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Therapeutic Effect of Electroacupuncture Based on Molecular Biological Studies

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Abstract

Acupuncture, which is one type of complementary and alternative medicine (CAM) have been used to cure many diseases and to maintain health, but an assessment of its efficacy is required. We have been investigating the molecular efficacy of electro acupuncture (EA), which is one type of acupuncture therapy. This short review introduces our molecular biological studies by using animal model: gene expression in mouse skeletal muscle which is regulated by EA and the therapeutic effect on muscle atrophy, identification of Aig1l gene which is induced in EA and approaches to predict the function of its protein. Our results showed that EA treatment suppresses myostatin expression, which leads to a satellite cell-related proliferative reaction, and repair in skeletal muscle and is effective for prevention of muscle atrophy. Our findings also suggest that the Aig1l gene may play a key role in the molecular mechanisms of EA efficacy.

Keywords: Electro acupuncture; Myostatin; Satellite cell; Atrophy prevention; Aig1l

Introduction

Acupuncture therapy has long been used to maintain health and cure many diseases as one of the routine clinical practices in eastern Asia (mainly Japan, China and Korea) and one of complementary and alternative medicine (CAM) in the world. Major acupuncture techniques utilize penetration of the skin by thin, solid metallic needles, which are manipulated manually or are stimulated electrically with a low-frequency microcurrent [1]. The electrical needle stimulation is called electroacupuncture (EA) [2]. EA is effective not only for pain but also for muscle problems, such as stiffness, exhaustion, and atrophy, in many patients including elderly people [3]. Previously, many investigations of acupuncture, have clarified a neural mechanism of its efficacy; for example, anesthesia produced by acupuncture induced endogenous opioids (β-endorphin and enkephalin) [4,5]. However, molecular mechanisms of other effects of acupuncture are as yet unclear. This article shows the molecular efficacy of EA along with the results based on the analysis of gene expression regulated by EA and the mice model. Due to space limitation, we would like to ask the readers to refer our reports for the details of our experiments and its results [1,6,7]. Ethical approvals were obtained from all the research institutions where we conducted animal experiments in this reports.The molecular efficacy of EA by using mice model scientific evidence of efficacy is as important for CAM research as it is for research in Western medicine. To identify the molecular characteristics of EA, identification of genes that are specifically modulated during the process of acupuncture would be an initial step toward elucidation of underlying mechanisms because many cellular and physiological processes are regulated at the transcription level of gene expression. As we reported in our study of EA and myostatin gene expression, we evaluated the differential expression of genes induced by EA in mouse skeletal muscle and then used RT-PCR to confirm the expression patterns of the differentially expressed genes [1]. Bioinformatics analysis of their transcription control regions showed that EA- inducible genes are related to cell differentiation, cell proliferation, muscle repair, and hyperplasia. These results suggested that EA treatment may induce cell proliferation in skeletal muscle. To verify this possibility, we used EA to stimulate mouse skeletal muscle daily for up to 1 mo and examined the long-term effects. As a result, the expression of the gene encoding myostatin, which is a growth repressor in muscle satellite cells [8], was suppressed by daily EA treatment for 1 wk; EA treatment for 1 mo resulted in more marked suppression of the gene (Figure 1A). Immunohistochemical analysis also showed that nuclei of muscle cells treated with EA for 1 mo, especially nuclei of satellite cells, reacted with anti-human PCNA (Figure 1B), which confirmed that EA caused a proliferative reaction in skeletal muscle. These molecular findings constitute strong evidence that EA treatment suppresses myostatin expression, which leads to a satellite cell-related proliferative reaction and repair in skeletal muscle. In this view of our study, it indicated that EA-induced myostatin gene suppression may help prevent muscle atrophy. We next used a hindlimb suspension model in a preclinical study to investigate the effect of EA on muscle atrophy [6]. Hindlimb-suspended (HS) rodents are a commonly used animal model for pathological studies of the loss of muscle mass, such as disuse muscle atrophy [9-11]. In our study, we compared the measures of muscle mass and myofibre diameter in the soleus muscle of HS mice and HS mice treated with EA (EA/HS). We also analysed changes in the expression of myostatin gene and ubiquitin ligase genes by using real-time quantitative PCR because they play key roles in hindlimb suspension. For proper internal control genes for real-time quantitative PCR, we analysed the expression of six housekeeping genes according to a previous report [12] because two factors intervene in our experiment, that are, HS and EA. However, no gene showed a constant expression level in both HS and EA/HS groups (Figure 2). We then chose three internal control genes from the six genes —β-actin, GAPDH, and transferrin receptor— by calculating the relative expression levels using the geometric mean of their expression levels. As a result of the comparison of gene expression in the mice with

