The alteration of A disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) in the knee joints of osteoarthritis mice

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Research article

Keywords: ADAMTS, Osteoarthritis, Knee joint, Articular cartilage, Subchondral bone

Posted Date: November 26th, 2019

DOI: https://doi.org/10.21203/rs.2.17731/v1

Version of Record: A version of this preprint was published on January 22nd, 2021. See the published version at https://doi.org/10.1080/01478885.2020.1861908.

Abstract

Background: To clarify the expression and distribution of ADAMTS1, ADAMTS2 and ADAMTS5 in knee joints of osteoarthritis (OA) mice.

Methods: OA was established via anterior cruciate ligament transection (ACLT) on the knee joints of C57BL/6J mice. The morphology change of OA was analyzed by Micro-CT. Histologic analysis was used to evaluate symptomatic change in articular cartilage and subchondral bone. Quantitative real-time PCR (qPCR) was used to analyze mRNA expressions of ADAMTS family in bone-related tissues and cells. Immunofluorescence staining was used to analyze the expressions and distributions of ADAMTS1, ADAMTS2, and ADAMTS5, as well as the condition of inflammation of OA.

Results: Cartilage deterioration, significant reduction of collagen and proteoglycan components in the cartilage matrix happened in ACLT-induced OA mice, along with increased inflammatory response and osteoclast activity. Among ADAMTS, the gene expression levels of ADAMTS1, ADAMTS2 and ADAMTS5 were ranked top 5 in cartilage/chondrocytes, osteogenic tissue/osteoblasts and cortical bone/osteocytes. After ACLT surgery, the expressions of ADAMTS1, ADAMTS2 and ADAMTS5 all increased in articular cartilage, growth plate and subchondral bone of knee joints.

Conclusion: The enhanced expressions of ADAMTS1, ADAMTS2 and ADAMTS5 after ACLT surgery provide a further understanding in degenerative change of OA.

Background

Osteoarthritis(OA) is a degenerative joint disease characterized by progressive degeneration and destruction of the articular cartilage, along with alterations in the subchondral bone, meniscus, synovium and surrounding joint tissues[1], leading to joint dysfunction or disability eventually. OA mainly occurs in middle-aged and elderly people, with a high incidence and more than 50% of the people over 65 years old develop the disease. Clinical features of OA include joint pain, limited joint movement, joint deformity and bone friction sensation, which severely decrease the quality of life of OA patients, and create an enormous social and economic burden.

It is generally agreed that OA has a multifaceted etiology related with many potential risk factors[2,3], however, its etiology and pathogenesis are not clearly understood so far. Cartilage dysfunction and destruction is critical in the pathogenesis of OA. Chondrocytes, as the only cell type in cartilage, play an important role in the maintenance of cartilage homeostasis via synthesizing and degrading extracellular matrix (ECM), including collagen and proteoglycan[1,4]. Collagen fiber network provides the tensile strength of articular cartilage, and the proteoglycan filled in the network has the hydrophilicity to bind water molecules and resist compressive force[5], which plays a favorable buffer role for the stress on articular cartilage. Once chondrocytes are exposed in the inflammatory environment of OA, its anabolism is inhibited and the catabolism is promoted, which accelerates the break down in cartilage. Proteoglycans

are degraded by relevant enzymes, and then the collagen fibrous network is destroyed, leading to irreversible progression in articular cartilage[3,5]. Aggrecan, the major component of proteoglycan, represents an early sign of cartilage destruction in osteoarthritis.

A disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) family, a novel group of 19 secreted proteinases, takes part in several physiological and pathological processes of tissues due to multiple functions. According to the structure and function, ADAMTS family is divided to four classification: 1) aggrecanases/proteoglycanases (ADAMTS1, 4, 5, 8, 9, 15 and 20); 2) procollagen N-propeptidases (ADAMTS2, 3 and 14); 3) the cartilage oligomeric matrix proteinases (ADAMTS7 and 12); 4) the von-Willebrand Factor proteinase (ADAMTS13); 5) unknow or orphan enzymes (ADAMTS6, 10, 16, 17, 18 and 19)[6]. As secreted proteases, ADAMTS are closely related to the degradation of extracellular matrix and contributes to osteoarthritis development by cleaving ECM.

Although ADAMTS family is gradually recognized to be an important mediator besides matrix metalloproteinase family (MMPs) in controlling degradation process in OA, the detailed investigation focused on ADAMTS family in the whole pathological joint is exclusive. The purpose of this study is to investigate the expression of ADAMTS family in the knee joints of OA mice. This may help to further understand the pathogenesis of OA.

