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Research paper

RNA sequencing reveals target genes of temporomandibular joint osteoarthritis in rats after the treatment of low-intensity pulsed ultrasound

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ABSTRACT

Purpose: To explore the potential molecular mechanism of low-intensity pulsed ultrasound (LIPUS) in the treatment of temporomandibular joint osteoarthritis (TMJ-OA), and identify the target genes for therapy of TMJ-OA.

Methods: Rat TMJ-OA was induced by unilateral occlusal trauma (UOT). At 8 weeks, the experimental group rats were treated by LIPUS for 4 weeks (5 days every week). The cartilage was examined by histological techniques. Gene expression profile in control, placebo and LIPUS-treated group were measured by RNA sequencing (RNA-Seq). Gene oncology (GO) and kyoto encyclopedia of genes and genomes (KEGG) annotated were performed and ten differentially expressed genes (DEGs) were further validated in another individual by quantitative real-time polymerase chain reaction (qRT-PCR). Per-2, a circadian rhythm gene, was further confirmed by western blot.

Results: TMJ-OA model was successfully established in rats through UOT. LIPUS played a positive role in attenuating the retrogression of cartilage. The cartilage lesion was determined by HE and Safranin-O staining. A significant and bran-new gene profile of 58 mRNAs was obtained from the RNA-Seq (LIPUS-treated/placebo) and generated approximately 30GB data. Annotation, functional classification and pathway of the data were analyzed based on GO and KEGG database and ten candidate DEGs were identified. Some of these genes were proved to be related to OA, such as matrix-degrading enzyme (ADAMTS-8), complement (C1qa, C3, C5aR1). Some were reported for the first time in TMJ-OA, such as circadian gene (Per-2, Dbp, Npas2 and Arntl). According to the results of qRT-PCR validation, the sequencing data was with a high degree of credibility. The circadian gene Per-2 was up-regulated by LIPUS in TMJ-OA on the mRNA and protein level.

Conclusion: This study reveals the potential therapeutic genes related to TMJ-OA. Especially the circadian Per-2 gene was detected up-regulated by the treatment of LIPUS. It provides us a precious, new target OA-related gene and further investigation of gene-function will provide us new insights in understanding the potential mechanical underling TMJ-OA.

1. Introduction

Osteoarthritis (OA) can cause severe pain and dysfunction in all joints, including the temporomandibular joint (TMJ) (Wang et al., 2015). Temporomandibular joint osteoarthritis (TMJ-OA) is a progressive joint disease primarily characterized by degradation of cartilage. Dysfunction of occlusion is a vital cause of temporomandibular joint disease. However, the exact pathogenesis of TMJ-OA remains unclear.

Low-intensity pulsed ultrasound (LIPUS) is a non-invasive, perceived safety and low cost treatment, which is an ideal candidate for the early protection of cartilage. LIPUS with low intensity under 100 mW/cm^2, having no thermal effect, is approved to treat the fracture by the FDA in 2000. Early clinical studies (Heckman et al., 1994) have

Abbreviations: LIPUS, low-intensity pulsed ultrasound; TMJ-OA, temporomandibular joint osteoarthritis; UOT, unilateral occlusal trauma; RNA-Seq, RNA sequencing; GO, gene oncology; KEGG, kyoto encyclopedia of genes and genomes; DEGs, differentially expressed genes; qRT-PCR, quantitative real-time polymerase chain reaction; MMPs, matrix metalloproteinases; ADAMTS, a disintegrin and metalloprotease with thrombospondin motifs

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demonstrated the promotion of LIPUS in the acceleration of the fracture-repair process. Cartilage has high mechanical reactivity and physiological mechanical stimulation has been proved to activate the anabolic of chondrocytes (Lammi, 2004; Sanchez-Adams et al., 2014). When ultrasound is propagating, the medium particles produce mechanical vibrations, which enable the cells to endure low-intensity, high frequency signal stimulation and subject the cells to certain functional loads. The mechanical stimulation produced by LIPUS can be transformed into a biological signal, which plays a specific effect through a complex biological signal transduction mechanism.

No study was performed to reveal the gene profile of LIPUS working on OA. In the present study, to investigate the effect of LIPUS working on OA, at first, we established a model of osteoarthritis with abnormal occlusal function and then treated by LIPUS. Then the cartilage samples were sequenced and RNA-Seq data was analyzed. Finally, we identified 58 mRNAs that are up- or down-regulated in LIPUS-treated cartilage versus placebo group. These genes may exhibit an important function in OA pathogenesis. To the best of our knowledge, this study is the first report of DEGs profile of the LIPUS-treated cartilage.

2. Material and methods

The research was carried out in accordance with Jinan Military General Hospital animal care regulations. Eight-week-old wistar rats, without any oral disease, were supplied by the Animal Center of Shandong University. The rats were raised in an ordinary environment with temperature of 24 °C, humidity of 55% ± 5% and with good ventilation.