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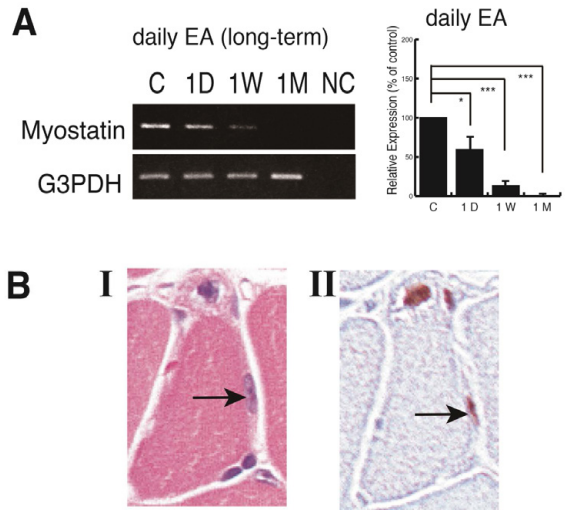

Figure 1: **(A)** The effect of long-term treatment of EA on skeletal muscle.(A) RT-PCR analysis of myostatin gene expression. G3PDH was used as a loading control. Lanes are as follows: lane C, control; lane 1D, EA stimulation for 1 day; lane1W, after daily EA stimulation for 1 wk; lane 1M, after daily EA stimulation for 1 mo.Relative transcript levels of myostatin are shown (means ± SD). *P < 0.05; NS, not significant.

(B) Histochemical and immunohistochemical analysis for EA-treated muscle. Hematoxylin and eosin (H&E)-staining is I; immunostaining is II. In serial sections, PCNA antibody reacted in satellite cells (I, indicated by the arrow), and nucleoli (II, indicated by the arrow) were found in the same nuclei by H&E staining.Original magnifications: ×100

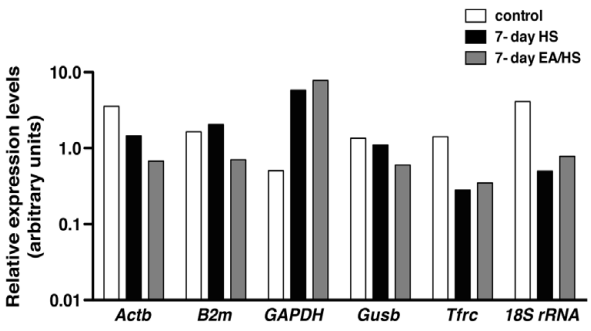

Figure 2: Logarithmic histogram of the expression levels of housekeeping genes.Actb, beta-actin; B2m, beta 2-microglobulin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Gusb, beta-glucuronidase; Tfrc, transferrin receptor; 18S rRNA, 18S ribosomal RNA. White bars, control mice; black bars, 7 days HS mice; gray bars, 7 days EA/HS mice.

7 days of HS and EA/HS, myostatin gene was induced in HS mice but its expression was significantly suppressed in EA/HS mice. In addition, the two ubiquitin ligase genes, MuRF-1 and MAFbx, were induced in HS mice but their expression was significantly suppressed in EA/HS mice (Figure 3A). Furthermore, HS mice had significantly reduced cross-sectional muscle fibre diameters when compared with control mice and EA/HS mice had significantly larger muscle fibre diameters than HS mice (Figure 3B). The result of the relative wet weight of the soleus muscle showed a significant reduction in HS mice, but a significantly higher in EA/HS mice (data not shown). All these findings suggest that EA prevented the muscle atrophy that was caused by hindlimb suspension. Aig1l gene induced by EA and the prediction of its function to elucidate the molecular efficacy of acupuncture, it is important to identify the regulated genes by acupuncture and to detect the function of the genes' proteins. In our study, we found an EA-induced gene in mouse skeletal muscle and named it acupuncture-induced 1-L (Aig1l) [1,7]. This gene is the only gene which was discovered in association with Oriental Medicine which includes acupuncture. We determined