Methods

ACLT-induced OA mice

The mice (C57BL/6J) were obtained from Dashuo Biological Institute (Chengdu, China) and all experimental procedures were approved and conducted by the Institutional Animal Care and Use Committees of Sichuan University.All experiments followed the guidelines for the ARRIVE guidelines. Anterior cruciate ligament transection (ACLT) surgery was operated to establish OA model in 4-week-old mice. A total of 20 male mice were randomly divided into Sham group (n = 10) and ACLT group (n = 10). In sham group, joint cavity was opened at right knee joint without cutting the anterior cruciate ligament, while ACLT surgery was performed on the right knee joint as ACLT group. Mice were sacrificed by CO_2 inhalation at 4 weeks after surgery, and samples of hind legs were dissected and fixed in 4% paraformaldehyde for 24 hours at room temperature, and then stored at 4°C.

Micro-computed tomography

Micro-computed tomography (μCT 50, Scanco Medical, Bassersdorf, Switzerland) was used to observe and evaluate the knee joint morphology of mice. Samples were scanned at 10 μm voxel size and then analyzed using the evaluation software provided by the manufacturer (Scanco Medical, Bassersdorf, Switzerland).

Hematoxylin and Eosin Staining

The acquired tissues were decalcified in 10% EDTA for 8 weeks. After the tissues were dehydrated and embedded in paraffin, paraffin tissue slices were sectioned at 8μm thickness by rotary microtome. The tissue slices were deparaffinized in xylene for 15 minutes, immersed in gradient ethanol for 3 minutes in each concentration, and hydrated in double distilled water (ddH_2O) for 10 minutes. The procedure above was repeated in all staining methods at the beginning of specific staining. In hematoxylin and eosin (H&E) staining, hematoxylin was applied for 5 min, followed by water rinse, differentiation with 1% hydrochloric acid ethanol and blue in ammonia-H2O. Eosin was stained for 1 min. After dehydrating in gradient ethanol, the slices were cleared in xylene for 5 minutes, and mounted in resinene.

Safranin O/Fast green Staining

The hydrated tissue slices were stained with Weigert's iron hematoxylin for 5 minutes and differentiated in 1% hydrochloric acid ethanol for 10 seconds. Fast green was applied to stain the background for 2 minutes. Double distilled water slightly washed the slices, and Safranin O was placed on the tissue slices for 15minutes. At last, the slices were processed by dehydration, vitrification, and sealed with resinene.

Masson's Trichrome Staining

Masson's trichrome staining, known as a three-color staining protocol, was operated strictly according to the official specification (Solarbio Life Sciences, Beijing, China). After the appropriate staining was observed under an optical microscope, the slices were dried, cleared and mounted in resinene.

Tartrate Resistant Acid Phosphatase Staining

Tartrate resistant acid phosphatase (TRAP)staining, for analyzing osteoclasts, was performed by the official protocal (Wako Pure Chemical Industries, Osaka, Japan). The hydrated tissue slices were stained with TRAP staining solution for 1h at room temperature and rinsed in double distilled water for 5 minutes. Alcian blue was applied to stain the background for 5 minutes. In the end, the slices were dehydrated, cleared and mounted in resinene.

Quantitative real-time PCR

Tissues and Cells in TRIzol (Invitrogen, Life Technologies, Grand Island, NY, USA) were disrupted using mortar with liquid nitrogen. Total RNA was isolated using the mRNA Selective PCR Kit (Takara, Japan). Complementary DNA was synthesized using SuperScript® III Reverse Transcriptase (Invitrogen, USA). Quantitative real-time PCR was performed in quadruplicates on a 7900HT sequence detector (Applied Biosystems, Palo Alto, CA, USA) using TaqMan Assay-on-Demand primers supplied by Applied

Biosystems. Gene of interest cycle thresholds were normalized to TATA-box binding protein (Tbp) housekeeper levels by the ΔΔCt method and displayed as relative copies per Tbp or relative expression normalized to experimental control groups.

Immunofluorescence Staining

Immunofluorescence was carried out using Vector kits DI1788 for green and DI1794 for red for polyclonal antibodies generated from rabbit (Vector Laboratories, Burlingame, CA) according to the manufacturer's instructions. The fluorescence secondary antibody was incubated (1:200) for 2 hours, and the slices were kept out of light from this step. PBS was used to rinse the slices for 10 minutes, and 40,6-diamidino2-phenylindole (DAPI) was applied for 10 minutes to show the cell nuclear. The images were collected by confocal laser scanning microscope (CLSM) after the slices were sealed by 50% glycerine.

Statistical analysis

The data was collected from at least three individual experiments, and then statistically analyzed via one-way analysis of variance. The difference was statistically significant when $P<0.05$.

