagnetic fields) regulate the expression of several genes (Aaron et al., 2004). PEMFs (pulsed EMFs) also have a number of well-documented physiological effects on cells and tissues, including the up-regulation of gene expression of members of the TGFβ (transforming growth factor β) superfamily (Fini et al., 2005). Moreover, RF-EMFs (radio frequency EMFs) affect the cellular stress response, comprising expression of hsps and stimulation of SAPKs (stress-activated protein kinases; reviewed in Gaestel, 2010).

We have previously reported an increased luciferase gene expression in cultured HeLa and BMK16 cell lines transfected with hsp70 promoter, when cells were treated with 60 Hz sinusoidal EMFs at 8 and 80 μT (Rodríguez-de la Fuente et al., 2009). In subsequent work (Heredia-Rojas et al., 2010), we demonstrated the induction of hsp70 expression by EMFs of similar characteristics in the INER-37 cell line; however, magnetic field exposure had no effect on the RMA E7 cells.

Contradictory results have also been reported by Reale et al. (2006), who observed that the effects of EMF exposure clearly differed with respect to the potentiation and inhibition of iNOS (inducible nitric oxide synthase) and MCP-1 (monocyte chemotactic protein-1) expression. Whereas iNOS was down-regulated both at the mRNA and protein levels, MCP-1 was up-regulated after overnight exposure of human monocytes to 1 mT/50 Hz extremely low-frequency sinusoidal EMFs.

The issue of EMFs effects on gene expression developed almost entirely from *in vitro* studies; however, *in vivo* studies can be a good model to establish the relationship between EMF exposure and gene expression. Recently, Cuccurazzu et al. (2010) demonstrated that 50 Hz/1 mT magnetic field exposure increased the transcription levels of pro-neuronal genes by using an *in vivo* hippocampal neurogenesis model in C57BL/6 mice.

In view of the continuing lack of agreement among researchers as to a definitive mechanism for the alteration of gene expression by EMF exposure in different biological models, we have undertaken an *in vivo* study to analyse the effect of 60 Hz sinusoidal magnetic field exposure at 80 μT in quadriceps muscle from BALB/c strain mice on the expression of the luciferase gene contained in a plasmid that includes the hsp70 promoter. This research is also an attempt to confirm the findings of Lin et al. (2001), who claimed that hsp70 promoter contains magnetic field response elements.

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Abbreviations used: EMF, electromagnetic field; ETS, E twenty-six; hsp70, heat-shock protein 70; iNOS, inducible nitric oxide synthase; MCP-1, monocyte chemotactic protein-1; pEMF, EMF plasmid; PEMF, pulsed EMF; NF-κB, nuclear factor κB; RLU, relative light units.

2. Materials and methods

2.1 Animals

BALB/c mice weighing 25–35 g (7–8 weeks old) were kept in a room with a 12 h light/12 h dark photoperiod, a temperature of 24–25℃ and relative humidity of 40–50%. Animals were housed 5 per cage and allowed free access to standard laboratory pellets and tap water. Mice were born and raised in our breeding colony. After a 10-day quarantine period, animals were randomly distributed into experimental (EMF exposed) and control groups. This research project fulfilled all requirements of the University's Animal Care and Use for Research Protocol, which is based on the National Guidelines for Ethics and Biosafety under the General Law of Health for issues regarding Health Research, Ministry of Health, México City.

2.2 Plasmid construction

The vector pNF-κB-luc (catalogue no. 631904, Clontech) was used after removal of the original promoter by using HindIII and Nhe1. The hsp70 promoter obtained from human whole blood using PCR was inserted. The resultant vector was called the pEMF (EMF plasmid). This gene construct also has the luciferase gene with a length of 1689 bp and a signal for polyadenylation of SV40 (simian virus 40) and the ampicillin-resistance gene.

