A B S T R A C T

Effects of mechanical stress on expression of extracellular matrix in rat TMJ disc cells

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[Introduction]

The TMJ disc plays a major role in distributing and absorbing the mechanical forces exerted by various orofacial functions and in facilitating smooth movements of the TMJ complex. It is a crucial component in both the normal functions and pathological processes of the joint (TMJ disorders; TMDs). The TMJ disc consists mainly of collagens proteoglycans and elastic fibers, which may play essential roles in tissue homeostasis and remodeling and in the pathology of the TMJ disc; however, little information is available on the adaptive responses of the TMJ disc to mechanical forces and the mechanisms responsible for TMD pathology. In this study, mRNA expressions of collagens, proteoglycans and tropoelastin in cultured cells derived from rat TMJ disc (referred here as TMJ disc cells) were examined to clarify the biological responses of the TMJ disc cells to mechanical stimulation.

[Materials and Methods]

1. TMJ disc cell culture

TMJ discs were extracted from 4-week-old male Wistar rats in a sterile manner. The discs were minced, transferred to a Petri dish, and cultured with minimum essential culture medium (MEM) supplemented with 10% newborn calf serum at 37℃ with 5% CO_2.

2. Application of Cyclic Uniaxial Stretching

Mechanical stretching was performed on flexible silicon membranes, which were coated with fibronectin to promote cell attachment on the silicone surface. TMJ disc cells were seeded on the membrane at an initial density of 1×10^5 cells/cm^2 and cultured until subconfluence of 70% for 3 days. After the subconfluence, uniaxial stretching was applied on the STB-140STREX Cell Stretch System with 10% elongation at a frequency of 1 cycle/min for 4 h and 12 h or left untreated (control group).

3. Total RNA isolation form TMJ disc cells

Total RNA was isolated from the cultured cells of 3 dishes per time point in each group.

4. Microarray hybridization and quantitative real-time PCR

The obtained RNA was transcribed into cDNA and then subjected to microarray hybridization. After microarray, each mRNA expression of extracellular matrix components which showed significant

differences between the 2 groups (type I collagen, versican, aggrecan, fibromodulin, lumican and decorin, tropoelastin), was further quantified using a real-time PCR.

5. Small interference RNA (siRNA) of fibromodulin

Expression of fibromodulin mRNA was suppressed by using siRNA. Expressions of fibromodulin and lumican mRNA were examined in stretched and non-stretched groups.

6. Statistical analysis

Statistical analysis was performed using SPSS 20 software. The data from the 2 groups were analyzed using the Mann-Whitney U test.

[Results]

1. A microarray based analysis

The mRNA levels of versican, aggrecan and fibromodulin increased after 4 h and 12 h of stretch. The type I collagen mRNA level increased after 4 h. The mRNA levels of lumican, decorin, asporin, tropoelastin and type III collagen decreased after 12 h, and the keratocan mRNA level increased after 4 h.

2. Quantitative RT-PCR analysis

After 4 h, the mRNA levels of versican, fibromodulin and type I collagen were 70%, 240% and 50% higher than those of non-stretched control in response to stretch, respectively, while the decorin mRNA level was 60% lower than that of control group. Stretch for 12 h resulted in 30%, 50% and 50 % increase in the mRNA levels of versican, aggrecan and fibromodulin, respectively. Stretch for 12 h resulted in 70%, 70% and 40% decrease in the mRNA levels of lumican, decorin and tropoelatin, respectively.

3. Influence of suppression of the fibromodulin mRNA expression on the lumican mRNA expression

To examine the interaction between two SLRPs, *i.e.*, fibromodulin and lumican, effect of suppression of the fibromodulin expression on the lumican mRNA expression was examined. Provided that the mRNA expression of fibromodulin was suppressed with siRNA for 12 h, the fibromodulin mRNA level

decreased down to 0.1 times as non-suppressed condition in non-stretched control and 0.4 times in stretched group, respectively. In contrast, the lumican mRNA level increased up to 1.7 times in stretched group, while it did not change significantly in non-stretched group.

[Discussion]

The previous histological studies indicated the relationships between mechanical stimulation and components and assembly of extracellular matrix components. For example, increased functional force resulted by growth and exercise induced an increase in the diameter, cross-sectional area and density of the collagen fibrils in the superficial digital flexor tendon of the mouse. In this study, cyclic tensile stimulation induced an increase in the mRNA level of type I collagen in the TMJ disc cells, which is in accordance with the previous in vitro studies in periodontal ligament cells, scleral fibroblasts and tendon fibroblasts. This result suggests that increase mRNA level of type I collagen may be an adaptive response of the TMJ disc cells against mechanical stimulation, and be involved in tissue integrity.

In this study, the mRNA level of versican increased after 4 h, and the mRNA level of aggrecan increased after 12 h. Versican and aggrecan belong to the modular proteoglycans, which contain a large core protein greater than 200 kDa in size with multiple structural domains, including an N-terminal hyaluronan binding domain, a GAG attachment domain, and various C-terminal functional domains. These proteoglycans have a lot of glycosaminoglycan (GAG) chains covalently attached to their core protein, which cause a swelling and expansion forces. These forces may antagonize traction of collagen fibers surrounding proteoglycan, and offer resistance to compressive force to organization. In this study, the increases in the both proteoglycans mRNA levels elicited by tensile stimulation were thought to be associated with enhancement of mechanical strength of the TMJ disc.

The previous studies indicated that fibromodulin and lumican have an ability to bind the same region of type I collagen, and regulate collagen fibrillogenesis. In addition, fibromodulin molecule competitively inhibits the binding of lumican molecule to collagen monomer, since it can bind to type I collagen monomer with higher affinity than lumican. In this study, the mRNA level of fibromodulin was increased after 4 h of stretch, and decreased down to the level of non-stretched group after 12 h, while the mRNA level of lumican decreased after 12 h. These results suggested the above-mentioned competitive relationship between the both proteoglycans. To clarify this hypothesis, suppression of fibromodulin mRNA expression with siRNA was applied in this study.

Interestingly, suppression of fibromodulin expression showed little effects on the lumican mRNA level in non-stretched control, however the lumican mRNA level increased significantly in stretched group. Therefore, mechanical stimulation may influence the inhibitory effect of fibromodulin on the lumican mRNA expression.

[Conclusion]

It was indicated that rat TMJ disc cells exhibited changes in mRNA expressions of various extracellular matrix components, such as collagens, proteoglycans and tropoelastin in response to tensile stimulation.