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Research report

The effect of electroacupuncture on proteomic changes in the motor cortex of 6-OHDA Parkinsonian rats

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ABSTRACT

Electroacupuncture (EA) has been reported to alleviate motor deficits in Parkinson's disease (PD) patients, and PD animal models. However, the mechanisms by which EA improves motor function have not been investigated. We have employed a 6-hydroxydopamine (6-OHDA) unilateral injection induced PD model to investigate whether EA alters protein expression in the motor cortex. We found that 4 weeks of EA treatment significantly improved spontaneous floor plane locomotion and rotarod performance. High-throughput proteomic analysis in the motor cortex was employed. The expression of 54 proteins were altered in the unlesioned motor cortex, and 102 protein expressions were altered in the lesioned motor cortex of 6-OHDA rats compared to sham rats. Compared to non-treatment PD control, EA treatment reversed 6 proteins in unlesioned and 19 proteins in lesioned motor cortex. The present study demonstrated that PD induces proteomic changes in the motor cortex, some of which are rescued by EA treatment. These targeted proteins were mainly involved in increasing autophagy, mRNA processing and ATP binding and maintaining the balance of neurotransmitters.

1. Introduction

Parkinson's disease (PD) is a representative neurodegenerative disorder as a consequence of a substantial loss of dopaminergic (DA) nigral neurons (Grayson, 2016). PD is accompanied by a series of behavioral disturbances (Sian et al., 1999). Physical therapies, which have been demonstrated to alleviate motor deficits, are attractive treatment options for PD (Deuschl et al., 2006; Khedr et al., 2007; Lee et al., 2013; Li et al., 2012). As an alternative to physical therapy for PD, 100 Hz electro-acupuncture (EA) therapy leads to subjective improvements of motor symptoms in PD patients (Huo et al., 2012). EA treatment has also been reported to reduce motor deficits in several rodent models (Deng et al., 2015; Lv et al., 2015; Yu et al., 2016). However, the mechanisms by which EA improves motor symptoms of PD remain unclear.

The proteomic technique provides a high throughput method for the quantitative assay of protein mixtures extracted from tissues or cells (Kim et al., 2010). Several proteomic studies have been performed in PD animal models to screen differentially expressed proteins induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and acupuncture treatment (Jeon et al., 2008; Kim et al., 2010). However, these studies have only focused on the proteome in the striatum and substantia nigra (SN). Recent studies have suggested that the motor cortex is an important therapeutic target for physical therapies (Filipovic et al., 2010; Li et al., 2012). The present study will investigate tissues from the bilateral motor cortex. Since mass spectrometry coupled to protein labeling by isobaric tags for the relative and absolute quantitation (iTRAQ) technique is more precise and reliable, it will be applied to replace the two-dimensional electrophoresis (2-DE) which has been used in the aforementioned proteomic studies (Qian et al., 2015; Zhang et al., 2016).

The aim of the present study is to identify the differentially expressed proteins in the motor cortex induced by 6-OHDA injection and EA treatment. The identified differentially expressed proteins in the motor cortex may serve as potential disease markers for early diagnosis and therapeutic targets for further treatments. Furthermore, the present study provides better understandings of the application of EA in 6-OHDA rat model and offers an alternative choice to current treatments.

2. Results

The results of TH IHC revealed that the expression of TH was significantly decreased in the lesioned substantia nigra (SN) and

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lesioned striatum of the 6-OHDA rats (Fig. 1A). Quantification of TH-positive neurons in the SN (Fig. 1B, $p = 0.000$ vs. sham) and TH-positive fibers in the striatum (Fig. 1C, $p = 0.001$ vs. sham) support that unilateral 6-OHDA rat model was well established. Spontaneous locomotion including FP move time (Fig. 2A, $p = 0.001$ vs. sham), FP distance (Fig. 2B, $p = 0.000$ vs. sham) and FP velocity (Fig. 2C, $p = 0.000$ vs. sham) was significantly impaired in 6-OHDA rats. The behavioral results show that FP spontaneous locomotion in the open field test was rescued by EA (Fig. 2A–C, $p = 0.019$, $p = 0.024$, $p = 0.021$ vs. model, respectively). Furthermore, 4 weeks of EA treatment also significantly improved the motor function of 6-OHDA rats measured by rotarod test (Fig. 2D, $p = 0.003$ vs. model).

4244 proteins were identified from SD rats, among which 4195 proteins were quantified. The expression of 36 proteins were decreased while 18 proteins were increased in the unlesioned contralateral motor cortex of model group when compared to sham group (Table 1). EA treatment up-regulated 30 proteins, and down-regulated 25 proteins in the unlesioned motor cortex of 6-OHDA rats (Table 2). The expression of 58 proteins was decreased while 44 proteins were increased in the lesioned motor cortex of model group when compared to sham group (Table 3). 60 proteins were up-regulated and 35 proteins were down-regulated in the lesioned motor cortex of 6-OHDA rats by EA treatment compared to non-treated PD control (Table 4). The differentially expressed proteins in the bilateral motor cortex of 6-OHDA rats compared with sham-operated rats were defined as PD-associated proteins. Differentially expressed proteins in the bilateral motor cortex of EA-treated 6-OHDA rats compared with untreated 6-OHDA rats were termed as EA-regulated proteins.