2.3 Electrotransfer of DNA

Quadriceps muscles of the mice were surgically exposed. Electrotransfection was carried out according to a previously described procedure (Liu and Huang, 2002), using a syringe electrode device. In brief, this syringe electrode system consists of 2 U-100 insulin syringes bonded together at a distance of 0.6 cm between the two needles. The needles themselves served as electrodes, and were connected to a square-wave electroporater BTX T820 (Genetronics). One of the needles loaded with 50 μg of the gene construct (pEMF), in 20 μl of 0.85% saline was inserted into the quadriceps parallel with the long axis of the muscle fibres. The polarity applied in the muscle can be either anodic or cathodic. The other needle was inserted into the gastrocnemius. Electric pulses were immediately applied as previously recommended (Aihara and Miyazaki, 1998), electrical contact with the leg skin being ensured by shaving the leg and applying conductive gel. A total of six electric pulses were applied during 20 ms each. Electric field strength, scored in terms of the ratio of the applied voltage to the distance between the electrodes, was on average 250 V/cm. Electroporation was done at room temperature. Immediately after being injected, mice were exposed to magnetic fields following the directions mentioned below.

2.4 EMF exposure conditions

Mice were exposed to a 60 Hz/80 μT EMF for 2 h daily for 7 days. A magnetic field exposure facility with identical characteristics to the one used in our previous work (Heredia-Rojas et al., 2004) was used. A coil was built by winding 552 turns of 1.3 mm diameter enamel-insulated copper wire to form a cylindrical solenoid with a radius of 13.5 cm and a length of 71 cm. This solenoid was connected to a step-down transformer and a variable transformer that was plugged into a 110 V AC source. Animals were placed in the middle of this solenoid where the EMF was homogeneous, and kept at a temperature of 24 ± 0.1℃ and 40% humidity. An equal number of sham-treated animals were used as controls and were placed in the same room into an EMF device of identical design as the one mentioned above, but it was switched off.

Magnetic flux density (rms) was measured using an axial Hall-effect probe (Bell FW 6010 gaussmeter). An oscilloscope (BK-Precision model 2120) was coupled to the system to monitor the resulting field. A 80 μT (rms)/60 Hz alternating sinusoidal EMF was generated. The EMF frequency content was nearly pure 60 Hz ($<$3% total harmonic distortion). The local temperature inside the solenoids with baffles present but without animals was measured setting the Bell FW 610 gaussmeter in Temp mode. The temperature value average was 24.0 ± 0.10℃ when the field was on and 23.9 ± 0.11℃ without current in the coils. No statistically significant differences were observed between two solenoids (Kolmogorov–Smirnov test for normality, followed by paired *t* test).

To keep the geometry of exposure, a plastic separator was placed inside the solenoid to allow the placement of uncaged mice in predetermined zones where the rms value of the EMF was 80 μT. A food container and a water bottle were also placed inside these compartments. Water and standard diet were offered *ad libitum.*

The magnetic field ambient background level was <0.4 μT. Moreover, the local geomagnetic field was also measured, setting the gaussmeter in DC mode and by using an axial high sensitivity Hall probe (Integrity Design IDR-321 geomagnetometer, Essex Junction). The average value was 20 μT within the exposure room.

2.5 Protein expression and luminescence assays

Mice were killed immediately after the last exposure. The quadriceps muscle was removed and 1 ml lysis buffer (0.1% Triton X-100, 2 mM EDTA and 0.1 M Tris/HCl, pH 7.8) added. The muscle was homogenized using a Tissue Tearor (model 985-370 Biospec Products), and centrifuged at 14 000 rev./min for 2 min. A 50 μl aliquot of the supernatant was analysed for activity according in a luciferase assay system (Promega), as described by Martin et al. (1996). Briefly, muscle lysates (20 μl) were added to 100 μl luciferin substrate, and luminescence measured using an MGM Instruments model Optocomp I Luminometer (serial no. 202379). Total protein was precipitated from 50 μl lysate with 10% trichloroacetic acid and dissolved in 0.1 M NaOH. Protein concentrations were determined by the method of Bradford (1976). Luciferase activity was adjusted for protein content by dividing the RLU (relative light units)/mg of protein (RLU by the protein concentration).

