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Molecular Basis Underlying the Biological Effects Elicited by Extremely Low-Frequency Magnetic Field (ELF-MF) on Neuroblastoma Cells

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ABSTRACT

Extremely low-frequency magnetic fields (ELF-MFs) may affect human health because of the possible associations with leukemia but also with cancer, cardiovascular, and neurological disorders. In the present work, human SH-SY5Y neuroblastoma cells were exposed to a 50 Hz, 1 mT sinusoidal ELF-MF at three different times, that is, 5 days (T5), 10 days (T10), and 15 days (T15) and then the effects of ELF-MF on proteome expression and biological behavior were investigated. Through comparative analysis between treated and control samples, we analyzed the proteome changes induced by ELF-MF exposure. Nine new proteins resolved in sample after a 15-day treatment were involved in a cellular defense mechanism and/or in cellular organization and proliferation such as peroxiredoxin isoenzymes (2, 3, and 6), 3-mercaptopyruvate sulfurtransferase, actin cytoplasmatic 2, t-complex protein subunit beta, ropporin-1A, and profilin-2 and spindlin-1. Our results indicated that ELF-MFs exposure altered the proliferative status and other important cell biology-related parameters, such as cell growth pattern, and cytoskeletal organization. These findings support our hypothesis that ELF radiation could trigger a shift toward a more invasive phenotype. J. Cell. Biochem. 112: 3797–3806, 2011.

KEY WORDS: 50-HZ MAGNETIC FIELDS; NEUROBLASTOMA; PROTEOME; HUMAN HEALTH; PROLIFERATION; DIFFERENTIATION

Since there is considerable public concern regarding the health effects of exposure to extremely low-frequency electromagnetic fields (ELF-MFs), such as those generated by high tension electrical distribution networks or from residential and occupational sources, there has been growing research interest placed into the mechanisms of interaction between ELF-MFs and living organisms [Kheifets et al., 2010]. Several studies suggested that there is a possible association between ELF-MFs and malignancies in childhood and adulthood such as leukemia and nervous system tumors as well as cardiovascular and neurological disorders [Wertheimer and Leeper, 1979; IARC, 2002; Håkansson et al., 2003; Draper et al., 2005; Davanipour et al., 2007]. A number of in vivo and in vitro investigations have shown a possible co-promoter capacity of ELF-MFs on tumor growth as well as the potential effects elicited by ELF-MFs on various cellular functions [Crumpton and Collins, 2004; Simkó, 2004; Santini et al., 2005; Wolf et al., 2005; Berg and Berg, 2006; Frahm et al., 2006; Lisi et al., 2006; Palumbo et al., 2006; Gottwald et al., 2007]. Unfortunately, the biological mechanisms by which these physical agents can be involved in a possible tumor-promoting or tumor-initiating action are still unclear.

A fundamental interaction mechanism between ELF-MFs and cells is also lacking, although several candidate mechanisms have been proposed; it has been suggested that the interaction between living organisms and ELF-MFs could interfere with reactive oxygen species (ROS) production and their half-life extension [Simkó and Mattsson, 2004; Simkó, 2007]. In accordance with this hypothesis, redox-related cellular changes following ELF-MFs exposure were reported in the last few years [Regoli et al., 2005; Wolf et al., 2005; Yokus et al., 2005; Zwirska-Korczala

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et al., 2005; Falone et al., 2007, 2008; Hashish et al., 2008; Di Loreto et al., 2009].

The human SH-SY5Y neuroblastoma cell line is currently used as a valid experimental model, as these cells exhibit many biochemical and functional properties of a neuronal cell type. In addition, SH-SY5Y cells proliferate in culture for long periods [Nicolini et al., 1998] and consequently, the SH-SY5Y cell line has been widely used in experimental neurological studies, including analysis of neuronal differentiation, metabolism, and function related to neurodegenerative and neuroadaptive processes [Xie et al., 2010]. We have previously demonstrated that short-term ELF-MF exposure improved cellular viability and triggered significant redox-based adaptive responses in human neuroderived SH-SY5Y cells. Specifically, we have found that a 50 Hz magnetic field induced a significant enhancement of the antioxidant defenses together with a major shift of redox homeostasis [Falone et al., 2007]. Standard biochemical techniques do not easily allow an extensive investigation of the broad spectrum of molecular events underlying the ELF-MF-induced alterations on neuronal cell biology and protein expression profile. Therefore, most of the cellular mechanisms by which ELF magnetic fields trigger biological responses remains uninvestigated. Nowadays, proteomic approach is widely used as a powerful research tool able to detect and characterize any change in protein expression during or following exposure to drugs, stress conditions, etc. [Chemale et al., 2010; Thompson et al., 2010].

At present, only few proteomic investigations aimed at identifying cellular events associated with the exposure to ELF-MFs have been carried out [Sinclair et al., 2006; Seyyedi et al., 2007] and none of these studies have attempted to identify in neuroderived cells the global changes in protein expression induced by ELF-MFs. The goal of our research is to establish how ELF-MF exposure at different times affects the cell biology modulating protein expression in a human neuroblastoma SH-SY5Y cells. This could gain importance as prolonged treatments seem to be useful experimental models in order to simulate actual exposure to electromagnetic pollution.

