Applying RNA sequencing technology to explore repair mechanism of Tuina on gastrocnemius muscle in sciatic nerve injury rats

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To the Editor: Peripheral nerve injury (PNI) refers to the injury of the peripheral nerve plexus, nerve trunk, or its branches under the action of external forces. Skeletal muscle-derived cells serve a strong secretory function while they can differentiate into Schwann cell-like cells or other cell types, so that it can effectively promote regeneration of peripheral nerves.[1] Using some specific acupuncture points with mild mechanical stimulation on the surface can have a positive effect on nerve repair.[2] Tuina helps relieve symptoms such as muscle spasms and pain caused by nerve damage. There are three points belonging to the Bladder meridian of foot Taiyang (BL) and the Gallbladder meridian of foot Shaoyang (GB), which usually are selected to carry out Tuina. According to the anatomical nerve position, Yinmen (BL37) is on the sciatic nerve trunk, Chengshan (BL57) is on the tibial nerve, and Yanglingquan (GB34) is on the common peroneal nerve; meanwhile, the three points are on the sciatic nerve or its branches. The three points are on the biceps femoris, gastrocnemius, and tibial anterior muscles. Tuina can promote recovery of pain perception and fine movement of hind limbs in sciatic nerve injury (SNI) rats, and it is related to genetic changes of dorsal root ganglion and nerve injury points; meanwhile, the differentially expressed genes (DEGs) were enriched in the biological processes related to the regulation of myocytes.[3] This study explored the effects of Tuina on the recovery of SNI rats' changes of hind limb muscle strength and the genetic changes of gastrocnemius muscle.

Twenty-seven male Sprague-Dawley (SD) rats of 6 weeks old (weights: 200 ± 10 g) were randomly assigned to the Sham group, SNI group, and Tuina group, respectively. The rats were subjected to fasting and water prohibition for 24 h before the surgery and were anesthetized by intraperitoneal injection of 1% pentobarbital sodium (0.35 mL/100 g). The rats were fixed in a prone position with a rat fixer and the right hip-femoral junction was prepared with iodophor, shaving, and re-sterilization. An incision of about 1 cm long was made in the skin along the direction of the sciatic nerve exposing the lower edge of the piriformis. The sciatic nerve was found, sterilized, sutured, and then sterilized layer by layer for the rats of the Sham group. For the rats in SNI and Tuina groups, the special hemostatic pliers were used to clamp 5 mm at the distal end of the sciatic nerve nodule for 5 s with full force (6 N) resulting in a length of about 2 mm injury point. The local area was flushed with saline following the same procedure as the Sham group. The hind limbs of the rats in the Sham group were able to move freely 1 day after the operation. Due to injury of the sciatic nerve in the rats of the SNI group and Tuina group, the knee joints of the rats were straightened with limited flexion and limping indicating that the animal pathological model succeeded.

The rats of the Sham group and SNI group were fed routinely withholding and controlling for 9 min every day during the intervention period. The rats of the Tuina group began treatments on the 7th day after the operation. Pointpressing, plucking, and kneading methods were administered quantitatively at Yinmen (BL37), Chengshan (BL57), and Yanglingquan (GB34) points on the affected side once a day. Each method of three and each point of three were used for 1 and 9 min in total for one rat per day. There were ten treatments following 1-day rest and ten treatments were repeated so that a total of 20 treatments were completed. In order to reduce animal stress response, petting and stroking animals for 9 min before the formal intervention every day.

An electrically inclined plate tester was used to detect the changes in the muscle strength of the rats' hind limbs. Six rats in each group were taken for behavioral testing and the test was measured at baseline before the surgery, on the days 0, 5, 10, 15, and 20 of the interventions. The heads of

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the rats were placed on the board toward the end and gradually increase the angle of the board after the rats calmed down. When the rats cannot stay in this position for 5 s, the critical angle of the protractor was recorded, and the average level of three measurements was taken. Nine rats in each group were injected intraperitoneally with 0.35 mL/100 g with 1% Pentobarbital Sodium for anesthesia after interventions. The tissue of the right gastrocnemius muscle was taken from the ice plate. The total RNA extracted from the tissue of three rats was mixed as a sample and each group had three biological replicates. SPSS 22.0 (IBM Corp, Armonk, NY, USA) was used to analyze data and the results were represented by mean ± standard deviation. Data were analyzed by oneway analysis of variance (ANOVA).