2.1. Experimental groups

The rats were assigned to 5 groups randomly (n = 33, Fig. 1): (1) Control group: no operation; (2) Recovery group: UOT for 8 weeks and then remove the resin, recover naturally for 4 weeks; (3) Placebo group: UOT for 8 weeks and then remove the resin, sham exposure with LIPUS for 4 weeks; (4) LIPUS-treated group: UOT for 8 weeks and then remove the resin, LIPUS treatment for 4 weeks; (5) UOT group: UOT for 12 weeks.

To evaluate the cartilage lesion and find an appropriate time point to perform the therapy of LIPUS, 8 weeks old rats were randomly divided into 4 groups according to the UOT time (i.e.,4, 8,12,16 weeks, n = 6). Control groups with the same age were set for the same four groups (n = 6).

2.2. Rat TMJ-OA model and LIPUS treatment

To induced TMJ-OA, the rats were anaesthetized with aether (SCR, Shanghai, China) in an anesthesia induction chamber. Metal-wire was pasted on the right maxillary first molar by resin and the occlusion was raised about 0.5 mm (Fig. 2). During all the operations, the mouth opening length was not > 1.5 cm. Every other day, under the condition of etherization, rats were inspected to ensure that the retention of the metal-wire.

The rats of LIPUS-treated group were treated with LIPUS after 8 weeks of UOT. LIPUS stimulations were generated by Cosmogamma US13 (Emildue, Della Canapa, Italy) with an intensity of 100 mW/cm^2 and a sinusoidal waveform of frequence 1.0 MHz. The pulse cycle frequency was 100 Hz and the duty cycle was 20%. After anesthesia with sodium pentobarbital (35 mg/kg), the areas of the right temporomandibular joints were shaved and rats were fixed in a home-made installation allowing full access to their joint regions (Fig.2). Ultrasound transducers (1 cm^2) were held by hand and attached to the joint region. To transmit the ultrasound energy effectively, the ultrasound transmission gel (Costoli, Italy) was applied between the transducer and the joint region. The exposure time was 20 min/day for 4 weeks.

2.3. Pathological examination

All the rats were sacrificed at the age of 20 weeks with excess sodium pentobarbital (> 70 mg/kg). The right condyles of the TMJs were dissected integrally, including the temporal bone, articular disc and the condylar. The samples were fixed in 4% paraformaldehyde (Solarbio, Beijing, China) for 24 h, decalcified by 10% EDTA (Solarbio, Beijing, China), and embedded in paraffin. Series sagittal sections (5 μm) were

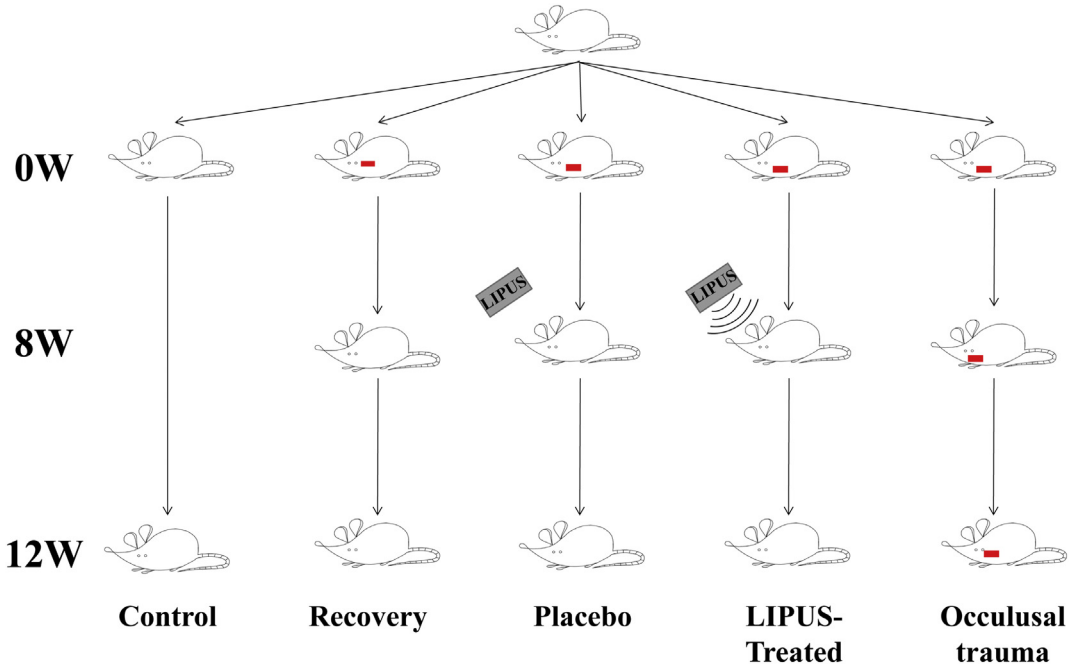

Fig. 1. Diagrammatic presentation of experimental groups.
The red squares represent the rats were treated by UOT. The gray squares represent LIPUS generator and the wave lines mean the machine is on working. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)