MATERIALS AND METHODS

CELL CULTURE

Human neuroblastoma (SH-SY5Y; Sigma–Aldrich, St. Louis, MO) cell line was seeded at 2×10^4 cells/cm^2 in vented-capped 25 or 75 cm^2 flasks and cultured as monolayer in a cell incubator (37°C, 5% CO_2 in moist air), using 10% (v/v) fetal bovine serum-supplemented RPMI-1640 medium containing 2 mM L-glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin (all supplied by Invitrogen Corporation, Carlsbad, CA), with medium renewal every two days. Cells were detached at sub-confluence (every 5 days) with trypsin–EDTA solution and, after Trypan Blue-based viable cell counting [Freshney, 1987], re-seeded for subsequent steps. Cells were not used over the 20th passage, as suggested by the supplier. All experiments were repeated four times.

ELF-MF TREATMENT

Cell exposure to ELF-MF was carried out in solenoids producing a highly homogeneous magnetic field at 50 Hz frequency and 1.0 mT flux density [Falone et al., 2007; Di Loreto et al., 2009]. ELF-MF treatment proceeded up to 15 days for all the experiments. The current flowing in the solenoid was monitored by an amperometer meter connected in series with the solenoid; the waveform and frequency of the current including the magnetic field intensity B, were monitored by a scope connected in parallel to a resistor, in series with the coil. Power line distribution allowed a 10% voltage stability. We mapped the B value inside the solenoid with axial Hall probe 912 Digital Gaussmeter, with an accuracy of 2%. The system allowed to achieve different magnetic field intensities by changing the current in the solenoid. Instruments used for generating and characterizing the magnetic field were supplied by RFL Industries. The temperature in cell cultures during treatments was controlled with an accuracy of 0.05°C and no magnetic field-induced heating was observed. Controls were cultured in identical electrically disconnected solenoids. Magnetic field intensity for control samples was regularly measured (<50 μT).

CELL VIABILITY AND PROLIFERATION

Control and treated SH-SY5Y cells were assessed for viability by measuring colorimetrically the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma–Aldrich) [Green et al., 1984]. Cells were seeded in quadruplicate in 24-well plates (1.5×10^4 cells/well) and grown for 5, 10, and 15 days (see Cell Culture Section). At each time point, 0.1 volume of MTT solution was added to wells and cells were incubated at 37°C for 3 h. The medium was removed from wells and DMSO was added to dissolve the purple formazan crystals. Plates were gently stirred in a gyratory shaker to enhance dissolution and subsequently read in a PerkinElmer Victor3 instrument (λ 570 nm, λ_{ref} 690 nm). Control and treated SH-SY5Ys were also assessed for cell proliferation by measuring colorimetrically the uptake of the thymidine analogue 5-bromo-2′-deoxyuridine (BrdU) (Cell Proliferation ELISA; Roche Applied Science, Indianapolis, IN) [Porstmann et al., 1985]. Cells were seeded in quadruplicate in 24-well plates (1.5×10^4 cells/well) and grown for 5, 10, and 15 days (see Cell Culture Section). At each time point, medium in wells was replaced with the labeling solution 1× and plates were incubated at 37°C for 1.5 h. Then, the medium in each well was replaced with the FixDenat solution and plates were incubated at room temperature for 30 min in the dark. Well contents with the freshly prepared anti-BrdU-POD-containing solution and plates underwent incubation at room temperature for 1.5 h in the dark. Then, plate wells were washed three times with the Washing buffer 1× and incubated for 5 min in the dark with the substrate solution. Multiwell plates were quickly stirred in a gyratory shaker and read in a PerkinElmer Victor3 instrument (λ 370 nm, λ_{ref} 690 nm).

IMMUNOCYTOCHEMICAL ANALYSIS

Cells were seeded in 24-well plates containing 12 mm round glass coverslips and grown for 5, 10, and 15 days (see Cell Culture Section). After two washes in PBS cells were fixed with freshly prepared 4% paraformaldehyde in PBS (10 min at room temperature), washed in PBS (5 min), and reacted with a appropriate primary antibody (diluted 1:100) in NET gel (150 mM NaCl, 5 mM EDTA, 50 mM Tris–HCl, pH 7.4, 0.05% NP-40, 0.25% carragenin Lambda

gelatin, 0.02 NaN_3) for 2 h at room temperature. After several washes, cells were incubated with an appropriate secondary antibody (diluted 1:150) in NET gel for 45 min at room temperature. After one wash with NET gel and one with PBS, samples were stained (5 min) with 0.5 μg/ml DAPI (in PBS), then washed in PBS, dried with ethanol (70%, 90%, 100%) and finally mounted in glycerol containing 1,4-diazabicyclo[2.2.2]octane to minimize fading. Negative controls were represented by samples incubated with the secondary antibody only. Slides were observed with a i50 microscope (Nikon) and images were acquired with a Cool-SNAPcf digital CCD camera (PhotoMetrics, Huntington Beach, CA). Digital acquisition, processing and analysis of fluorescence were performed by Meta Image Series 7.5 (MetaMorph, Metafluor, MetaVue) software obtained from Molecular Devices.