Results

Degeneration and destruction of the articular cartilage in ACLT-induced OA mice

At four weeks after ACLT surgery, significant degeneration occurred in the articular cartilage tissue. MicroCT showed wear and tear on the surface of articular cartilage of the knee joint at four weeks after surgery, and decreased volume of bone tissue (BV/TV), including femoral head, tibial head, meniscus, and meniscus, compared with the Sham group (Figure 1A). The surface of articular cartilage in the Sham group was smooth and intact. However, not only the surface of articular cartilage was rough, but also the meniscus was worn and cracked in the ACLT group. In addition, the thickness of articular cartilage layer and the chondrocyte number were significantly reduced, along with abnormal vacuolar changes and uneven cartilage matrix staining at four weeks after surgery (Figure 1B). In order to further detect the changes of cartilage matrix, we used Safranin O staining and Masson's trichrome staining. By the Safranin O staining, the proteoglycan was stained with red, which shows significantly decreased content of proteoglycan in the ACLT group, while the calcified cartilage layer with lighter staining increased significantly in thickness, and collagen with green staining also became slightly lighter in color, reflecting decreased content of bone collagen as well (Figure 1C). Masson's trichrome staining showed the blue-stained collagen and the red-stained content was increased was decreased in the ACLT group (Figure1D). In this stain method, collagen fibers, mucus and cartilage were stained blue, while the cytoplasm, muscle, cellulose and neuroglia were stained red, and the cell nuclei were stained dark blue.

Inflammatory reaction in the joints of ACLT-induced OA mice

Inflammatory reaction is one of key features of osteoarthritis. Inflammatory reaction promotes degeneration and destruction of the articular cartilage, and also enhances bone resorption in the subchondral bone. With immunofluorescence, we manifested inflammatory cells by detecting respective markers and determine the condition of inflammation. F4/80 (marker for macrophages) and CD3 (marker for T cells) were increased in the bone marrow of the femur at four weeks after surgery, indicating the presence of acute inflammation (Figure 2A). IL–1β and TNFα concentrations, analyzed by ELISA, were also measured to further characterize the inflammatory response. In ACLT group, the concentration of IL–1β increased by approximately 1.80-fold compared with the Sham group, and that of TNFα increased by approximately 1.35-fold (Figure 2B). Osteoclasts were demonstrated by TRAP staining. Osteoclasts were distributed on the surface of bone tissue, and stained red by TRAP. Osteoclasts increased in the ACLT group and was 2.4 times as much as that in the Sham group (Figure 2C).

mRNA expressions of ADAMTS family in bone related tissues and cells

By qPCR (Figure 3), expression of ADAMTS5 was strongest in chondrocyte, osteoblast, osteocyte and cartilage. Expression of ADAMTS2 was strongest in osteogenic tissue. While expression of ADAMTS1 was strongest in cortical bone. Overall, the expressions of ADAMTS1, ADAMTS2 and ADAMTS5 were relatively strongest. Thus, it is representative for analyzing ADAMTS family via detecting the protease expression of ADAMTS1, ADAMTS2 and ADAMTS5 in knee joints of ACLT-induced OA mice.

ADAMTS1 expression in the knee joints of OA mice

The panorama images of protease expression through the entire knee joint were acquired (Figure 4). Then, we focused on three areas: the articular cartilage area, the subchondral bone area and the growth plate area. In articular cartilage, ADAMTS1 was weakly expressed in Sham group and slightly increased in ACLT group, but mainly located in the middle layer of articular cartilage (red boxed area in Figure 4). In the subchondral bone, ADAMTS1 was distributed in the extracellular around bone cells, and the expression was significantly increased in the ACLT group (yellow boxed area in Figure 4). In addition, ADAMTS1 expression in the ACLT group also showed an increasing trend in the cartilage matrix of growth plate (cyan boxed area in Figure 4).

ADAMTS2 expression in the knee joints of OA mice

From the panorama images, it was obvious shown that ADAMTS2 was highly expressed in the ACLT group (Figure 5). Although ADAMTS2 was slightly expressed in all layers of articular cartilage in the Sham group, its expression significantly increased in the ACLT group (red boxed area in Figure 5). ADAMTS2 expression also significantly increased in subchondral bone (yellow boxed area in Figure 5) and chondrocytes of growth plate (cyan boxed area in Figure 5).

