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RESEARCH ARTICLE

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Preliminary Correlation Between Warm Needling Treatment for Knee Osteoarthritis of Deficiency-Cold Syndrome and Metabolic Functional Genes and Pathways

Conge Tan[1], Jianguo Wang[2], Wenzhe Feng[1], Weijun Ding[3], Miqu Wang[3]*

[1]*Diagnosis Laboratory, Shaanxi University of Traditional Chinese Medicine, Xianyang, China*
[2]*School of Mechanical Engineering, University of Western Australia, Crawley, Australia*
[3]*Genetics Laboratory, Chengdu University of Traditional Chinese Medicine, Chengdu, China*

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Abstract
The warm needling technique used in traditional Chinese medicine has been shown to be effective in the treatment and prevention of knee osteoarthritis (OA), but the biological mechanisms behind this action have not been well explored. This study investigated the molecular mechanisms behind warm needling using cDNA microarray technology, thus providing further scientific evidence for its efficacy. Ten patients with knee OA of deficiency-cold syndrome were selected for 2 weeks of warm needling treatment. This treatment involved stimulating the selected acupoints using needles with a burning moxa stick on their handle for 40 minutes per session. Pain intensity and accumulated clinical scores of deficiency-cold syndrome were assessed pretreatment and posttreatment using a 40-factor questionnaire of OA with deficiency-cold syndrome. Four patients with the best therapeutic efficacy were selected for cDNA microarray testing. Among the four patients, 41, 246, 57 and 70 differentially expressed genes were obtained, with more than 50% of these differentially expressed genes functionally linked to primary, cellular and energy metabolism pathways. This work demonstrates that the molecular mechanism behind warm needling treatment may be associated with the regulation of metabolism-related genes and pathways.

1. Introduction

Warm needling treatment is a particular form of acupuncture, combining acupuncture together with moxibustion by stimulating acupoints with a burning moxa stick on the handle of the acupuncture needle. Warm needling treatment has a long history in China. Many researchers have explored how acupuncture and moxibustion work and have investigated the change in the body after treatment. Studies have also been performed at the molecular level. For example, acupuncture has been found to induce expression of the *FOS* gene (for c-Fos) in spinal cord injury [1], the *IL1A* gene in allergic rhinitis [2] and bone cancer [3], and heat shock protein 70 in aging rats [4]. More recently, cDNA microarray technology has

*Corresponding author. Genetics Laboratory, Chengdu University of Traditional Chinese Medicine, Chengdu 610072, China.
E-mail: wangmiqu1@yahoo.com.cn

been used to target differential expression of genes induced by acupuncture. Kim et al [5] identified gene alterations in animal studies due to electroacupuncture. Li and Zhang [6] found electroacupuncture at the Fenglong acupoint could regulate the expression of genes related to cholesterol metabolism, signal transduction, transcription regulation, the cell cycle, cell adhesion, immunity and stress. Guo et al [7] used cDNA microarray to explore gene expression related to the protective effects of electroacupuncture against cerebral ischemia, finding that major upregulated genes were related to signal transduction, the cell cycle, metabolism, the stress response, and DNA repair. However, these studies have not identified a dominant category of gene, nor has an interaction between these different gene categories been identified.

Acupuncture is an effective treatment for knee osteoarthritis (OA), relieving not only local pain and stiffness but also reducing whole body symptoms [8,9]. According to the four diagnostic methods of traditional Chinese medicine (TCM), general symptoms of knee OA are mainly manifested by aversion to cold, cold pain and stiffness in the knee joint, huddling up during sleep, relief from pain by warmth or pressure, clear and profuse urine, loose stools, a pale, white furry tongue, and a deep, slow and feeble pulse. These symptoms all belong to the clinical manifestations of deficiency-cold syndrome, which occurs with the highest frequency in knee OA [10]. The view of TCM is that deficiency-cold syndrome is caused by the declined *yang-qi* and the body's failure to generate heat for warmth. Warm needling treatment could reactivate the *yang-qi* through moxibustion and needling, thus reducing the deficiency-cold symptoms of knee OA.

The biological mechanisms behind warm needling treatment of the OA patient with deficiency-cold syndrome has not been identified, with the effects of warm needling treatment mostly evaluated using clinical observations [11,12]. Studies into the genetic background of either OA or deficiency-cold syndrome have been performed. Some genes found to play a role in the development of OA include IL-1, TNF-α, IL-6, TGF-β, matrix metalloproteinases [13–15], growth arrest and DNA damage-inducible protein 45beta [16], thrombospondin motifs 5 and apolipoprotein L-I [17]. These genes are mainly involved in cell growth, apoptosis and bone remodeling [18–20]. Gene expression in deficiency-cold syndrome has also been explored, with studies indicating that differentially expressed genes were mainly associated with metabolism-related gene regulation [21–25]. Our preliminary studies also suggested that differential expression of genes between OA patients with deficiency-cold syndrome and controls were mainly associated with metabolism [26]. Given this observation, we adapted cDNA microarray technology to analyze the molecular mechanisms involved with warm needling treatment.

2. Materials and Methods

2.1. Samples

Ten patients with highly accumulated clinical scores for knee OA of deficiency-cold syndrome were selected from Dabao town, Pengzhou, Sichuan Province, China. Patients were selected according to knee OA diagnostic criteria revised by the United States Rheumatology Institute in 1995, and the classification standard for deficiency-cold syndrome [27,28]. All patients accepted 2 weeks of warm needling treatment. The peripheral blood was collected from each of them before treatment began and after treatment finished. Only four blood samples of patients showing the greatest efficacy were further processed for cDNA microarray tests.

2.2. Diagnosis questionnaire for knee OA of deficiency-cold syndrome

The questionnaire was designed using the comprehensive manifestations of both knee OA and deficiency-cold syndrome. The questionnaire was also established with referencing of the World Health Organization's quality of life questionnaire (WHO-100), the America Health Condition Questionnaire (SF-36) and the Western Ontario and McMaster Universities Index of Osteoarthritis [29]. This 40-factor questionnaire was developed and ascertained through the analysis of data collected from epidemiological studies. The 40 factors can be divided into main symptoms (cold pain in knee joint, cold limbs, aversion to cold, curling body up for warmth) and supplementary symptoms (stiffness of the knee joint, cold lumbar pain, clear and copious urine, pale facial complexion, pale tongue, and slow pulse).

Each factor was given a qualitative and quantitative score. The qualitative score was graded as 1 for positive and 0 for negative. The quantitative score was graded with a four-point grading system [27]: none, light, medium and severe, which was correspondingly scored as 0, 1, 2, and 3. For example, a quantitative score of 1 was given if the waist and the back occasionally felt cold and no extra clothes or a blanket were required. A score of 2 occurred when the patient often felt cold and needed extra clothes or a quilt. Patients feeling very cold and needing thick clothes or a blanket were given a score of 3. Adding the qualitative and quantitative scores for each main symptom gave the qualitative total score for main symptoms and the quantitative total score