2.6 Experimental design

To evaluate EMF effects on luciferase gene expression in quadriceps muscle of pEMF-electrotransferred mice, four groups

were used: (a) non-electrotransferred mice as a negative control and without EMF treatment, (b) pEMF electrotransferred mice, but not exposed to EMFs, (c) mice injected with the commercial expression vector pNF-κB-luc, but not exposed to EMFs and (d) pEMF-electrotransferred mice, exposed to EMFs at 80 μT 2 h daily for 7 days. Three animals were used for each group.

2.7 Statistical analysis

Statistical differences were calculated among the groups by using ANOVA for normal distributions and the correspondent parametric Tukey test for establishing individual differences. The normality of the data was estimated by means of Kolmogorov–Smirnov test ($P<0.05$). All analyses used SPSS for Windows, version 15.0. Differences were considered significant when $P<0.05$.

3. Results

Figure 1 shows the arithmetical means of RLU/mg of protein from pEMF electrotransferred mice exposed to magnetic fields at 80 μT compared with non-electrotransferred and pEMF-electrotransferred mice, but without EMF treatment. A control of pNF-κB-luc electrotransferred mice without EMF exposure was also included. These results indicated a statistically significant difference between the groups. The mice treated with EMFs showed increased gene expression compared with the controls. The pNF-κB-luc electrotransferred mice showed no increase in luciferase gene expression compared with the controls and EMF-exposed mice. In terms of percentage, EMF-exposed mice showed a considerable increase (87.6%) in luciferase gene expression when compared with pEMF non-exposed ones.

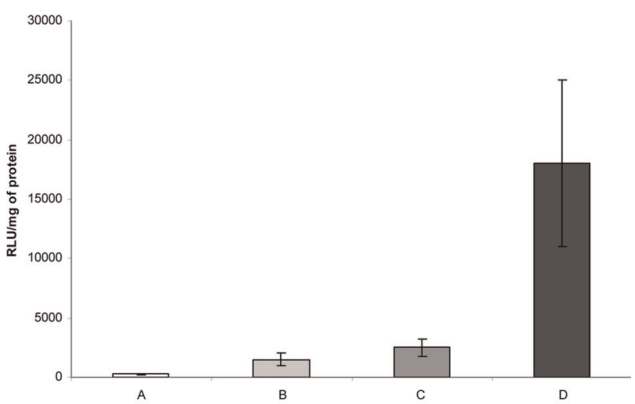

Figure 1 Effect of 60 Hz sinusoidal EMF at 80 μT on the luciferase gene expression in quadriceps muscle of BALB/c mice

The animals were exposed to EMFs 2 h daily for 7 days: (A) non-electrotransferred mice as a negative control and without EMF treatment, (B) pEMF electrotransferred mice but not exposed to EMF, (C) mice injected with the commercial expression vector pNF-κB-luc, not exposed to EMF, and (D) pEMF-electrotransferred mice exposed to EMFs. Three animals were used for each group. Bars represent means $\pm$ S.D.

4. Discussion

The diversity of biological effects attributed to EMFs is large; however, we chose to confine our *in vivo* experimental investigation to a single parameter, the very sensitive gene-biomarker of stress proteins and the possible expression by extremely low-frequency magnetic field exposure. The design of the experiment allowed us to see the effect of 60 Hz EMF exposure at 80 μT on the expression of hsp70 promoter in quadriceps muscle of mice. We previously found activation of hsp70 promoter in cultured HeLa and BMK16 cell lines (Rodríguez-de la Fuente et al., 2009) and in INER-37 cells (Heredia-Rojas et al., 2010) after exposure to 60 Hz EMFs of similar characteristics to the fields used in the present study. The vector here, called pEMF, included the three nCTCTn sequences previously described for the induction of hsp70 expression by magnetic fields. We also replicated the work of Lin et al. (2001), who claimed that gene activation by EMFs requires the so-called EMF response elements, such as the ones contained in the gene construct we used.