ADAMTS5 expression in the knee joints of OA mice

ADAMTS5 expression increased not only in articular cartilage, subchondral bone and growth plate, but also in meniscus chondrocytes (Figure 6). In articular cartilage, ADAMTS5 was expressed on the surface of cartilage in the Sham group, but significantly increased in the ACLT group (red boxed area in Figure 6). In subchondral bone, ADAMTS5 was rarely expressed in the Sham group, but its expression increased in the ACLT group (yellow boxed area in Figure 6). Similarly, ADAMTS5 significantly increased in chondrocytes and cartilage matrix of growth plates in the ACLT group (cyan boxed area in Figure 6).

Discussion

In this study, we successfully established OA model via anterior cruciate ligament transection (ACLT) on the knee joints in C57BL/6J mice. We multi-perceptively showed the symptom of early stage of OA. It was reported that the expressions of ADAMTS1 and ADAMTS5 significantly increased in the synovial fluid of OA patients[9], suggesting that the ADAMTS family may play a key role in osteoarthritis. However, there is no detailed study on the expression and distribution of ADAMTS family in ACLT-induced OA knee joints.

ADAMTS1 was firstly found in the gene screening of mouse tumor research[10]. ADAMTS1 mRNA was expressed in both articular cartilage and growth plate cartilage of normal mice. It belongs to aggrecanases and has weak ability of cartilage matrix degradation, whose substrates also include other extracellular matrix proteins such as versican, brevican and type I collegen[11,14]. However, the previous study showed that aggrecan and degraded products contents of cartilage in the ADAMTS1 knockout mice manifested no obvious difference compared with the normal mice[12]. On the other hand, overexpression of ADAMTS1 could have an influence on bone mineral density. In addition, ADAMTS1 was considered as a potential mediator in bone remodeling[13]. It was found that ADAMTS1 was expressed in osteoblasts and up-regulated where new bone was forming[15], which is consistent with our results. Another study showed that ADAMTS1 promoted type I collagen degradation and osteoblast proliferation[16]. According to our study results, ADAMTS1 expression increased in subchondral bone of osteoarthritis, which may be related to abnormal bone remodeling changes of subchondral bone in osteoarthritis.

As a procollagen N-proteinase, ADAMTS2 is involved in the transformation of procollagen into collagen. Only processed procollagens can transform to normal collagen monomer, and finally forms normal collagen fibers[17]. ADAMTS2 is capable of cleaving procollagen type Ⅰ, Ⅱ and Ⅲ. Both TNFα and TGFβ

could promote secretion of ADAMTS2. In our results, the expression of ADAMTS2 significantly increased in articular cartilage, subchondral bone and growth plate in osteoarthritis, indicating its significant effect on osteoarthritis.

ADAMTS5, also named as aggrecananse2, the most efficient aggrecananses, mainly performs function in degrading aggrecan, which has attracted much attention in the exploration of the pathological mechanism in osteoarthritis. Through the study of OA, it was found that ADAMTS5 was the main aggrecan degrading enzyme[18,19]. According to our results, ADAMTS5 was significantly enhanced in the articular cartilage of OA knee joints, which was consistent with its strong aggrecan degradation ability.

In previous study, many efforts were made to find out the role of MMPs family on pathological process of OA[20–23]. However, the situation of degradation process in OA was much complexed if only analyzed via MMPs family. Thus, we will further explore the role of ADAMTS family on OA, which may take part in several physiological and pathological process of OA due to multiple functions. In summary, this study provided the expression and distribution patterns of ADAMTS1, ADAMTS2 and ADAMTS5 in knee joints of ACLT-induced OA mice, and it will be the benefit of the in-depth study for detailed role and mechanism of ADAMTS protease family in OA. Further research is still needed to find out the mechanism of ADAMTS family in the initial stage and development of OA.

Conclusion

In this study, we successfully established the OA model by ACLT in mice and revealed its pathological changes, including morphology and inflammation. Besides, we uncovered the high expression level of ADAMTS family in bone related tissues and cells. Based on this, we further demonstrated the ADAMTS1, ADAMTS2 and ADAMTS5 were all up-regulated at the articular cartilage, subchondral bone and growth plate of OA mice by immunofluorescence staining.

Abbreviations

OA: Osteoarthritis; ADAMTS: A disintegrin and metalloproteinase with thrombospondin motifs; ACLT: anterior cruciate ligament transection; BV/TV: The ratio of Bone volume to Tissue volume; TRAP: Tartrate resistant acid phosphatase; qPCR: Quantitative real-time PCR. CLSM: confocal laser scanning microscope.

Declarations

Acknowledgments

The authors would like to thank all staff from the participating this work.

Authors’ contributions

Conception and design of the study: LW, DZ, JS, YC, LC. Collection of data: CC, YL, JG, LL. Analysis and interpretation of data: GZ, LZ, CP. Drafting the article: JX. Critically revision of the article: XZ. Co-authors agreed with the finalized submission. All authors read and approved the final manuscript.

