Distribution of ADAMTS5 and Tenascin-C in the Synovial Tissues around the tendon in the Rotator Cuff Injury

INTRODUCTION:
ADAMTS5 is a secretory type enzyme to cleave Glu-Ala-Ala of interglobular domain of aggrecan belonging to ADAMTS family [1]. Recent studies have demonstrated that ADAMTS5 could be an important factor for acceleration of the cartilage degeneration [2]. The large proteoglycans including aggrecan of target molecule by ADAMTS5 have been reported to be one of major component in tendon as well as cartilage, especially in the compressed zone such as insertion to bone. In this part, tendons such as the Achilles and rotator cuff (RC) are susceptible to chronic and spontaneous ruptures in the absence of previous symptoms. The fact made a suspicion that large proteoglycan breakdown could involve in pathology of chronic tendinopathy with the load exposed at daily activity.

Furthermore, tenascin-C (TN-C), which was reported to be promoter of the gene in various pathological conditions in response to mechanical stress, was significantly up-regulated in subacromial bursa (SAB) of the patients with rotator cuff tear or tendinitis.

The purposes of this study are histologically and immunohistochemically 1) to determine the expression and distribution of ADAMTS5 and TN-C of subacromial bursa and glenohumeral joint (GH) in the patients with RC injury, 2) to clarify change of immune labeling in these molecules with the progression of pathology and 3) to analyze whether TN-C expression is related to NF-κB, gene regulator of inflammatory molecules.

MATERIALS AND METHODS:
Subjects: The study group consisted of 17 patients (6 women and 11 men), which were surgically treated for RC injury in 14 cases or recurrent shoulder instability in 3 cases. The synovium of SAB and GH specimens were obtained and analyzed. The specimens from instability were served as controls. All patients signed an informed consent document, and the study was approved by the institutional review board.

Histological analysis and grade: The specimens were fixed in 10% formalin and embedded in paraffin. The sections cut into 4-μm-thick sections were stained with HE. Each microscopic field was graded into 3 groups according to the most outer layer in the lining as shown in Fig.1A-C. Then, the classified pattern in each section was calculated because there were regional variation for the classification.

Immunohistochemical analysis: Primary antibodies were used on serial sections with anti-ADAMTS5 rabbit polyclonal antibody and anti-TN-C mouse monoclonal antibody. The reaction products were visualized in 0.15mg/ml DAB solution containing 0.003% hydrogen peroxide. Counter stain was hematoxylin.

Double immunofluorescence staining: Anti-ADAMTS5 rabbit polyclonal antibody and anti-TN-C mouse monoclonal antibody was reacted with AlexaFlour488 conjugated anti-mouse IgG and AlexaFlour594 conjugated anti-rabbit IgG, respectively. Furthermore, Anti-TN-C rabbit polyclonal antibody and anti-NF-κB (clone: 12H11) mouse monoclonal antibody was reacted with AlexaFlour488 conjugated anti-mouse IgG and AlexaFlour594 conjugated anti-rabbit IgG, respectively. Nucleus was detected with bisbenzimide (Hoechst-33342) staining.

RESULTS:
We observed expression of ADAMTS5 and TN-C in the synovium of both SAB and GH, and the immunoreactivities were higher in the specimens obtained of RC tear than that of shoulder instability (Fig. 1D-I). In the specimens of instability, ADAMTS5 was weakly expressed only in the most outer layer of synovial lining. On the other hand, expression of ADAMTS5 of the RC injury was strongly shown in the lining cells as well as endothelium of vessels (Fig. 1D-F). Regarding to the expression of TN-C, there was no or little expression in the specimens of shoulder instability. In the specimens from RC injury, the expression of TN-C was strongly observed in connective tissues under the synovial lining and tunica media within vessel walls (Fig.1G-J). In contrast to the expression of ADAMTS5, TN-C was weakly expressed in the lining. Double immunofluorescence staining also showed diffuse expression of TN-C in connective tissues while ADAMTS5 was highly expressed in synovial lining (Fig.3A).

Furthermore, histological grading was likely to relate with the expression of ADAMTS5 and TN-C. In fact, there was the most frequently severe pattern in the specimens of RC injury (Fig.2), in which intense immunostaining for these molecules were noted. In addition, the lacunas were seen around the cells immunolabeled for ADAMTS5.

Relationship between TN-C and activated form of NF-κB was also analyzed in double immunofluorescence staining, demonstrated that NF-κB was highly expressed in nuclei and cytoplasm of lining cells in the neighborhood of the strongly immunolabeled area in TN-C (Fig.3B,C). ADAMTS5 and TN-C were frequently severe pattern in the specimens of RC injury.

DISCUSSION:
ADAMTS5 was not only immunolocalized in human arthritic synovium but also degradation products for this molecule were identified by analysis of synovial fluid samples in osteoarthritis of the knee joints, indicated that ADAMTS5 must play a major role of degradation of cartilage [2, 3]. Likewise, parts of tendons subjected to compression such as RC reveal the presence of fibrocartilage with rich in aggrecan and type II collagen. The present study clearly showed that ADAMTS5 was highly expressed in the synovium around tendon obtained from the patients with RC injury, especially in severe group. These findings suggested that ADAMTS5 generated in synovium could exogenously cleave aggrecan in RC tendon with result in progression of tendinopathy. TN-C was also markedly increased in the stroma of thickened synovium in RC injury. TN-C reappears in association with wound healing or inflammatory processes, although the expression is restricted in normal adult tissues. Jones et al. have demonstrated that tenascin-C gene expression can be mechanosensitive and that it occurs on most occasions at the level of the gene promoter [4]. In fact, there was the expression of activated form of NF-κB in the lining cells similar to the expression of ADAMTS5, while TN-C was strongly expressed in the connective tissue. Additionally, the synovium of RC injury include many parts of severe pattern according to histological grading. Taken together, TN-C up-regulated in response to mechanical stress appeared to induce synovial thickening by increase of various inflammatory mediators through NF-κB pathway. The proliferation of lining cells as the result, the unbalance between the synthesis and degradation can be caused by increase of exogenously ADAMTS5 proteinase to the tendon with the progressive pathology of RC injury.

SIGNIFICANCE:
The present study showed that there was expression of ADAMTS5 in the synovial lining while TN-C injury was strongly expressed in the connective tissues. This immunohistological findings suggested that thickened lining by up-regulation of TN-C appeared to increase exogenously ADAMTS5 proteinase to the tendon in the progressive pathology of RC injury.

REFERENCES: