Activation of BMP signaling in a modified model of shoulder rotator cuff tear arthropathy

Tomohiro Iuchi[1,2], Toshiro Ijuin[1], Yusuke Masuda[1,3], Hiroki Tawaratsumida[1], Shingo Maeda[4], Noboru Taniguchi[1,2,3,4]
[1]Department of Orthopaedic Surgery, [2]Department of Medical Joint Materials, [3]Department of Locomotory Organ Regeneration,
[4]Department of Bone and Joint Medicine, Kagoshima University, Kagoshima, Japan
Email: toushou01.6707@gmail.com

Disclosures: Tomohiro Iuchi (N), Toshiro Ijuin (N), Yusuke Masuda (N), Hiroki Tawaratsumida (N), Shingo Maeda (N), Noboru Taniguchi (N)

INTRODUCTION: Rotator cuff tear is one of the major causes of severe shoulder joint pain, affecting more than 60% of the elderly population over the age of 80[1]. Massive rotator cuff tears can result in severe osteoarthritis (OA) of the glenohumeral joint and humeral head, known as rotator cuff tear arthropathy (CTA). CTA can appear as early as 5 years after a rotator cuff tear[2] and is characterized by significant deformity of the glenohumeral joint, such as acetabularization, femoralization of the humeral head, a collapse in the humeral head subchondral bone accompanying pannus-like fibrous cells, and bone loss[3]. It has been reported that bone morphogenetic protein (BMP)-2 expression is increased in cartilage in knee OA[4–5], suggesting its involvement in OA pathogenesis, although the expression status in CTA remains elusive. The existing CTA animal models exhibit only minor joint changes and a delayed onset of arthropathy (12 weeks in rats or 45 weeks in mice, which is approximately equivalent to 8 or 36 years of human life)[6–7]. 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)[8]. In this study, we examined the state of BMP signaling during the onset and progression of CTA in our mCTA model and clinical samples of CTA.

METHODS: The animal study was approved by the Institutional Animal Care and Use Committee of Kagoshima University (#MD21024, #MD22038, #MD22072, #MD23009). A total of 14 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 experiment using human clinical samples was approved by the Ethics Committee on Epidemiological Studies, Kagoshima University (Epidemiology #220018). The human samples were obtained from patients who underwent reverse shoulder arthroplasty (CTA case) or from patients who had fractured their humerus (non-CTA case) at Kagoshima University Hospital in Kagoshima, Japan. Informed consent was obtained from all the patients who participated in this study prior to sample collection. The humeral head samples were fixed with 10% formaldehyde in PBS for 24 hours, followed by decalcification with K-CX (FALMA, Tokyo, Japan), dehydration, and embedding in paraffin. Subsequently, 4-µm-thick sections were prepared. Histological analysis was performed using Safranin O staining, immunohistochemical staining, and bone histomorphometry. Statistical comparisons were made using the unpaired Student's t-test.

RESULTS SECTION: Safranin O staining of the rat mCTA humeral head showed destruction of the articular cartilage and depression of the subchondral bone (Fig. 1). Fibroblastic pannus-like cells were continuously present from the bone marrow through the collapsed subchondral bone to the articular cartilage. Not only articular chondrocytes but also pannus-like cells showed increased expression of typical OA-related markers such as IL-1β, IL-6, TNF-α, ADAMTS5, MMP-3, and MMP13. Bone histomorphometry demonstrated bone volume loss in the subchondral bone. Cathepsin K-positive osteoclasts were present at the border between the pannus-like fibrous cells and depressed subchondral bone. The pannus-like cells were positive for the osteoblast master transcription factor Runx2 and expressed Rankl, the osteoclast differentiation initiator. BMP-2 was strongly detected in the pannus-like cells and clustered hypertrophic chondrocytes in the degenerated articular cartilage, and p-(phosphorylated, activated) Smad1 and Smad5, the downstream BMP signal mediators, were stained in these cells (Fig. 2). In human CTA humeral head articular cartilage, p-Smad1 and p-Smad5 were strongly positive in the clustered hypertrophic chondrocytes, whereas unaffected cartilage cells showed only a weak signal for them. At the site of subchondral bone collapse, fibroblastic pannus-like cells, lining osteoblasts, osteocytes, and osteoclasts, showed strong expression of p-Smad1/5 (Fig. 3).