Funding Statement

This work was financially supported by the National Natural Science Foundation of China (grant numbers 81371136, 81430011 to Xuedong Zhou, and grant numbers 81600840, 81771047 to Jing Xie), and Basic- and Basic Applied- Research Project of West China Hospital of Stomatology, Sichuan University (grant number RD–02–201912)

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

The mice (C57BL/6J) were obtained and all experimental procedures were approved by the Institutional Animal Care and Use Committees of Sichuan University (approval number SYXK()2018–185).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

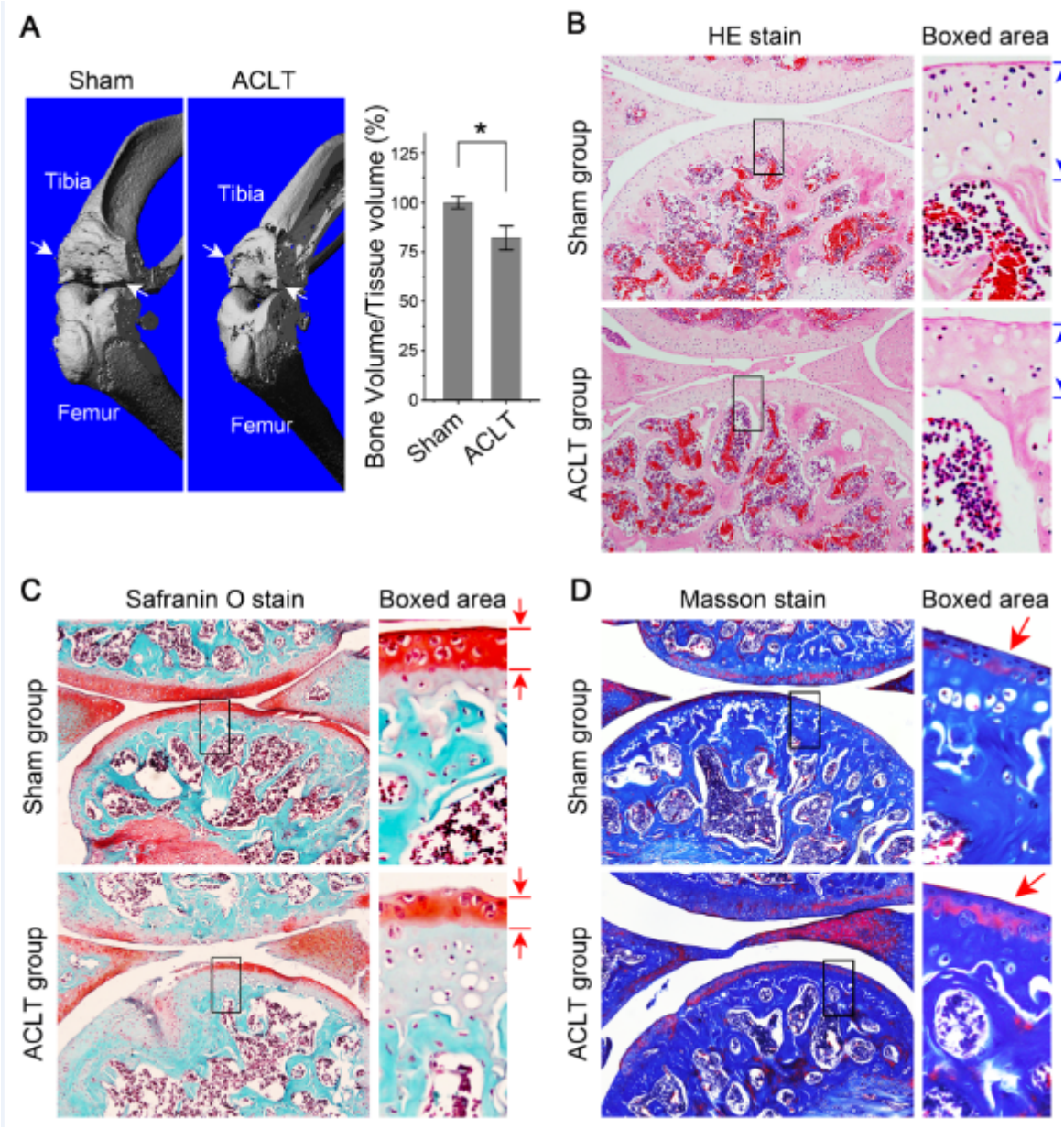

Figure 1

Symptoms of joint in OA mice at four weeks after ACLT. (A) Micro-CT showing the destruction of articular cartilage and meniscus at four weeks postoperatively. The ratio of Bone volume to Tissue volume (BV/TV) decreases in the ACLT group. The data is averaged from three different sets of experiments. The difference is statistically significant compared with the Sham group. (n = 3, *P < 0.05). (B) H&E staining showing the morphological changes of joint surface. Particularly, the thickness of articular cartilage layer is thinner, and the chondrocytes is less. (C) Representative safranin O staining indicating the proteoglycan loss in articular cartilage four weeks post ACLT surgery (n = 3). (D) Representative masson's trichrome staining indicating the changes in collagenous fibrous tissue four weeks post ACLT surgery (n = 5).