PROTEOME ANALYSIS

After ELF-MF treatment, in three different exposure times (5, 10, and 15 days, respectively) SH-SY5Y cells were harvested and resolved by 2D-PAGE according to procedures described previously [Angelucci et al., 2010]. In order to create a master gel representative of all conditions analyzed, 3 different gel runs for each control and treated samples were performed and then subjected to image analysis with Image Master 2D platinum software (GE Healthcare formerly Amersham Biotech, Uppsala, Sweden). The image analysis allowed to distinguish three kinds of protein spots related to independent experimental variables such as the differences due to cellular aging. Proteins of interest were excised from the gel, digested with trypsin [Gharahdaghi et al., 1999] and analyzed using the peptide mass finger printing (PMF) approach with a MALDI–TOF–MS. The results from the PMF were employed to search the human NCBIn protein database by Mascot search engine, which compares the experimentally determined tryptic peptide masses with theoretical peptide masses calculated for protein from these databases. Search parameters are as follows: type of search, peptide mass fingerprint; enzyme, trypsin; fixed modification, carbamidomethylation (Cys); variable modifications, oxidation (Met); mass values, monoisotopic; peptide charge state, 1+; maximum missed cleavages, 1; and peptide mass tolerance, 100 ppm. All protein identification carried out by MALDI–TOF–MS were validate by Post Source Decay (PSD) analysis. Confirmation of the proteins identified in the SH-SY5Y neuroblastoma cell line after ELF-MF exposure was made by Western blot analysis in the 1D and 2D mini system. An equal protein amount of 30 μg for 1D from each total cellular lysate was loaded. For 2D Western blot analysis 40 μg of protein were separated on 7 cm pH 4–7 IPG strip (GE Healthcare formerly Amersham Biotech). Primary antibody sources and dilutions were: rabbit polyclonal antibodies against ACTG 1:300 (Abcam, Cambridge Science Park, UK), ADK5 1:100 (Abcam), PRDX2 1:2,000 (Abcam), PRDX6 1:1,000 (Abcam), RAB3D 1:1,000 (Abcam), SPIN1 1:1,000 (ProteinTech Group, Chicago). Mouse monoclonal antibody against PROF2 1:2,000 (Abcam), ROPN1 1:2,000 (Abcam), TCPB1:1,000 (Abcam). Mouse polynoclonal antibody against MTPN 1:1,000 (Abcam), THTM 1:1,000 (Abcam), PRDX3 1:1,000 (ProteinTech Group). Signal detection was performed with an ECL plus kit (GE Healthcare formerly Amersham Biotech) and visualized by autography on Biomax light film (Sigma Chemical, St. Louis, MO). All blot experiments were performed at least three times.

RESULTS

CELL COUNTS, VIABILITY, AND PROLIFERATION

A significant increase in cell count, viability, and proliferation rates were observed as a consequence of the exposure to ELF-MF at all time points considered (Fig. 1). Indeed, significant elevations of Trypan blue-excluding cell number (about +40%, $P < 0.01$ vs. time-matched controls; Fig. 1A), MTT-related signal (about +25%, $P < 0.001$ vs. time-matched controls; Fig. 1B) and BrdU uptake (about +25%, $P < 0.05$ vs. time-matched controls, Fig. 1C) were detected in neuroblastoma cells for 5 days with MF. SH-SY5Y cells

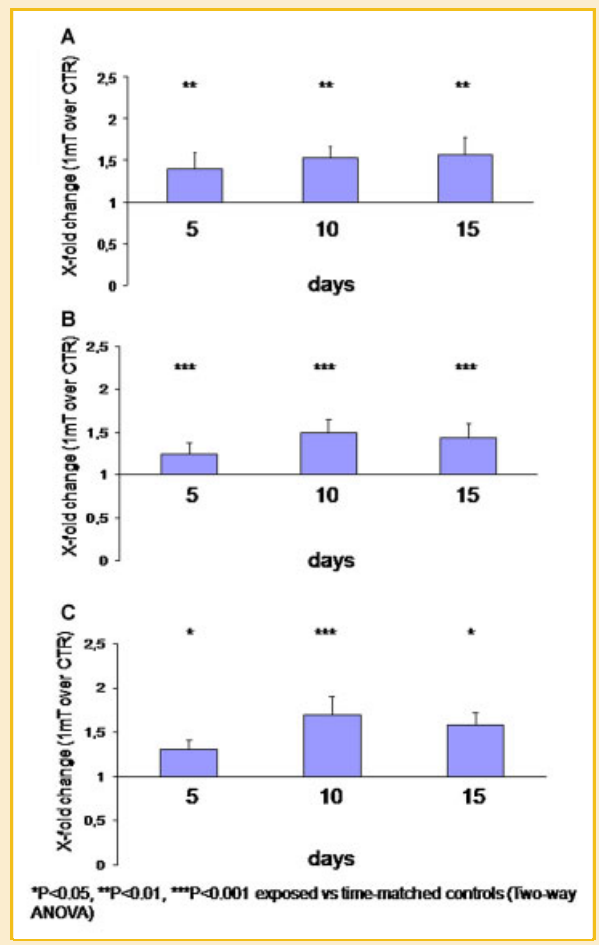

Fig. 1. Effects of 5, 10, and 15 days of sinusoidal ELF-MF exposure (1 mT, 50 Hz) on cell counts (panel A), cell viability (panel B), and cell proliferation (panel C) of SH-SY5Y human neuroblastoma cells. Significant increases in cell counts, viability, and proliferation rates were observed as a consequence of exposure to ELF-MF at all time points considered. Values are given as means treated over control X-fold changes ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ exposed versus time-matched controls (two-way ANOVA). [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

exhibited a more pronounced increase in cell counts ($P < 0.01$) and viability ($P < 0.001$) (about +50% for both indexes; Fig. 1A,B) and replicative activity (about +60%, $P < 0.001$; Fig. 1C) after 10 days of ELF-MF exposure, when compared to time-matched unexposed cells. No further increase in cell number, viability, and proliferation rates were observed after 15 days of exposure, though statistical differences were still detected between treated and time-matched unexposed controls (cell count, $P < 0.01$; MTT, $P < 0.001$; BrdU, $P < 0.05$) (Fig. 1A–C).

