TGF-β signaling is dysregulated in the cartilage and the subchondral bone of a rotator cuff tear arthropathy model

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INTRODUCTION: Shoulder joint pain is one of the three major musculoskeletal pains, along with lower back pain and knee joint pain. One of its main causes is rotator cuff tear and the subsequent development of glenohumeral joint osteoarthritis (OA), called rotator cuff tear arthropathy (CTA). CTA can appear as early as 5 years after a rotator cuff tear[1] and is characterized by OA change of humeral head accompanying a collapse in the subchondral bone with pannus-like fibrous cells[2]. The molecular mechanism underlying CTA progression is completely unknown. Transforming growth factor (TGF)-β plays a protective role in maintaining the cartilage quality thereby preventing cartilage degeneration of OA. Conversely, in the subchondral bone, TGF-β promotes proliferation of mesenchymal stem cells (MSCs) and subsequent formation of bone marrow osteoid islets which results in the initiation of cartilage degeneration[3]. To date, there have been no reports mentioning the activity of TGF-β signaling in shoulder OA, and it remains unclear whether the knowledge from knee joint OA can be directly applied to this context. Recently, we developed a modified CTA rat model (mCTA) that reproduces critical features of CTA in 4 weeks (equivalent to approximately 3 years of human life)[4]. In this study, we examined the state of TGF-β signaling in our mCTA model.

METHODS: The animal study was approved by the Institutional Animal Care and Use Committee of Kagoshima University (#MD21024, #MD22038, #MD23009). A total of 5 adult male Sprague-Dawley rats were included in this study. Under general anesthesia, we created a mCTA model by resecting the rotator cuff tendon, long head of biceps tendon, and the superior part of the capsuloligamentous complex on the right shoulder, while the left shoulder underwent a sham operation with a deltoid incision only. Animals were sacrificed at 4 weeks after surgery. The humeral head samples were fixed with 10% formaldehyde in PBS for 24 hours, followed by decalcification, dehydration, and embedding in paraffin. Subsequently, 4-μm-thick sections were prepared. Histological analysis was performed using Safranin O staining and immunohistochemical staining (for TGF-β1, TGF-β2, TGF-β3, phosphorylated (p)-Smad3, and Smad2/3). Statistical comparisons were made using the unpaired Student's t-test.

RESULTS SECTION: Our rat mCTA humeral head showed destruction of the articular cartilage and depression of the subchondral bone. Fibroblastic pannus-like cells were abundantly present around the collapsed subchondral bone. While TGF-β1 and TGF-β3 exhibited only weak staining in the cartilage cells, TGF-β2 displayed strong expression in both the sham control and mCTA model, with comparable intensity observed between the two. When examining which cells in the articular cartilage was activated by this TGF-β signaling using immunohistochemistry of p-Smad3, the downstream signaling mediator, it was observed that in the mCTA group, staining intensity in the superficial layer chondrocytes was reduced compared to the deep layer cells (Fig. 1). The pannus-like fibrous cells at the subchondral bone collapse site clearly expressed TGF-β2 and p-Smad3 (Fig. 2). In the subchondral bone, staining of p-Smad3 was observed in osteocytes, and this was significantly increased in the mCTA group (Fig. 3).

DISCUSSION: In rat humeral head, among the three TGF-β types, TGF-β2 exhibited the highest expression in the cartilage; this profile might be specific to the shoulder joint. Since the expression of p-Smad3 decreased in superficial layer chondrocytes in the mCTA group, similar to the evidence in knee OA, it is suggested that TGF-β signaling in CTA may act protectively on cartilage and weaken with OA progression. TGF-β2 and p-Smad3 were strongly expressed in pannus-like cells, indicating the strength of this signal. We speculate that this cell population, similar to MSCs responding to TGF-β, might contribute to the pathology of the subchondral bone by forming bone marrow osteoid islets. Similar enhancement of the TGF-β signal was observed in osteocytes in the subchondral bone, suggesting that, in CTA, there might also be a promoting effect on the OA changes of the TGF-β signal. Regarding the TGF-β type I receptors, in knee joint OA, it is known that as the condition progresses, there is a switch from ALK5 to ALK1, meaning that downstream signaling transitions from Smad2/3 to Smad1/5, leading to further degeneration of both cartilage and subchondral bone[5]. Our colleague Iuchi T. is preparing to present findings of Smad1/5 activation in our mCTA model at this meeting. Therefore, it will be necessary to investigate the expression of ALK1 and ALK5 in our future research.

SIGNIFICANCE/CLINICAL RELEVANCE: In this study, we have, for the first time, shed light on the status of the TGF-β signaling in shoulder joint OA. From the findings of this research, inhibiting the TGF-β signal with, for example, TGF-β type I receptor inhibitors, may lead to the suppression of subchondral bone lesions. However, there is a possibility of opposite effects on articular cartilage. Since this study utilizes a quadrupedal animal model, further validation using clinical specimens is crucial for understanding the changes in the TGF-β signaling in non-weight-bearing joint OA, CTA, and we are currently pursuing this avenue of research.

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IMAGES:

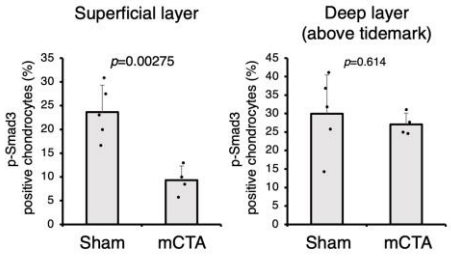

Fig. 1: p-Smad3 was decreased in superficial layer chondrocytes of humeral head cartilage in the mCTA model.

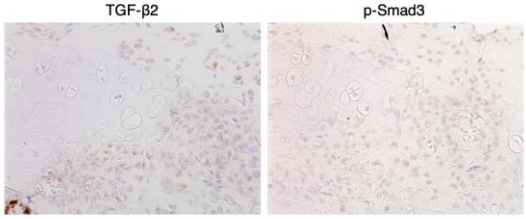

Fig. 2: Strong expression of TGF-β2 and p-Smad3 in pannus-like fibrous cells at the site of subchondral bone collapse in the mCTA model.

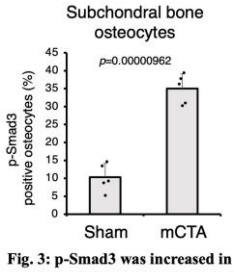

Fig. 3: p-Smad3 was increased in osteocytes in the subchondral bone of humeral head in the mCTA model.