The Secreted Aggrecanases From Synovium In Rotator Cuff Tear Participate In Progression Of Cartilage Degradation In The Shoulder Joint

Takahiro Iino\textsuperscript{1}, Masaya Tsujii, MD, PhD\textsuperscript{1}, Toru Wakabayashi, MD, PhD\textsuperscript{2}, Naoki Kokubu, MD\textsuperscript{1}, Hirokazu Yokoyama, MD\textsuperscript{1}, Takuya Nakanishi, MD\textsuperscript{1}, Masahiro Hasegawa, MD, PhD\textsuperscript{1}, Akihiro Sudo, MD PhD\textsuperscript{1}.
\textsuperscript{1}Department of Orthopaedic Surgery, School of Medicine, Mie University, Tsu, Japan, \textsuperscript{2}Department of Orthopaedic Surgery, Toyohashi Esaki Hospital, Toyohashi, Japan.


Introduction: Cuff tear arthropathy is difficult disorder to treat caused by the massive rotator cuff tear with the limitation of shoulder joint motion and severe pain. The pathophysiology of this disease is unclear although this condition is an obvious osteoarthritis in the shoulder joint with osteophyte, bone cyst, synovial swollen around the joint and cartilage degeneration. A number of studies demonstrated that aggrecanses, ADAMTS4 and ADAMTS5, generated in synovium as well as cartilage could be an important factor for acceleration of the cartilage degeneration. ADAMTS4 was reported to depend on NFκB signal, but ADAMTS5 was not \cite{1}. This report suggested that the expression of ADAMTS4 was induced by inflammation. On the other hand, ADAMTS5 is unlikely to be necessarily caused by inflammation. Furthermore, recent study reported that participation of miR140 was found on the pathogenesis of the OA. In fact, the miR140-/- mouse induced the early onset of spontaneous OA-like changes in articular cartilage of the age-related model \cite{2}. Additionally, this microRNA was reported to down-regulate ADAMTS5 in the study using transfection-technique of ds-miR140 to chondrocyte \cite{3}. These results made us hypothesis that these ADAMTSs could involve in the pathological acceleration of cuff tear arthropathy, and pathology of synovium around rotator cuff might be cause of cartilage degradation as well as rotator cuff tear because the favorite site of tear was composed of fibrocartilage with many aggrecans similar to the cartilage. The purpose of this study was to investigate expression and localization of ADAMTS 4, ADAMTS5, mature miR140s, and activated NFκB in around tendon of the patients with rotator cuff tear or recurrent dislocation of the shoulder joint.

Methods: Subjects: The study group consisted of 17 patients (6 women and 11 men), which were surgically treated for RC injury in 13 cases and recurrent shoulder dislocation in 4 cases with their mean age of 63.2 and 32.3 years (range, 21 to 69 years), respectively. The synovium of subacromial bursa (SAB) and glenohumeral joint (GH) were obtained during arthroscopic surgery and analyzed as follows. Furthermore, the size of rotator cuff tear was medium: 4 cases, large: 7 cases, and massive: 2 cases. Classification: The tear was divided into four categories according to arthoroscopic findings (1-3 cm of tear size as medium: 4 cases; 3-5 cm as large: 7 cases; greater than 5 cm as massive tear: 2 cases). Moreover, we histologically classified lining cell layer of synovium in 3 grades. Normal grade was 1 layer. Mild grade was about 2-3 layers, and the lining cells had lacuna between the extracellular matrix and the layer of itself. Severe grade was more than about 4 layers, and the cytoplasm had eosinophil-rich (Figure.3-A, B, C).
Immunohistochmical analysis: Primary antibody was used on serial sections with anti ADAMTS4 antibody and anti ADAMTS5 antibody. The reaction products were visualized in DAB solution. The activated NFκB was stained by immunofluorescence. The antibody was visualized by Alexa488.

In situ hybridization: Distribution of miR140-5p and -3p were examined by in situ hybridization using miRCURY LNA detection probe. The samples were hybridized by LNA probe labeling double-DIG. Thereafter, the sections were incubated with anti-DIG antibody conjugated POD. After sensitization of POD, the reaction products were visualized in DAB solution.

**Results:** The ratio of length of severe area in rotator cuff was higher than that in recurrent dislocation. Especially, the synovium of SAB from the patient with massive tear had significantly more severe area than that from the patients with the other condition (p<0.05). In addition, the ratio tended to be high with the increase of arthroscopically injured size of rotator cuff (Figure.1-C,D). Immunohistochemical analysis showed that the expression of ADAMTS5 and ADAMTS4 were mainly localized on the lining cells with predominance in severe area of lining cells (Figure.2). Furthermore, the expression of activated NFκB could be observed in the lining cells expressing ADAMTS4. With regard to distribution of mature miR140s, in situ hybridization clearly showed labeling in the majority of the cells within the fibroblasts and vessels (Figure.3-L, P). It was a noteworthy finding that there were few expression in cells of the most outer layer of synovial lining with severe thickness (Figure.3-K, O). In addition, this area with few or no expression of mature miR-140s paradoxically labeled clear expression of ADAMTS5 (Figure.3-G, K, O).

**Discussion:** This study showed that the ratio of length on severe area was increased in the synovium around rotator cuff with large sized tear, especially of SAB in massive tear. We know that there were synovial thickening within SAB and shoulder joint of the patients with rotator cuff tear as well as osteoarthritis. Expression of ADAMTS4 and ADAMTS5 in the synovium around rotator cuff of the shoulder joint was strongly upregulated on the severe area according to histological grading regardless arthroscopic tear size. Furthermore, expression and distribution of NFκB and mature miR140s were investigated in this study. NFκB and ADAMTS4 were expressed on the same area of synovium, especially in advanced stage of rotator cuff tear, suggested that expression of ADAMTS4 could be induced by inflammation with advancement of pathology of rotator cuff. Furthermore, the mature miR140s with an ability of down-regulation of ADAMTS5, were clearly immunolabeled in vascular endothelial cells and fibroblasts of connective tissues, while this microRNAs were unlabeled in the most outer layer of synovial lining cell with clear expression of ADAMTS5. Some authors also suggested that the massive tears accelerate pathology to cuff tear arthropathy, and the molecular factor was not always derived from degenerative cartilage [4][5]. And ADAMTS4 and ADAMTS5 are secretion type enzymes [6]. Taken together, this study suggested that the increased secretion ADAMTSs by hyperplasia synovium cause progression of both more cartilage degradation as well as the extension of the tear size through the connection of two cavities in rotator cuff. Given the proliferation of severe lining cells as the clinical result, these molecules can cause more severe degradation for joint's cartilage and tendon's fibrocartilage. This study suggested a new possibility of treatment strategy for the acceleration of cartilage degeneration complicated with rotator cuff tear.

**Significance:** This study investigated the expression of ADAMTSs in the synovium around the rotator cuff tendon. Furthermore, the localization of NFκB and mature miR140s was shown for supporting the expression of these aggrecanases. These results suggested that the increased secretion ADAMTSs by hyperplasia synovium cause progression of both more cartilage degradation as well as the extension of
the tear size through the connection of two cavities in rotator cuff. Accordingly, this study suggested a new possibility of treatment strategy for the acceleration of cartilage degeneration complicated with rotator cuff tear.

(Figure 1) The ratio of synovial lining cells each tear-size.

(Figure 2) Localization of ADAMTSs on lining cells.
(Figure 3) Localization of mature miR140s in synovium.