MORPHOLOGICAL ANALYSIS

The immunocytochemical results of SH-SY5Y cells obtained using DAPI and antibody against alpha-tubulin are reported in Figure 2. In the 5–10–15 day control samples, the DAPI staining reveals that cells are clustered, while treated cells appear to be more widely distributed, occupying all the available space. This event was not time dependent (Fig. 2A). As can be seen in Figure 2B the ELF treated cells show a higher and more widespread expression level of alpha-tubulin compared to control cells. In these last cells, it appears that the alpha-tubulin expression level is more detectable in the periphery of cell clusters (Fig. 2B). Treatment seemed to induce a spatial orientation of cells (Fig. 2B). The detailed view of alpha-tubulin expression did not reveal any significant change in its intracytoplasmic organization and distribution in both different experimental conditions (Fig. 2C).

As can be seen in the supplemental Figure S1, profilin 2 shows a cytoplasmic and nuclear distribution in both treated and untreated cells. However, the ELF-MF exposure seems to increase the profilin 2 expression in the nucleus compared to the cytoplasmic compartment.

PROTEOME CHANGES INDUCED BY ELF-MF EXPOSURE

In order to evaluate changes of protein pattern induced by ELF-MF, the master gels resulting from each sample in all treatment conditions examined (Fig. 3) were compared with the synthetic gel (data not showed) built by using 2D maps obtained for all SH-SY5Y untreated cell sample preparations. $2{,}200 \pm 187$ common protein spots were detected by matching analysis. 300 ± 26 of these spots were digested and analyzed by PMF. 86 unique proteins distributed on 2D map having a molecular mass ranging from 12 to 180 kDa and with an isoelectric point in the 4–7 pH range were assigned and listed in Table S2 (Supplementary data file).

In Figure 4, we reported a representative functional distribution of the control and treated SH-SY5Y common protein spots. Functional distribution analysis reveals that the proteins belonging to cellular organization, proliferation, and defense mechanism groups significantly increased after treatment from 20% to 28% added together.

Using matching analysis between the T10 treated cells and the control sample, we identified three new proteins: ropporin-1A (ROPN1), peroxiredoxin-2 (PRDX2), and actin cytoplasmic 2 (ACTG) (Table I). Their identification was confirmed by PSD and Western blotting experiments (Fig. 5). These three proteins were still expressed after 15 days treatment even though ROPN1 and PRDX2 increased in their levels whereas ACTG remained constant (Fig. 5A). Furthermore, six additional new proteins, that is, peroxiredoxin-3 (PRDX3), peroxiredoxin-6 (PRDX6), T complex protein 1 subunit beta (TCPB),

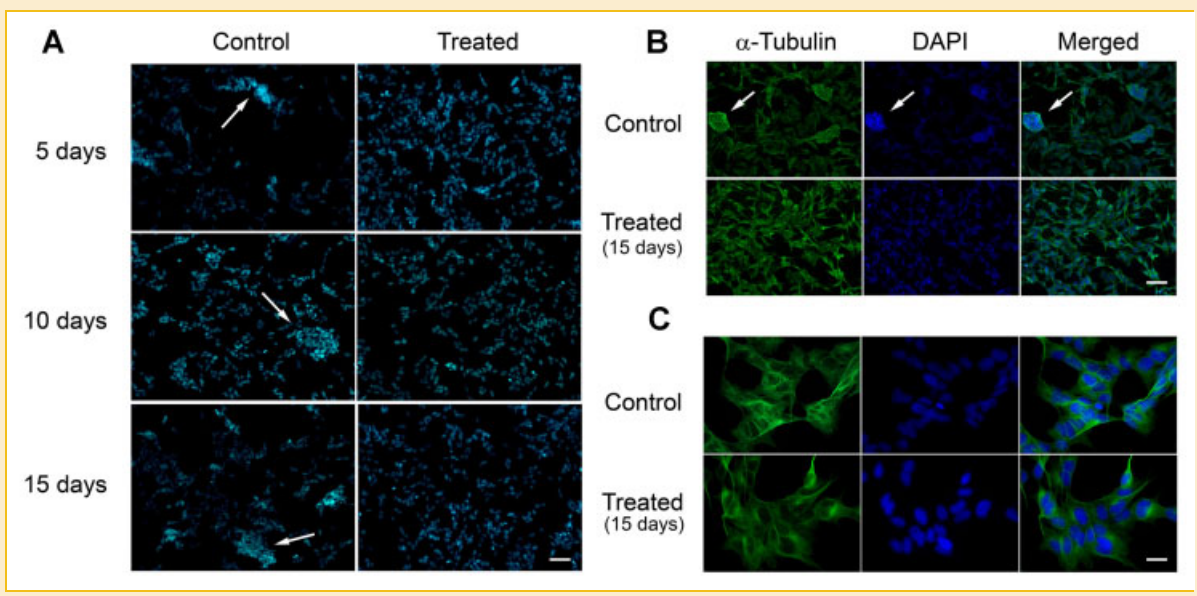

Fig. 2. Immunocytochemical analysis of SH-SY5Y after ELF-MF exposure. Control culture conditions induced cell cluster formation (panel A, scale bar 150 μm). Treated cells were more widely distributed compared to control cells, occupying all the available space (panel A). This event was not time dependent (panel A). Treated cells showed a higher and more widespread expression level of alpha-tubulin compared to control cells. Under control conditions, high expression levels were detectable in the periphery of cell clusters (panel B, scale bar 150 μm). Treatment seemed to induce a spatial orientation of cells (panel B). The detailed view of alpha-tubulin expression did not reveal any significant change in its intracytoplasmic organization and distribution in both different experimental conditions (panel C, scale bar 40 μm). [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

