Gene analysis of rat chromaffin cells ELF MF differentiated using microarrays.

Tatiana Olivares-Bañuelos[a], Lina Riego[b], Alicia Gonzalez[c] and Rene Drucker-Colin[a].

a. Departamento de Neurociencias.
b. Unidad de Servicio de Microarreglos de DNA.
c. Departamento de Genética Molecular, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, México.

Correspondence: Dr. René Drucker-Colín, Depto. de Neurociencias, Instituto de Fisiología Celular, UNAM, Apartado. Postal 70-600, 04510 México, D.F., México
Phone: 525-550-6662; **Fax:** 525-550-0904; **Email:** drucker@servidor.unam.mx

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Chromaffin cells have been extensively used as a model for the study of differentiation. Several reports have shown that such cells can change from an endocrine phenotype to a sympathetic neuron-like type when stimulated with either Nerve Growth Factor (NGF) or Extremely Low Frequency Magnetic Fields (ELF MF, 60 Hz, 0.7 mT, 4 hours/day, 7 days)[1-3].

We have recently demonstrated that ELF MF-neuron-like differentiated cells predominantly form dopaminergic cells[4] and develop neurites with a large number of tyrosine hidroxylase positive vesicles as well as augmented neurofilaments[5]. It has also been shown that this differentiation process largely depends on L-type Ca^{2+} channels[6].

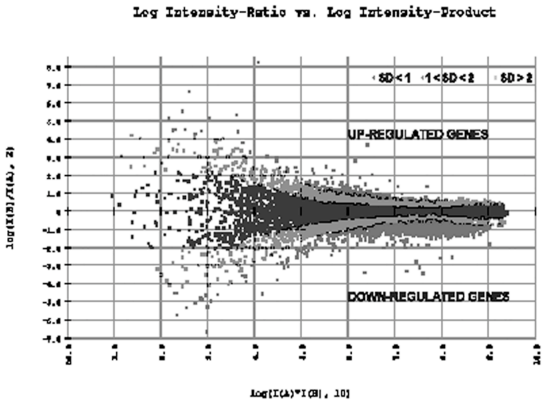
Thus, it can be expected that Electromagnetic Fields (EMF) stimulation must result in the induction of a variety of biochemical processes and changes in gene expression leading to short and long-term consequences on cellular behavior[7-9]. The mechanisms through which magnetic fields induce functional modifications have not been understood. The aim of the present work is to analyze the gene expression pattern obtained by ELF MF induction during chromaffin cells differentiation using microarray hybridization.

Microarrays are typically used to measure mRNA abundance, and this kind of experiments often provides a lot of information that cannot be completely analyzed in a single study [10].

RESULTS AND DISCUSSION

ELF MF treatment elicits a complex chromaffin cells trans-differentiation process that should imply important modifications in gene expression. As Figure 1-A shows, Wistar neonate rats' chromaffin cells cultures ELF MF-differentiated (experimental group) present differences on gene expression pattern compared with untreated chromaffin cells (control group). After filtering and analyzing four microarray slides using the TIGR-MIDAS software[11], with two standard deviation score we found that 170 genes modify their expression level between control and experimental group.

A



B

GENES DIFFERENTIALY EXPRESED ON CHROMAFFIN CELLS ELF MF TREATED	
Bone morphogenetic protein 4 (UR)	Pleiomorphic adenoma gene-like 1 (UR)
Cyclin G1 (UR)	Ribosomal protein L24 (UR)
Discoidin domain receptor family, member 1 (UR)	Ribosomal protein L5 (UR)
GTP cyclohydrolase 1 (UR)	Solute carrier family 12, member 2 (UR)
Nogo-A (UR)	Thioredoxin reductase 2 (UR)
Protein phosphatase 1, catalytic subunit, beta isoform (UR)	Transgelin 3 (UR)
Amyloid beta (A4) precursor protein-binding, family A, member 3 (X11-like 2) (UR)	Tumor protein, translationally-controlled 1 (UR)
Biglycan (UR)	v-akt murine thymoma viral oncogene homolog 1 (UR)
Cysteine rich protein 1 (UR)	Vesicle associated protein (UR)
Global ischemia induced protein GIIG15B (UR)	Vesicle-associated membrane protein 3 (UR)
Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta (UR)	Growth associated protein 43 (DR)
Insulin induced gene 2 (UR)	Inositol polyphosphate multikinase (DR)

Figure 1. Differences between chromaffin cells untreated and chromaffin cells ELF MF differentiated using microarray analysis. A) Graphic of local variation as a function of intensity is used to identify differentially expressed genes by calculating an intensity-dependent Standard Deviation-score using the TIGR-MIDAS software. In this R-I plot, array elements are grey scale-coded depending on whether they are less than one standard deviation (SD) from the mean (black), between one and two SD (grey), or more than two SD from the mean (grey light). Zones of both up and down regulated genes are indicated. **B)** Table of known identified differentially expressed genes on chromaffin cells after ELF MF treatment. Both up (UR) and down (DR) regulation are indicated.

Of these genes, 24 are of known function and 146 correspond to ESTs sequences. We observed a mayor number of genes down regulated (22 genes) during ELF MF chromaffin differentiation (Fig. 1-A); in contrast with only 2 genes up regulated. Similar observations were made by Angelastro[12] on PC12 cells NGF-differentiated; they report only a 2% of genes up regulated during this process using SAGE method.

We observed that the genes with known function participating on ELF MF cellular differentiation are mainly involved on banding, cellular adhesion, catalytic activity and signal transduction (Figure 1-B). On PC12 cells have been reported genes that regulate the expression of adhesion molecules, membrane receptors and protein phosphorylation[13-15]. We found two up regulated genes: cyclin G1, involved on cellular processes as mitosis; and, bone morphogenetic protein 4, which participate on cellular development. Bone morphogenetic proteins (BMPs) are shown to promote NGF-induced neuronal differentiation on PC12 cells. Also, the addition of BMP4 or BMP6 robustly increased the neuritogenic effect on NGF within 2 days on PC12 cultures[16].

Our results indicate that ELF MF treatment elicits a peculiar transcriptional response where genes are manly down regulated; meanwhile few are up regulated, like BMP4. This observation supports the proposition that the large repulsive forces generated by ELF MF could determine a physical modification of the DNA structure of the region[17], which could either favor or hinder transcription. This suggests that the differentiation process elicited by ELF MF implicates changes in cellular programs that must turn off expression of complete sets of genes. Some other genes are over-expressed, thus the combination of the two may be responsible for the process.

The identification of the ESTs genes whose regulation is specifically increased by ELF MF treatment will surely shed light on the nature of the signal transduction pathway needed for ELF MF-dependent differentiation.

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