DISCUSSION: In both our rat mCTA model and human humeral head samples, p-Smad1/5 was strongly detected in pannus-like cells and articular chondrocytes, indicating an upregulation of the BMP signal. Similarly, osteoblasts and osteoclasts exhibited a similar pattern, suggesting that enhanced BMP signaling may be implicated in the high bone turnover around the collapsed subchondral bone. Therefore, one of the molecular pathogeneses of CTA changes appears to be an upregulation of the BMP signal, which could also be considered an exacerbating factor.

SIGNIFICANCE/CLINICAL RELEVANCE: The upregulation of the BMP signal in degenerated cartilage and subchondral bone in both the Rat mCTA model and human humeral head suggests that inhibiting this, for instance with a BMP type-I receptor inhibitor compound, could potentially lead to the suppression or treatment of CTA progression. We are currently exploring this possibility.

REFERENCES:

1. Teunis T., *et al*., *J Shoulder Elbow Surg* **23**, 1913-1921, (2014)
2. Chalmers P.N., *et al*., *J. Shoulder Elbow Surg* **25**, 1749-1755, (2016)
3. Neer C.S., *et al*., *J. Bone Joint Surg. Am*. **65-A**, 1232-1244, 1983
4. Nakase T., *et al*., *Osteoarthritis Cartilage* **11**, 278-284, (2003)
5. Liu Y., *et al*., *Med Sci Monit* **21**, 363-370, (2015)
6. Kramer E.J., *et al*., *J. Shoulder Elbow Surg* **22**, 1702-1709, (2013)
7. Zingman A., *et al*., *J. Orthop. Res* **35**, 506-514, (2017)
8. Ijuin T., *et al*., *Osteoarthr Cartil Open* **5**, 100389, (2023)

IMAGES:

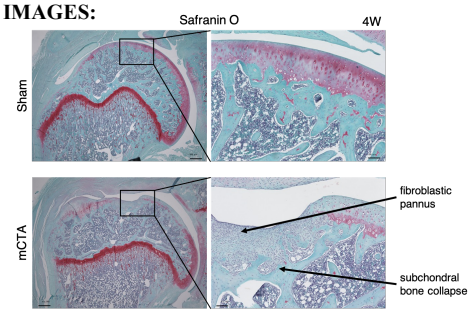

Fig. 1: Evaluation of mCTA model by Safranin O staining

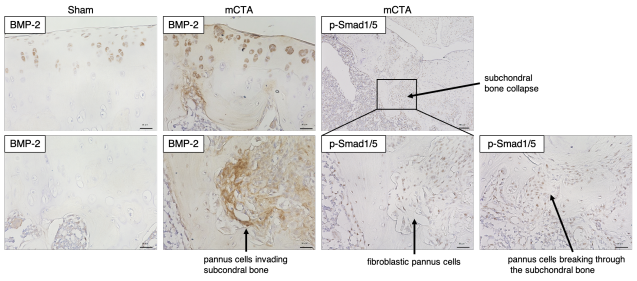

Fig. 2: Immunohistochemical staining (BMP-2, phospho–Smad 1/5) of mCTA model

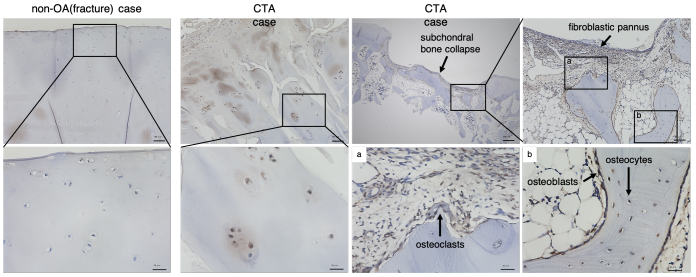

Fig. 3: Immunohistochemical staining (phospho–Smad 1/5) of humeral head