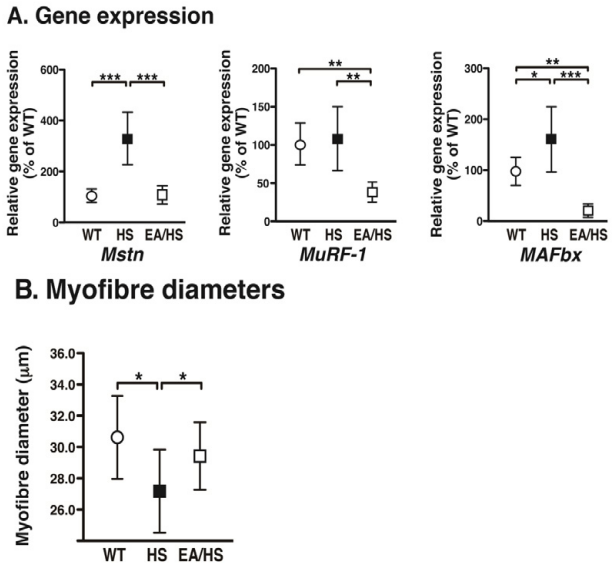

Figure 3: Effect of EA on hindlimb suppression.
(A) Gene expression as related to hindlimb suppression and EA effect. Real-timequantitative PCR analysis of expression for Mstn , MuRF-1, and MAFbx genes in WT,HS, and EA/HS mice. Relative transcript levels are shown (means ± SD).
(B) Changes in myofibre diameters after EA. The effect of EA on prevention of muscle atrophy was10 shown by changes in myofibre diameters in three groups. Open circle, WT mice; closed squares, HS mice; open squares, EA/HS mice. *P < 0.05; **P < 0.01; ***P < 0.005.

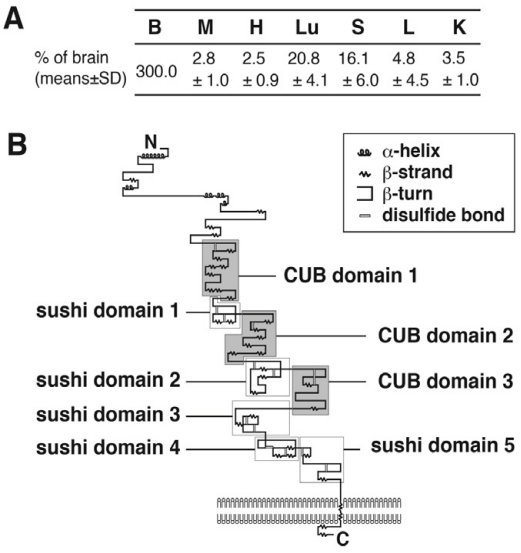

Figure 4: Tissue distribution of Aig1l gene and the secondary structure of the protein.
(A) Tissue distribution of Aig1l gene as detected by real-time quantitative PCR analysis
(n = 5).B, Brain; M, muscle; H, heart; Lu, lung; S, spleen; L, liver and K, kidney.
(B) The secondary structure of Aig1l protein predicted by Pfam database and Jpred program. Aig1l protein is predicted to be a one-pass transmembrane protein, which has three CUB and five sushi domains.

the complete cDNA sequence of Aig1l and registered it with the GenBank genome database (GenBank accession no. DQ167195). The sequence of Aig1l is also registered as seizure related 6 homolog like (Sez6l) gene. The tissue distribution of Aig1l gene expression revealed the greatest expression in the brain, as evidenced by real-time quantitative PCR analysis (Figure 4A). The rest of other tissues had very weak gene expression (Figure 4A). In our study for the Aig1l protein, we have predicted its secondary structure by bioinformatics analysis via Pfam database (http://pfam.xfam.org/) and JPred4 program (http://www.compbio.dundee.ac.uk/jpred/). The results indicated that Aig1l includes a one-pass transmembrane protein, which has CUB and Sushi domains (Figure 4B). These domains were reported its association with neurotransmission and protein-protein interaction [13-15]. Since protein domains are key to understand the function of the protein, further analysis of Aig1l focused on neurotransmission and protein-protein interaction may reveal the functional importance of this gene. Thus a detailed functional analysis of Aig1l protein is necessary and is worth continuing the study. Aig1l protein may play a key role for the molecular mechanism of the therapeutic effect of EA. Indeed, future research into such area is required.

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