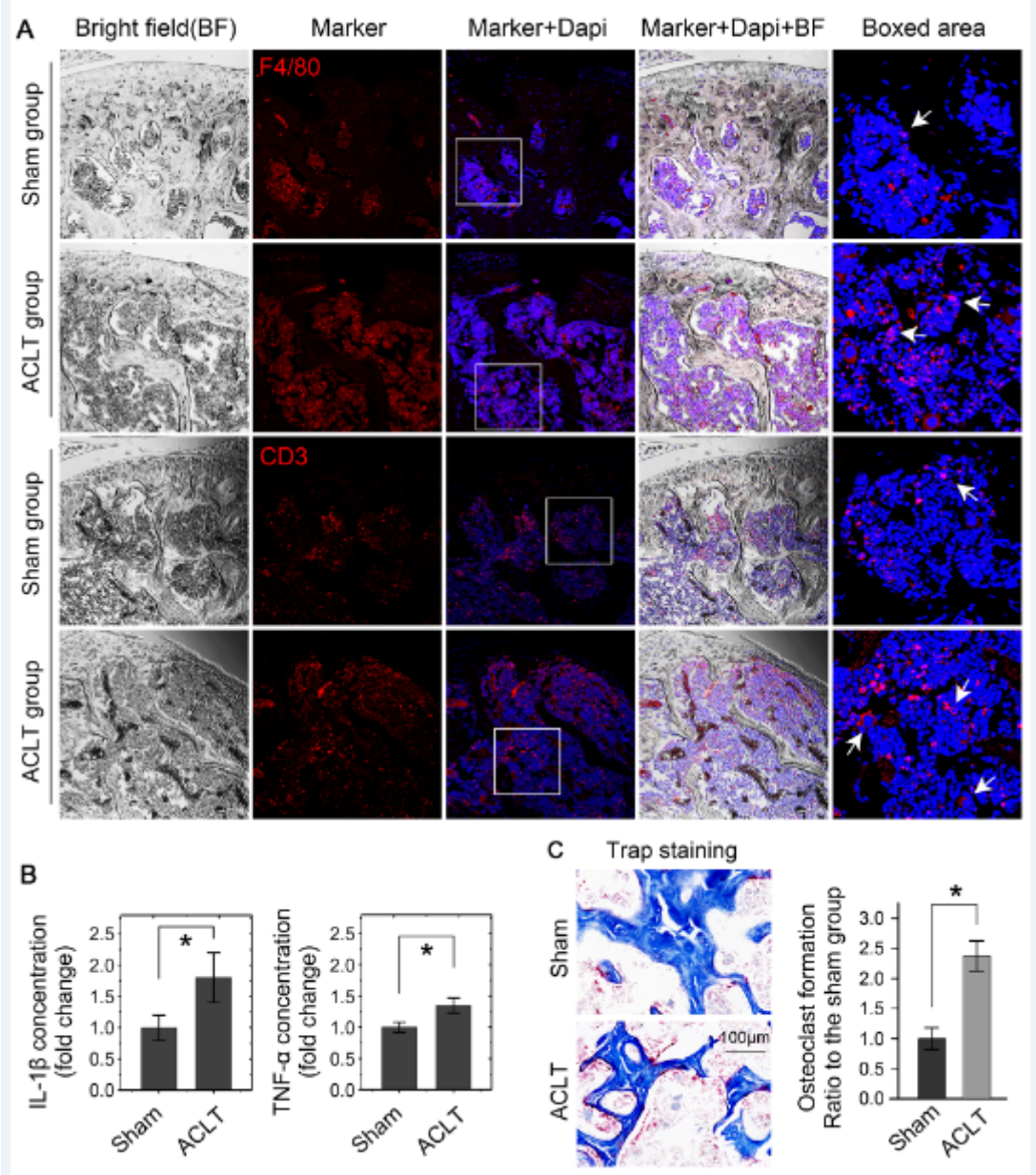

Figure 2

Inflammation and osteoclasts all increase at four weeks after ACLT. (A) Immunofluorescence staining showing F4/80 (marker of macrophages) and CD3 (marker of T cells) all enhanced at four weeks after surgery, indicating increased inflammatory cell response. (B) Concentrations of IL-1β and TNFα in serum of mice all increased, detected by enzyme-linked immunosorbent assay (n = 3, *P < 0.05). (C) Tartrate resistant acid phosphatase (TRAP) staining showing the number of osteoclasts increased in the ACLT group, indicating the overactive bone resorption of subchondral bone (n = 3, *P < 0.05).