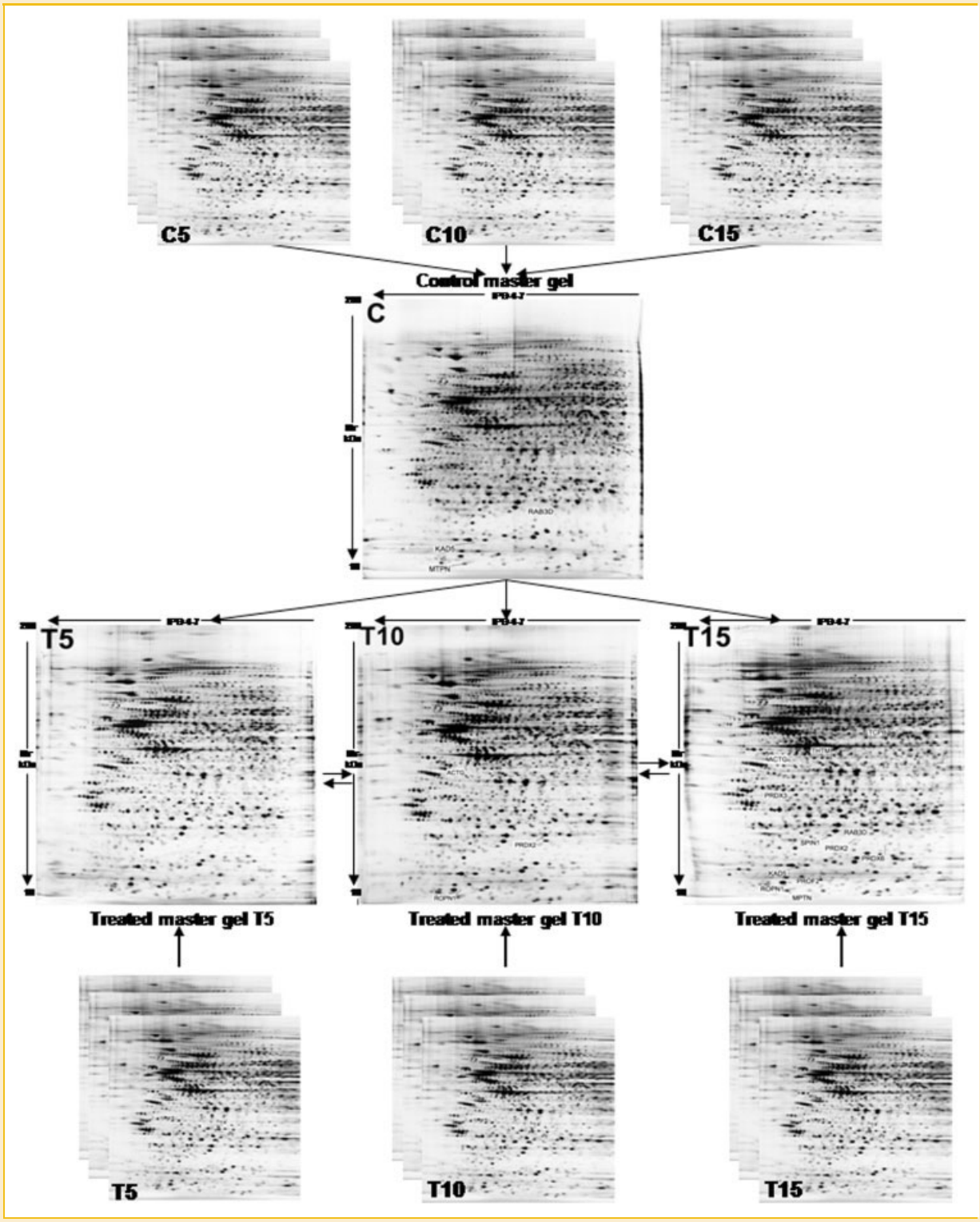

Fig. 3. Proteome analysis of human SH-SY5Y neuroblastoma cells. Master gel comparison between untreated and treated samples with 1 mT magnetic field (50 Hz) at three different times 5, 10, and 15 days. Each 2D map is the result obtained from three independent technical replicates and three biological replicates. C, control gel; T5, 5-day treatment gel; T10, 10-day treatment gel; T15, 15-day treatment gel. New and differentially expressed proteins are indicated in the gel with abbreviation name. All identifications were carried out by PMF.

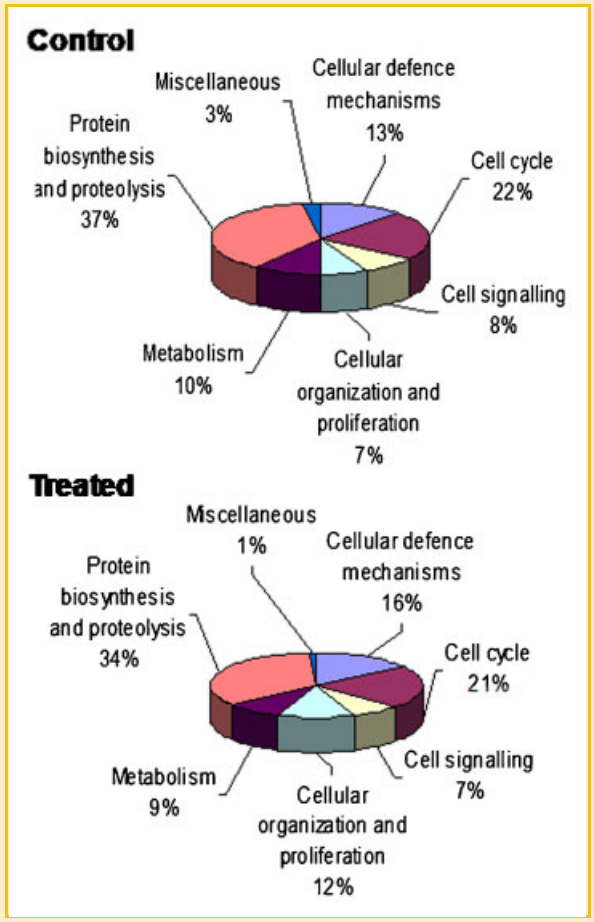

Fig. 4. SH-SY5Y common protein spots during ELF-MF treatment. Values represent the percent distribution of proteins classified by biological process of gene ontology terms into functional categories.