GO analysis was performed to evaluate the function of differentially expressed proteins. This helps investigators to translate the proteomic data into the structural and functional meaning. The GO annotation contains cellular component, molecular function and biological process. The most common cellular components of PD-associated proteins were extracellular matrix part, nucleoplasm, nuclear speck, nuclear body, extracellular region and mitochondrial membrane in the unlesioned motor cortex (Fig. 3A). EA-regulated proteins in unlesioned motor cortex were mainly involved in the extracellular region, membrane raft, membrane part, mitochondrial outer membrane translocase complex, dense core granule, plasma membrane, cell periphery, cell surface and external side of plasma membrane (Fig. 3A). In addition, PD-associated proteins in lesioned motor cortex were enriched in catalytic step 2 spliceosome, spliceosomal complex, mitochondrial inner membrane, organelle inner membrane, U12-type spliceosomal complex, terminal bouton, heterotrimeric G-protein complex, mitochondria respiratory chain, endocytic vesicle membrane (Fig. 3A). EA-regulated proteins in lesioned motor cortex, were enriched in catalytic step 2 spliceosome, cell part, nucleus, spindle, inclusion body, cell surface and external side of plasma membrane (Fig. 3A).

The most common molecular functions of PD-associated proteins were cation binding, metal ion binding, cytochrome *c* oxidase activity, heme-copper terminal oxidase activity, oxidoreductase activity, RNA 7-methylguanosine cap binding, iron ion binding, RNA cap binding and ARF GTPase activator activity in unlesioned motor cortex (Fig. 3B). EA-regulated proteins in unlesioned motor cortex were enriched in molecular functions such as glycoprotein binding, non-membrane spanning protein tyrosine kinase activity and protein tyrosine kinase activity (Fig. 3B). Meanwhile, the most prevalent molecular functions of PD-associated proteins in the lesioned motor cortex were as follows: phosphotransferase activity, protein domain specific binding, GTP binding, guanyl nucleotide binding, guanyl ribonucleotide binding, SH3 domain binding, chromatin binding, heme-copper terminal oxidase activity and oxidoreductase activity (Fig. 3B). The most prevalent molecular functions of EA-regulated proteins in unlesioned motor cortex were as follows: non-membrane spanning protein tyrosine kinase activity, protein tyrosine kinase activity, ubiquitin binding, protein methyl-

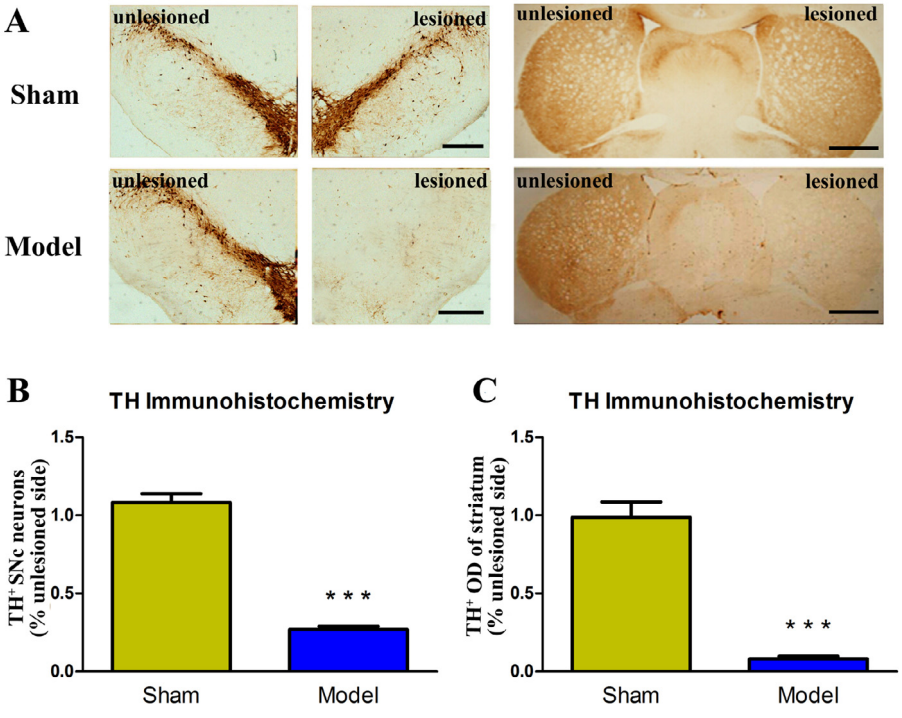

Fig. 1. There was a significant reduction of TH expression in the SN and striatum of 6-OHDA rats compared to sham-operated rats. (A) Representative photomicrographs of the SN under 10× magnification and striatum under 5× magnification. The scale bar = 200 μm. The damage extent of TH-positive neurons in the lesioned SN (B) and TH-positive fibers in the lesioned striatum (C) were quantificated as compared to unlesioned side. ***$p < 0.001$ *vs.* sham; N = 6 in each group.