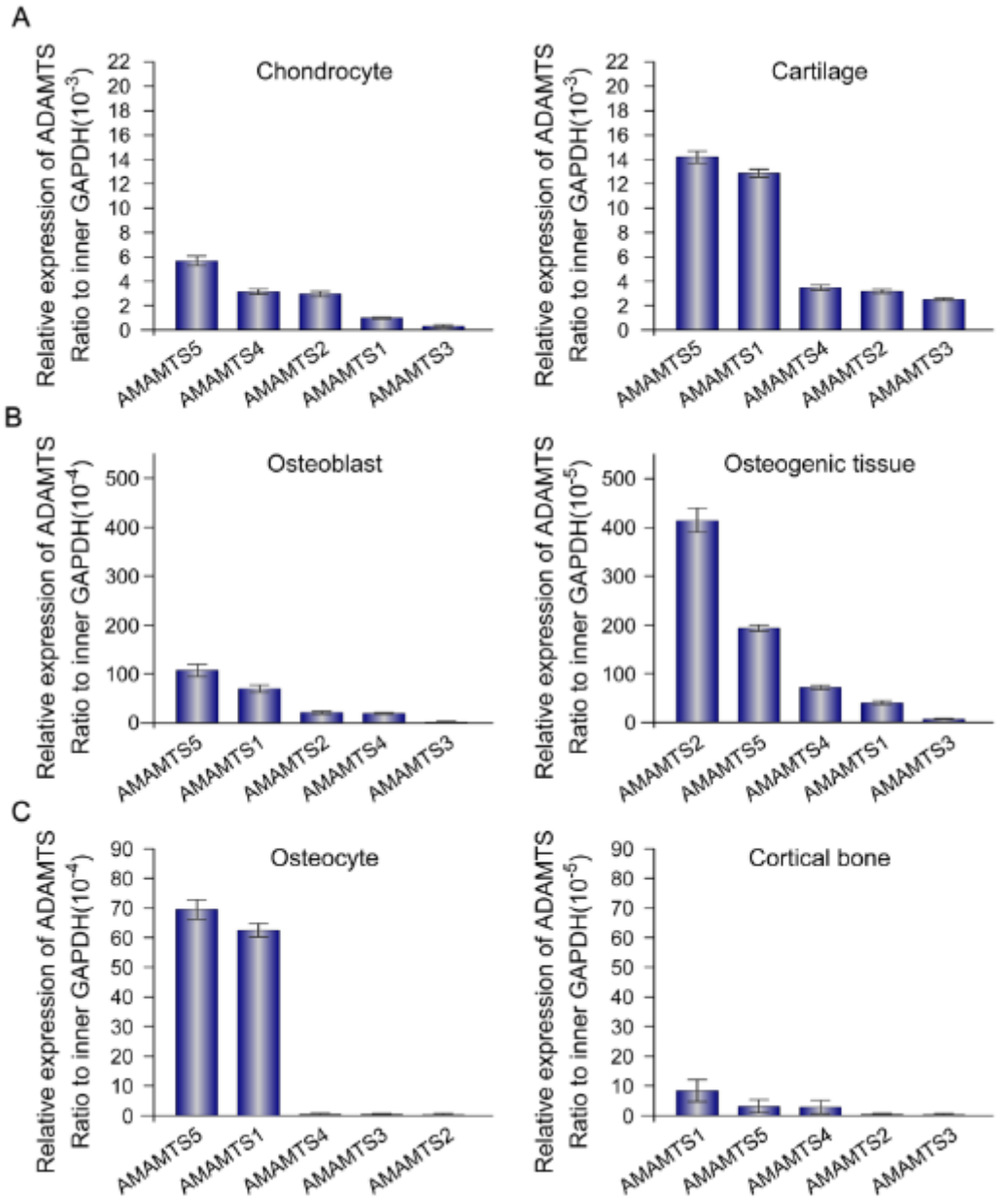

Figure 3

mRNA expressions of ADAMTS family in bone-related tissues and cells. Overall, the expressions of ADAMTS1, ADAMTS2 and ADAMTS5 are ranked top 5 in cartilage/chondrocytes, osteogenic tissue/osteoblasts and cortical bone/osteocytes. (A) mRNA expressions of ADAMTS family in chondrocyte and cartilage. (B) mRNA expressions of ADAMTS family in osteoblast and cancellous bone. (C) mRNA expressions of ADAMTS family in osteocyte and cortical bone. These results were based on three independent experiments (n = 3).