The angle of the inclined plate of the rats in each group at baseline was not statistically significant. Compared with those in the Sham group, the inclined plate angle of the rats in the SNI group and the Tuina group decreased significantly before intervention (7 days after the operation) but there was no significant difference between the SNI group and the Tuina group. Compared with those in the SNI group in the tenth intervention, the inclined plate angle of the rats in the Tuina group increased significantly ($P < 0.05$) and increased very significantly ($P < 0.01$) in the 15th and 20th interventions, but there were still significant differences compared with those in the Sham group [Supplementary Figure 1A, http://links.lww.com/CM9/A912]. The saturation curve results showed that the sequencing results were reliable [Supplementary Figure 2, http://links.lww.com/CM9/A912]. Veen's results showed that there were a total of 44 DEGs between the Sham group and Tuina group. Four genes in the Sham group and 20 genes in the Tuina group had significant differences compared with the SNI group [Supplementary Figure 1B, http://links.lww.com/CM9/A912]. The gene ontology (GO) function of DEGs included cellular process, single-organism process, metabolic process, regulation of the biological process, response to stimulus cell part, cell, organelle, binding, etc [Supplementary Figure 1C, http://links.lww.com/CM9/A912]. GO functions were enriched in regulation of complement activation, regulation of response to stimulus, regulation of cellular senescence, regulation of Wnt signaling pathway involved in digestive tract morphogenesis, etc [Supplementary Figure 1D, http://links.lww.com/CM9/A912]. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways included Amino acid metabolism, Lipid metabolism, Metabolism of cofactors and vitamins, Xenobiotics biodegradation and metabolism, immune system, nervous system, immune diseases, neurodegenerative diseases, etc [Supplementary Figure 1E, http://links.lww.com/CM9/A912]. KEGG was enriched in cytokine-cytokine receptor interaction, chemical carcinogenesis, AMPK signaling pathway, etc [Supplementary Figure 1F, http://links.lww.com/CM9/A912].

The inclined plate test results indicated that Tuina promoted recovery and regeneration of SNI, promoted the establishment of connections between injured nerves and target organs, and delayed gastrocnemius atrophy to a certain extent. The DEGs results showed that ankyrin repeat domain 1 (Ankrd1) had the largest up-regulated expression differential in GO enrichment and ectodysplasin A2 receptor (Eda2r) had the largest differential up-regulated expression in KEGG enrichment. Interleukin 12 receptor subunit beta 2 (IL12rb2) had the largest differential expression in GO function and KEGG pathway enriched down-regulated expression. Ankrd1 was a protein that participated in the regulation of muscle fiber type conversion. The constituent cell components of Ankrd1 included Band I and Tuina intervention promoting SNI rats motor function changes may adjust the structure of myofilaments, regulate skeletal muscle differentiation and respond to muscle strain, stabilize the gastrocnemius morphology, and promote repairing of injured nerves through mRNA expression of Ankrd1 in the gastrocnemius muscle's Band I. IL12rb2 played an important role in PNI repair. Previous research indicated that Eda2r promoted the repair of injured nerves by regulating the nuclear factor (NF)-κB, C-Jun kinase (JNK), and p53 signaling pathways related to analgesia.

Tuina promoted recovery of hind limb muscle strength in SNI rats which may be achieved by improving gastrocnemius gene expression and its DEGs involved multiple biological functions and pathways. Tuina promoted repair of SNI rats may be related to the gastrocnemius's cellular process, single-organism process, response to stimulus, etc; it was achieved through cytokine-cytokine-receptor interaction, AMPK, and other signaling pathways, and possibly by regulating the gene expression ofAnkrd1, IL12rb2, and Eda2r in the gastrocnemius muscle.

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Conflicts of interest

None.

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