profilin-2 (PROF2), spindlin-1 (SPIN1), and 3-mercaptopyruvate sulfurtransferase (THTM) were identified in T15 (Fig. 5B). Their identification was validated by PSD and Western blotting experiments as can be seen in Figure 5B. We also found that three proteins, namely myotrophin (MPTN), adenylate kinase isoenzyme 5 (KAD5), and ras-related protein rab-3D (RAB3D) increased in their expression only after 15 days of treatment (Fig. 5A). These differentially expressed proteins, once assigned by PMF were validated by immunoblotting analysis (Fig. 5A).

DISCUSSION

Epidemiological and experimental evidence suggests that chronic exposures to ELF-MFs may be associated with increased risks of tumor development [Crumpton and Collins, 2004; Simkó, 2004; Santini et al., 2005; Wolf et al., 2005; Berg and Berg, 2006; Frahm et al., 2006; Lisi et al., 2006; Palumbo et al., 2006; Falone et al., 2007; Gottwald et al., 2007]. Our group previously established that ELF-MF exposure improves cellular viability and induces significant adaptations in the redox-related biochemical machinery of the human neuroderived SH-SY5Y cell line [Falone et al., 2007]. The findings of this proteomics-based research have demonstrated that ELF-MF exposure triggered significant changes in the protein global profile of SH-SY5Y cells. In particular, the expression levels of common proteins spots involved in cellular defense mechanisms, organization, and biogenesis increased as a consequence of ELF-MF treatment (Fig. 4).

The most interesting and relevant effect of ELF-MF on proteome of treated cells is the expression of nine new proteins, five belonging to cellular organization and proliferation group (ACTG, TCPB, ROPN1, PROF2, and SPIN1) and four belonging to cellular defense mechanism group (PRDX2, PRDX3, PRDX6, and THTM).

TCPB, PROF2, and ROPN1 are important proteins involved in the regulation of actin maturation and thus in the cytoskeleton arrangement which is considered a determinant factor for tumor cellular motility and metastatic potential [Hall, 2009]. PROF2 is a small actin binding protein which regulates the actin polymerization–depolymerization dynamics, whereas TCPB is a cytosolic chaperonin involved in the promotion of the correct folding of tubulin and actin [Roobol et al., 1995]. The presence of PROF2 following ELF-MF treatment in SH-SY5Y suggests it has a putative

TABLE I. MS Identification of Newly and Differentially Expressed Protein of SH-SY5Y After ELF-MFs Exposure

Protein	Abbr. name	AC[a]	Score[b]_SC[c] (%)	Theoretical M_r_p*I*	Experimental M_r_p*I*
Actin, cytoplasmic 2[d]	ACTG	P63261	316_57	41,792_5.31	40,556_5.16
Adenylate kinase isoenzyme 5[e]	KAD5	Q9Y6K8	183_59	22,087_5.38	19,722_5.15
3-Mercaptopyruvate sulfurtransferase[f]	THTM	P25325	260_57	33,178_6.13	46,395_5.57
Myotrophin[e]	MTPN	P58546	91_21	12,894_5.27	15,790_5.19
Peroxiredoxin-2[d,g]	PRDX2	P32119	122_30	21,891_5.66	21,495_5.66
Peroxiredoxin-3[f]	PRDX3	P30048	103_21	27,692_7.6	32,082_5.33
Peroxiredoxin-6[f]	PRDX6	P30041	341_83	25,034_6.00	20,877_5.98
Profilin[f]	PROF2	P35080	222_49	15,046_6.55	17,496_5.40
Ras-related protein Rab-3D[e]	RAB3D	O95716	160_60	24,267_4.76	23,352_5.90
Ropporin-1A[d,g]	ROPN1	Q9HAT0	149_53	23,892_5.52	16,577_5.17
Spindlin-1[f]	SPIN1	Q9Y657	130_51	29,600_6.46	21,902_5.40
t-Complex protein 1 subunit beta[f]	TCPB	P78371	121_31	84,972_6.01	577,940_6.00

[a]Entry code number from NCBI database.
[b]Score is $-10\log(P)$, where P is probability that the observed mach is a random event, it is based on NCBI database using the MASCOT searching program as MALDI-TOF data. Sequence coverage means the ratio of portion sequence covered by matched peptide to the full length of the protein sequence.
[d]New proteins expressed after 10 days of treatment.
[e]Proteins differentially expressed after 15 days of treatment.
[f]New proteins expressed after 15 days of treatment.
[g]Proteins up-regulated after 15 days of treatment.