The interaction between EMFs and DNA is poorly understood, but because it is well documented that electric and magnetic fields interact with membrane components (Blank, 1995a, 1995b; Luben, 1995), it is possible that EMFs could be transduced by the membrane via an enzyme cascade into a biochemical messenger to the nucleus that leads to changes in DNA. On the other hand, it is known that magnetic fields are generally unattenuated by cell membranes, and therefore membrane processes may not be necessary for the signal to reach the DNA in the nucleus and stimulate a response (Blank and Goodman, 1997). These authors have also proposed that EMFs can alter moving charges in the DNA (Blank and Goodman, 1999). Conducting electrons in DNA were found by Porath et al. (2000), who made direct measurements of electrical transport through DNA. Conduction in DNA depends on specific structures, since different DNA sequences have varying electrical conductivities (Meggers et al., 1998). In view of these findings, EMFs could theoretically interact preferentially with specific DNA sequences and the EMF response elements in the hsp70 promoter, we used such sequences. Our human hsp70 promoter between -273 and -1 included all the sites involved in magnetic-field-induced hsp70 expression, as noted by Lin et al. (1999).

On the other hand, Blank and Goodman (2008) hypothesized a mechanism based on charge transfer in DNA. Electrons move in DNA, and specific DNA sequences are associated with the response to EMF. Interaction with electrons could displace electrons in H-bonds that hold DNA together leading to chain separation and initiating transcription. However, direct EMF effects on DNA are also possible through changes in chromatin conformation. Recently, Matronchik and Belyaev (2008) demonstrated that extremely low-frequency magnetic fields at low intensities affect conformation of nucleoids in bacterial *Escherichia coli* cells and even human lymphocytes.

We have found an increased luciferase gene expression in quadriceps muscle of mice exposed to 60 Hz EMFs compared with their control groups. These results agree with those of Mucci

et al. (2001), who showed *in vivo* evidence of the effect of EMFs on ETS (E twenty-six)-oncogene expression. They observed an increase in ETS1 mRNA and protein expression in mice exposed to 50 MHz radiation modulated at 16 Hz. Moreover, Goto et al. (2006) found that the Ntan1 gene was significantly increased approximately 1.5–2-fold in the hippocampus in 12-day-old or newborn mice that were successively exposed to magnetic fields at 100 mT for 2 h for four times per day.

In contrast, Jasti et al. (2001) observed no effect in cytokine gene expression in rat spleen after exposure to PEMFs. These results suggest that power-frequency EMFs are not a universal stressor like other physical agents, e.g. heat. Non-thermal induction of hsps, i.e. EMF induction, may occur as Cotgreave (2005) suggested, and it appears pleiotropic for many other regulatory events. However, many of these studies are flawed by inconsistencies in their exposure models, cell types and the independent reproducibility of the findings.

There have been many attempts to develop a coherent explanation for the phenomena of EMF interactions with genes. Several studies (Smith et al., 1987; Lednev, 1991; Blanchard and Blackman, 1994; Zhadin and Barnes, 2005) have proposed that magnetic field effects on ions, protein–ion complexes and membranes could account for indirect EMF effects on gene expression. A physical mechanism is suggested for a resonant interaction of weak EMFs with biological systems. An ion inside a Ca^{2+}-binding protein is approximated by a charged oscillator. This, in turn, can affect the interaction of the ion with the surrounding ligands. Finally, this mechanism could act as a gate to control the movement of the ion across the cell membrane, affecting several cellular processes including gene expression. Following this viewpoint, many natural frequencies should exist in the cells of all kinds of living forms, dependent upon the cell elements, structures, components, alignment, etc.

In conclusion, our *in vivo* study suggests that the sinusoidal 60 Hz EMF can modify luciferase gene expression by activation of the hsp70 promoter in quadriceps muscle of mice. However, further studies using a variety of biological models under different experimental conditions are required to help clarify the controversy concerning the mechanism of altered gene expression induced by magnetic fields.

Author contribution

Abraham O. Rodríguez-De la Fuente performed the gene construct, electrotransfection of DNA into muscle and magnetic field exposure. Juan M. Alcocer-González designed the experiment and critically revised the manuscript. J. Antonio Heredia-Rojas did the magnetic field exposure facilities, wrote the manuscript and updated literature. Cristina Rodríguez-Padilla did protein expression and luminescence assays. Laura E. Rodríguez-Flores performed the magnetic field exposure and measurements, critically revised the manuscript and updated literature. Martha A. Santoyo-Stephano did the statistical analysis and updated literature. Esperanza Castañeda-Garza prepared the experimental animals, critically revised the manuscript and did the statistical analysis. Reyes S. Taméz-Guerra did the protein expression and luminescence assays.

