Complete Stress Deprivation from the Rabbit Patellar Tendon Induces Fibroblast Apoptosis In Vivo via Activation of JNK and p38 Mitogen-activated Protein Kinase

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Introduction: It has been demonstrated that if no stress is applied to the autograft after ligament reconstruction, the graft strength decreased remarkably. The effect of stress deprivation on tendon and ligament tissues has been an important focus in the field of biomechanics. We previously clarified that complete stress deprivation drastically deteriorates the mechanical properties of the patellar tendon within a week, using an originally developed rabbit model in which the patellar tendon is completely shielded from stress without immobilizing the knee. We reported that stress deprivation dramatically increases the number of cells within the patellar tendon. Recently, We demonstrated that stress deprivation induces the over-expression of TNF-alpha and IL-1beta in fibroblasts in the patellar tendon. However, little is known about the mechanisms of the effect of stress deprivation. Apoptosis is an important component of the maintenance of homeostasis in many adult tissues. Recently, Yuan et al. reported that excessive apoptosis occurs in degenerating rotator cuff tendon. However, few in vivo studies have been conducted to clarify the relationship between the mechanical stimuli and apoptosis of fibroblasts in the tendon and ligament. Based on several studies using cultured cells, we hypothesized that complete stress deprivation from rabbit tendon may induce activation of Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (p38) in fibroblasts, and that activation may be followed by apoptosis of the fibroblasts. The purpose of this study is to test these hypotheses.

Materials and Methods: 1. Study design
A total of 36 rabbits were used in this study. In each animal, the right patellar tendon (PT) was completely shielded from stress (SS) by the established technique, in which the PT was relaxed by stretching a flexible stainless-steel wire installed between the patella and the tibial tubercle. Then, the animals were randomly divided into 2 groups, SS and Sham Groups, and only in Sham Group, the stainless steel wire was cut immediately. All animals allowed unrestricted activities, and sacrificed at 0, 1, 2, 4, 7, and 14 days after the treatment. The patella-patella tendon-tibia complex was sectioned for histological evaluations. The left PTs was used for nor- 

tal control data. Morphological changes of patellar tendon were evaluated with hematoxylin and eosin staining.

2. TUNEL assay
DNA fragmentation in cells was detected with the TUNEL method.

3. Immunohistochemical evaluations
We performed immunohistochemistry with monoclonal antibody to phospho-JNK, phospho-p38, and active Caspase-3.

Results: Histological appearance of the midsubstance of the stress-shielded PT showed no changes at 1 to 7 days as compared to that in the sham and normal control. The stress-shielding dramatically increase the number of cells with a round-shaped nucleus and decreased the longitudinally aligned collagen fibers at 14 days. In the SS group, some cells positively stained with the TUNEL method at 1 day after SS, and the number of such cells gradually increased by 4 days. The number of TUNEL positive cells were not difference between normal control and sham group. But in the SS groups, the number of positive cells were significantly increased at 1–4 days after SS, compared to normal and sham groups. Then, the number decreased at 7 and 14 days. Concerning cells with expression of active caspase-3, we observed the similar tendency. Namely, the number of active caspase-3 positive cells increased by 4 days, and then decreased.

Expression of phospho-JNK and phospho-p38 was observed in SS models, similar to the results of TUNEL and active caspase 3 staining. The expression of phospho-JNK and p38 was rarely observed in normal control and sham groups. However, we observed some differences of upregulation between TUNEL, active caspase 3 and phospho-JNK, p38.

In TUNEL and active caspase 3 staining, the number of positive stained cells increased 2 to 4 days, and reached maximum, at 4 days. On the other hand, phos- 

pho-JNK and p38 have already rise to 1 day, and it continued to rise 2 to 4 days at the same degrees.

It suggest that JNK and p38 were activated at the upstream of caspase 3, and expressed at early stage of apoptosis signaling pathway in stress-shielded patellar tendon.

Discussion: The TUNEL assay and active caspase-3 immunostaining in the present study demonstrated that completely stress deprivation from the PT induced fibroblast apoptosis in vivo, which started at 1 days and reached a peak within 7 days. This study also showed that the complete stress deprivation induced activation of JNK and p38 in fibroblasts within a few days prior to the induction of the apoptosis. Some in vitro studies reported that activation of JNK and p38 play important roles in inducing apoptosis in cultured cells with the fibroblast origin. Therefore, the present study suggested that complete stress deprivation from the rabbit patellar tendon induces fibroblast apoptosis in vivo via the JNK and p38 pathway.

Fig.1 TUNEL positive cells
positive cells/contour/section

Fig.2 active caspase 3 positive cells
positive cells/contour/section

Fig.3 phospho-JNK positive cells
positive cells/contour/section

Fig.4 phospho-p38 positive cells
positive cells/contour/section

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