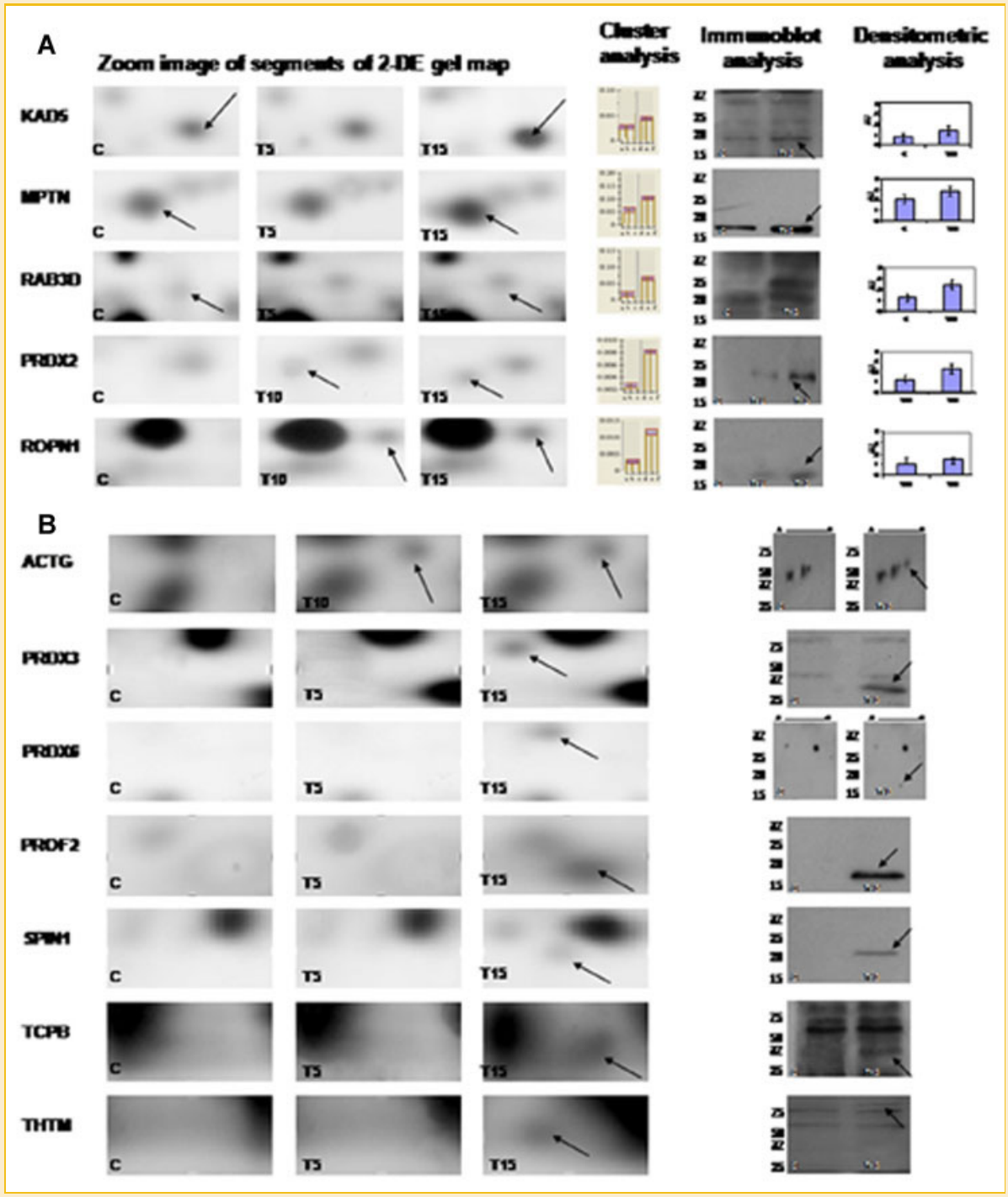

Fig. 5. Zoom image of newly and differentially expressed protein of SH-SY5Y after ELF-MF exposure. Panels A and B: Segments of 2DE gel map from control and treated cells. The arrows indicate the differentially and new expressed proteins. Cluster analysis display the relative spot volume of proteins compared with the total amount of the protein in the gel. Panel A: 1D gels probed with antibodies raised against KAD5, MPTN, PRDX2, RAB3D, ROPN1, and differentially expressed proteins with relative densitometric analysis. Panel B: 1D blot analysis raised against PRDX3, PROF2, SPIN1, TCPB, and THTN and 2D blot analysis raised against ACTG and PRDX6. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

role in other cellular processes such as transcriptional activity or membrane trafficking connected to cellular changes occurring in malignant transformation [Witke, 2004]. It is also believed that PROF2 can mediate a more efficient nucleation and elongation of actin filaments and this could be associated with alterations in cell shape and enhanced capacity of cell migration [Jonckheere et al., 1999; Cooper, 2000].

Another protein that regulates the actin cytoskeleton organization is the GTP-binding protein Rho, ROPN1. This protein appears only after 10 days of ELF-MF treatment and its expression is

enhanced at 15 days. It has been suggested that ROPN1 induces actin re-organization by modulating the myosin light chain phosphorylation [Fujita et al., 2000]. Various cellular processes such as cell adhesion and motility have been correlated to its functional cellular role [Fujita et al., 2000]. It is noteworthy that ROPN1 was found over-expressed in hematologic tumor cells [Li et al., 2007].