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References

Aaron RK, Boyan BD, Ciombor DM, Schwartz Z, Simon BL. Stimulation of growth factor synthesis by electric and EMF. Clin Orthop Relat Res 2004;419:30–7.

Aihara H, Miyazaki J. Gene transfer into muscle by electroporation *in vivo*. Nat Biotechnol 1998;16:867–70.

Blanchard JP, Blackman CF. Clarification and application of an ion parametric resonance model for magnetic field interactions with biological systems. Bioelectromagnetics 1994;15:217–38.

Blank M. Electric and magnetic field signal transduction in the membrane Na,K-ATPase. Adv Chem 1995a;250:339–48.

Blank M. Electric stimulation of protein synthesis in muscle. Adv Chem 1995b;250:143–53.

Blank M, Goodman R. Do electromagnetic fields interact directly with DNA? Bioelectromagnetics 1997;18:111–5.

Blank M, Goodman R. Electromagnetic fields may act directly on DNA. J Cell Biochem 1999;75:369–74.

Blank M, Goodman R. A mechanism for stimulation of biosynthesis by electromagnetic fields: charge transfer in DNA and base pair separation. J Cell Physiol 2008;214:20–6.

Blank M, Goodman R. Electromagnetic fields stress living cells. Pathophysiology 2009;16:71–8.

Bradford M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.

Cotgreave IA. Biological stress responses to radio frequency electromagnetic radiation: are mobile phones really so (heat) shocking? Arch Biochem Biophys 2005;435:227–40.

Craig EA. The heat shock response. Crit Rev Biochem 1985;18:239–80.

Cuccurazzu B, Leone L, Podda MV, Piacentini R, Riccardi E, Ripoli C et al. Exposure to extremely low-frequency (50 Hz) electromagnetic fields enhances adult hippocampal neurogenesis in C57BL/6 mice. Exp Neurol 2010;226:173–82.

Fini M, Giavaresi G, Carpi A, Nicolini A, Setti S, Giardino R. Effects of pulsed electromagnetic fields on articular hyaline cartilage; review of experimental and clinical studies. Biomed Pharmacother 2005;59:388–94.

Gaestel M. Biological monitoring of non-thermal effects of mobile phone radiation; recent approaches and challenges. Biol Rev Camb Philos Soc 2010;85:489–500.

Goodman R, Lin-Ye A, Geddis MS, Wickramaratne PJ, Hodge SE, Pantazatos S et al. Extremely low frequency electromagnetic fields activate the ERK cascade, increase hsp70 protein levels and promote regeneration in Planaria. Int J Radiat Biol 2009;9:1–9.

Goto Y, Taniura H, Yamada K, Hirai T, Sanada N, Nakamichi N et al. The magnetism responsive gene Ntan1 in mouse brain. Neurochem Int 2006;49:334–41.

Heredia-Rojas JA, Caballero-Hernández DE, Rodríguez-De la Fuente AO, Ramos-Alfano G, Rodríguez-Flores LE. Lack of alterations on meiotic chromosomes and morphological characteristics of male germ cells in mice exposed to a 60 Hz and 2.0 mT magnetic field. Bioelectromagnetics 2004;25:63–8.

Heredia-Rojas JA, Rodríguez-de la Fuente AO, Alcocer-González JM, Rodríguez-Flores LE, Rodríguez-Padilla C, Santoyo-Stephano MA et al. Effect of 60 Hz magnetic fields on the activation of hsp70 promoter in cultured INER-37 and RMA E7 cells. *In Vitro* Cell Dev Biol Animal 2010;46:758–63.

Jasti AC, Wetzel BJ, Aviles H, Vesper DN, Nindl G, Johnson MT. Effect of a wound healing electromagnetic field on inflammatory cytokine gene expression in rats. Biomed Sci Instrum 2001;37:209–14.

