Abstract

Effects of mechanical load and estrogen
in cultured cells from superficial layer of mandibular
condyle

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【Introduction】

Temporomandibular joint (TMJ) osteoarthritis (OA) is a degenerative condition characterized by the deterioration of articular tissues and bony alterations in the mandibular condyle. The cartilage in the mandibular condyle, rich in extracellular matrix, exhibits viscoelastic properties against extension and compression forces and provides lubrication against frictional forces in the joint. However, when the mechanical load surpasses the physiological limits on the articular surface of the TMJ, it disrupts the metabolic equilibrium of articular cartilage, leading to degeneration and destruction.

On the other hand, epidemiological studies on TMJ-OA indicate a higher incidence among women, and there is a demonstrated correlation between blood estrogen levels in TMJ-OA patients and the pathogenesis of TMJ-OA. Therefore, it is suggested that estrogen plays a role in the pathogenesis of TMJ-OA. However, the effects of estrogen on articular cartilage remain inconsistent, with some studies reporting protective effects and others reporting detrimental consequences such as cartilage destruction and degeneration. In addition, it remains unclear how the underlying mechanisms of these effects.

Recent advancements in molecular biology have identified key genes and biomarkers in knee osteoarthritis (knee-OA). However, differences in the type, quantity, composition, and structure of estrogen-related receptors in knee and temporomandibular joints, along with variations in resistance to mechanical loading and molecular regulatory pathways, suggest the need for further exploration. Notably, research on osteoarthritis has predominantly focused on knee-OA, with limited attention given to TMJ-OA.

Therefore, the purpose of this study to elucidate the effects of mechanical loading and estrogen in rat cultured cells from superficial layer of mandibular condyle (CSLMC) and comprehensively analyze genes associated with the onset of TMJ-OA.

【Materials and Methods】

1. Preparation of Rat Primary CSLMC

Superficial layer of mandibular condyle was harvested from 8-week-old female Wistar rats. Cells were isolated using the Outgrowth method, resulting in primary CSLMC.

2. Application of Mechanical loading and estrogen

Compressive forces of 10.0 g/cm^2 and the addition of 10^{-6} mol/L (1.0 μM) estrogen were applied individually or simultaneously to CSLMC. The experimental groups were designated as the Loading group, Loading and Estrogen group, Estrogen group, and Control group, with each consisting of three samples (n=3).

3. RNA-seq analysis

CSLMC in each group were collected after three hours, and DNA-free total RNA was extracted for RNA-seq analysis (n=3).

4. Protein-protein interaction (PPI) network construction and hub gene analysis

The PPI network was constructed using the online STRING tool, followed by MCODE cluster analysis. hub genes were identified using Cytoscape (ver3.9.1) and the Maximal Clique Centrality (MCC) method.

5. qRT-PCR analysis

qRT-PCR analysis involved the same RNA extraction method as RNA-seq. The SYBR Green PCR protocol was used for the cDNA obtained, with primer sets for β-actin (internal standard) and each gene. Quantitative analysis of mRNA expression was performed (n=6).

6. Western blotting analysis

Western blot analysis of the 4 extracellular matrix core proteins COL1A1, COL3A1, LAMC1, and LAMB1 was performed. ImageJ software was used for analysis.

7. Statistical processing

Statistical analysis was performed using the Mann-Whitney U test and the Tukey method. P values were denoted as * for $P < 0.05$, and ** for $P < 0.01$, with $P < 0.05$ considered to be significant.

【Results and Discussion】

1. Identification of hub Genes

PPI analysis identified 10 hub genes, including collagen type I alpha 1 chain (COL1A1), collagen type Ⅲ alpha 1 chain (COL3A1), collagen type Ⅳ alpha 1 chain (COL4A1), collagen type Ⅴ alpha 1 chain (COL5A1), collagen type Ⅳ alpha 2 chain (COL4A2), collagen type Ⅳ alpha 5 chain (COL4A5), laminin subunit gamma 1 (LAMC1), heparan sulfate proteoglycan 2 (HSPG2), laminin subunit beta 1 (LAMB1), and laminin subunit alpha 3 (LAMA3).

2. Effect of Mechanical Load on hub Genes

Mechanical loading of CSLMC resulted in the expression of genes of the matrix metalloproteinase (MMP) family and a disintegrin and metalloproteinase with thrombospondin type I motifs (ADAMTS) family, which are substrate proteases. ADAMTS) family genes were increased. COL1A1, COL3A1, COL4A1, COL4A2, COL5A1, COL4A5, LAMC1, LAMB1, and LAMA3 mRNA expression was decreased in the Loading group compared to the Control group. These results suggest that mechanical loading in CSLMC may cause a loss of mechanical properties by inhibiting the synthesis of extracellular and pericellular matrices.

3. Effect of estrogen on hub genes

Gene expression of MMP and ADAMTS family members was increased in the Loading group relative to the Control group. In the Estrogen group, gene expression of several MMP and ADAMTS families was increased in the Control group. MMP13 and ADAMTS5 were both significantly upregulated. COL3A1, COL4A1, COL4A2, COL4A5, LAMC1, and LAMB1 mRNA expression was decreased in the Estrogen group versus the Control group. These results suggest that Estrogen may inhibit the synthesis of extracellular and pericellular matrices as well as mechanical loading in CSLMC.

4. The combined effect of mechanical load and estrogen on hub genes

The mRNA expression of COL1A1, COL3A1, COL4A1, COL4A2, COL5A1, COL4A5, LAMC1, LAMB1 and LAMA3 was increased in the Loading and Estrogen

group. Combining mechanical stress and estrogen may suppress the inhibition of extracellular and pericellular matrix synthesis. Protein expression of COL1A1, COL3A1, LAMC1, and LAMB1 also showed the same trend as mRNA expression, suggesting that COL1A1, COL3A1, LAMC1, and LAMB1 may be genes related to mechanical stress and estrogen in CSLMC.

【Conclusion】

Mechanical loading and estrogen addition to CSLMC increased gene expression of matrix proteases and inhibited the synthesis of extracellular and pericellular matrices, respectively. However, simultaneous mechanical loading and estrogen addition decreased gene expression of matrix proteases and inhibited inhibition of extracellular and pericellular matrix synthesis. COL1A1, COL3A1, LAMC1, and LAMB1 were shown to be genes related to mechanical loading and estrogen in CSLMC and may be associated with the development of TMJ-OA.