SPIN1 is localized in the inter-phase nucleus and mitotic chromosomes [Zhao et al., 2007]. Even though its biological function is at present poorly understood, it appears involved in cell cycle regulation. However, human ovarian cancer cells show an increase of expression level when compared to normal ovarian tissue [Yuan et al., 2008]. In addition, Zhang et al. [2008] demonstrated that over-expression of SPIN1 seems to induce chromosomal instability during gametogenesis, promoting cancer development. ELF radiation is thought to significantly alter redox balance. Some of us have already reported that a 50 Hz, 1 mT magnetic field exposure positively modulates antioxidant enzymatic defense and significantly increases the levels of reduced glutathione in SH-SY5Y cells [Falone et al., 2007]. In the present study, 10- and 15-day treatments triggered the expression of new isoforms of peroxiredoxin, that is, PRDX2, PRDX6, PRDX3, and THTM. PRDXs are a group of antioxidant thioredoxin-dependent peroxidases that have many functions such as cellular protection against oxidative stress (reducing H_2O_2, organic peroxides, and peroxinitrite), modulation of H_2O_2-mediated intracellular signaling cascades and regulation of cell proliferation (involving c-Myc-mediated cell trasformation and apoptosis) [Kim et al., 2000; Wonsey et al., 2002; Wood et al., 2003; Immenschuh and Vogt, 2005; Cao et al., 2007].

Literature data report an over-expression of PRDXs in several human cancers (mesothelioma, bladder, breast, and brain cancers) during tumorigenesis [Kinnula et al., 2002; Chang et al., 2007; Zhang et al., 2009], tumor recurrence or progression [Quan et al., 2006], and cancer therapies [An and Seong, 2006; Pak et al., 2011].

According to recent studies by Chang and co-workers, PRDX6 has a high concentration in the metastatic breast and lung cancer cell, confirming the functional role of this enzyme to promote cellular invasiveness and metastatic potential in these cancer cells [Chang et al., 2007; Ho et al., 2010]. In addition, abnormal expression of PRDX6 associated with malignant transformation in oligodendroglioma suggests that PRDX6 may be considered a putative molecular predictive factor [Park et al., 2008].

The final antioxidant protein induced following the 15-day treatment is THTM, an enzyme participating in the formation of sulfane sulfur-containing compounds that transfer their sulfur atoms to various acceptors affecting cell proliferation [Toohey, 1989; Jurkowska and Wrobel, 2008; Jurkowska et al., 2011].

As commonly accepted, extensive reprogramming of proteome expression can initiate and sustain major modifications in several aspects of cell behavior. The comparative proteomic approach we used in this study revealed that a prolonged exposure to a 50 Hz, 1 mT ELF-MF is able to activate the expression of several new proteins, some of which are thought to be linked to increased cell proliferation and biogenesis, as well as to cytoskeleton re-arrangement and enhanced cellular motility, and to malignant progression. These intriguing findings led us to investigate how ELF-MF-induced changes in proteome expression could affect major cell biology characteristics.

This study has revealed that the proliferative activity of SH-SY5Y cells was profoundly enhanced by ELF-MF treatment. The MF-induced perturbation of proliferative behavior exhibited a time-dependent profile and both the BrdU- and MTT-based assays indicated a plateau effect after 10 days of MF exposure. Cell proliferation is thought to be strictly linked to malignant potential. Zhao et al. showed a major reduction in tumor development when suppression of SH-SY5Y cellular division was achieved within a mouse model of human neuroblastoma xenograft, thus demonstrating that cell proliferation is a key factor controlling neoplastic growth [Zhao et al., 2008].

Immunocytochemical investigations revealed that ELF-MF triggered major alterations in cell growth behavior and induced evident morphological cellular modifications, that is, markedly clustered cellular aggregations in non-treated cells when compared to uniformly distributed monolayered exposed cells. Nevo et al. [2008] brilliantly showed that local and metastatic variants of neuroblastoma cells show distinct cell morphologies and growth aspects, and demonstrated that SH-SY5Y local variants resemble the cultured parental cell lines with aggregate-growing neuroblastic phenotype; in contrast, the metastatic variants were found to grow mostly in monolayer. As reported by several authors, the antimetastatic activity of certain anticancer drugs is thought to be strongly related to the ability to inhibit cell proliferation and cell migration [Voigt and Zintl, 2003; Timeus et al., 2008; Ferrari-Toninelli et al., 2010]. Our data are in accordance with the results obtained by Pirozzoli et al. [2003], regarding the ELF-MF-induced increase in proliferation rates that counteract differentiative processes. Other authors have suggested that a 50 Hz, 1 mT sinusoidal ELF-MF radiation may significantly alter the invasive properties of non-nervous tumor spheroids [Santini et al., 2006]. Intriguingly, our investigation revealed that several behavioral functions and aspects regarding cellular biology were affected by ELF-MF before significant changes in proteome expression were revealed. A possible explanation for this fact may be that ELF-MF exposure initially modified critical cell behaviors through alterations of functional properties of existing proteins and enzymes within cells; on the other hand, the prolonged treatment upon ELF magnetic field caused dramatic changes in proteome profile, and these molecular modifications could be linked to functional and behavioral shifts.

In summary, our results suggest that the 50 Hz, 1 mT ELF-MF triggered a likely phenotypic shift toward a more undifferentiated state. Proteins whose over-expression is related to high malignant potential, drug resistance, cytoskeleton re-arrangement, and enhanced defense against oxidative stress were found in ELF-MF-treated samples, together with higher proliferative activity. Our findings seem to strengthen the hypothesis of an association between exposure to ELF-MFs and malignant diseases [Wertheimer and Leeper, 1979; IARC, 2002; Draper et al., 2005], providing some molecular details underlying the effects elicited by ELF radiation on cellular systems.

Our investigation identified a number of probable cellular targets through which ELF-MFs exposure elicits time-dependent major

changes in cell biology and behavior. As well, we revealed that a number of ELF-MF-induced molecular alterations could be associated with less differentiated cellular phenotype and with enhanced malignant potential.

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