Lednev VV. Possible mechanism for the influence of weak magnetic fields on biological systems. Bioelectromagnetics 1991;12:71–5.

Li JL, Liu HL, Zhang XR, Xu JP, Hu WK, Liang M et al. A phase I trial of intratumoral administration of recombinant oncolytic adenovirus overexpressing HSP70 in advanced solid tumor patients. Gene Ther 2009;16:376–82.

Lin H, Blank M, Goodman R. A magnetic field-responsive domain in the human HSP70 promoter. J Cell Biochem 1999;75:170–6.

Lin H, Blank M, Rossol-Haseroth K, Goodman R. Regulating genes with electromagnetic response elements. J Cell Biochem 2001;81:143–8.

Liu F, Huang L. A syringe electrode device for simultaneous injection of DNA and electrotransfer. Mol Ther 2002;5:323–8.

Luben RA. Membrane signal-transduction mechanisms and biological effects of low-energy electromagnetic fields. Adv Chem 1995;250:437–50.

Martin CS, Wight PA, Dobretsova A, Bronstein I. Dual luminescence-based reporter gene assay for luciferase and beta-galactosidase. Biotechniques 1996;21:520–4.

Matronchik AY, Belyaev IY. Mechanism for combined action of microwaves and static magnetic field: slow non uniform rotation of charged nucleoid. Electromagn Biol Med 2008;27:340–54.

Meggers E, Michel-Beryele ME, Giese B. Sequence dependent long range hole transport in DNA. J Am Chem Soc 1998;120:12950–5.

Mucci N, Ianni A, Ursini CL, Arzani D, Bhat NK, Navarra P et al. *In vivo* modulation of ETS genes induced by electromagnetic fields. In Vivo 2001;15:489–94.

Planelló R, Martínez-Guitarte JL, Morcillo G. Effect of acute exposure to cadmium on the expression of heat-shock and hormone-nuclear receptor genes in the aquatic midge *Chironomus riparius*. Sci Total Environ 2010;408:1598–603.

Porath D, Bezryadin A, De Vries S, Dekker C. Direct measurement of electrical transport through DNA molecules. Nature 2000;403:635–8.

Reale M, De Lutiis MA, Patruno A, Speranza L, Felaco M, Grilli A et al. Modulation of MCP-1 and iNOS by 50 Hz sinusoidal electromagnetic field. Nitric Oxide 2006;15:50–7.

Rodríguez-de la Fuente AO, Alcocer-González JM, Heredia-Rojas JA, Balderas-Candanosa I, Rodríguez-Flores L, Rodríguez-Padilla C et al. Effect of 60 Hz electromagnetic fields on the activity of hsp70 promoter: an *in vitro* study. Cell Biol Int 2009;33:419–23.

Said Ali K, Ferencz A, Nemcsók J, Hermesz E. Expressions of heat shock and metallothionein genes in the heart of common carp (*Cyprinus carpio*): effects of temperature shock and heavy metal exposure. Acta Biol Hung 2010;61:10–23.

Smith SD, McLeod BR, Liboff AR, Cooksey K. Calcium cyclotron resonance and diatom mobility. Bioelectromagnetics 1987;8:215–27.

Wu BJ, Morimoto RI. Transcription of the human hsp70 gene is induced by serum stimulation. Proc Natl Acad Sci USA 1985;82:6070–4.

Wu BJ, Kingston RE, Morimoto RI. Human hsp70 promoter contains at least two distinct regulatory domains. Proc Natl Acad Sci USA 1986;83:629–33.

Yang FF, Xu XN, Hu D, Shi F, Qu Y. Recombinant heat shock protein 70 induced by adenovirus protects neurons and glial cells against hypoxia/reoxygenation injury. Zhongguo Wei Zhong Bing Ji Jiu Yi Xue 2008;20:681–4.

Zhadin M, Barnes F. Frequency and amplitude windows in the combined action of DC and low frequency AC magnetic fields on ion thermal motion in a macromolecule: theoretical analysis. Bioelectromagnetics 2005;26:323–30.

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