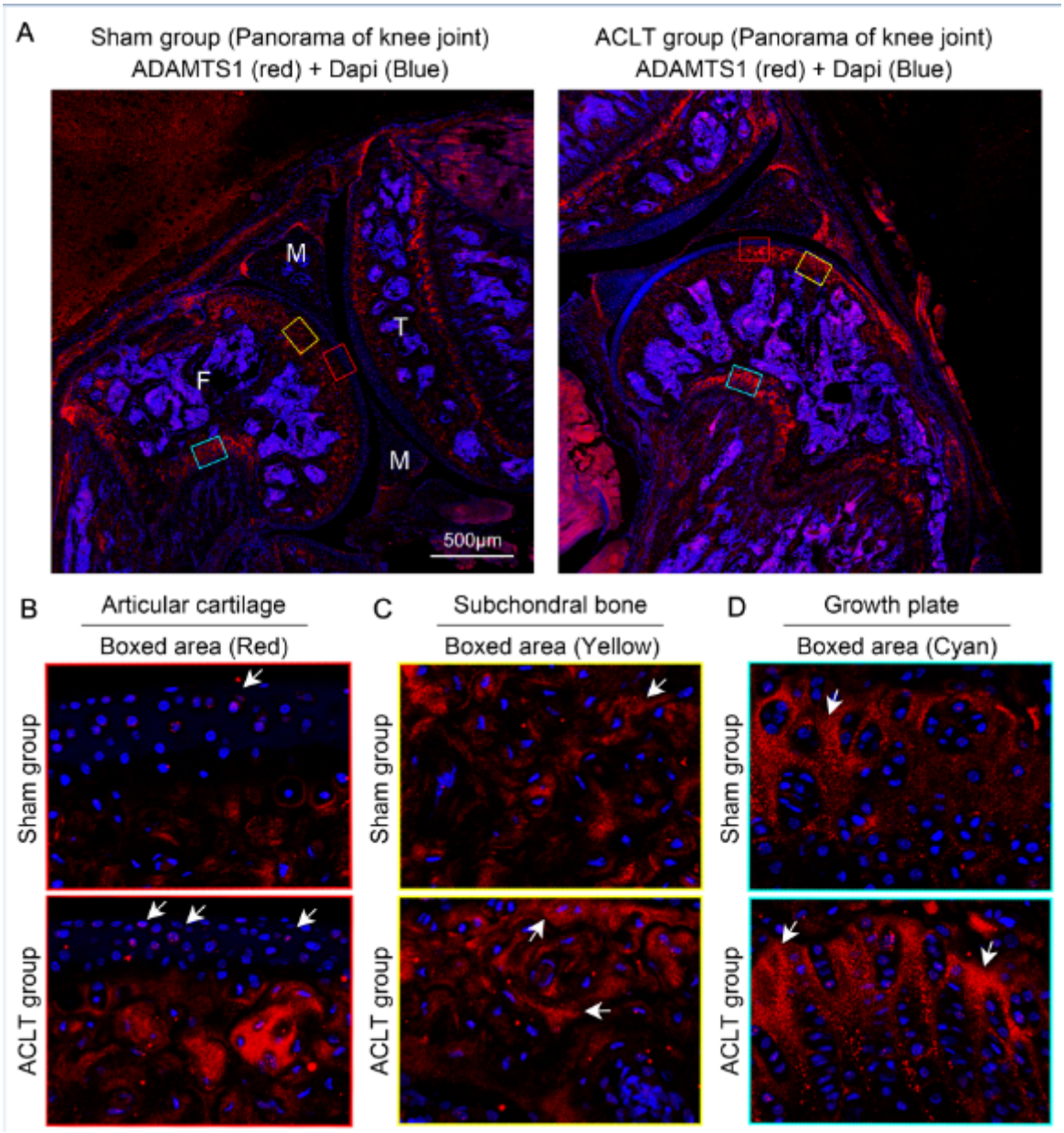

Figure 4

The expression and distribution of ADAMTS1 in the knee joint of mice four weeks post ACLT surgery. (A) Representative panoramic images showing the detailed expression changes of ADAMTS1 by immunofluorescence captured with CLSM. Magnified boxed areas showing the changes of ADAMTS1 in the regions of articular cartilage (B), subchondral bone (C) and growth plate (D).

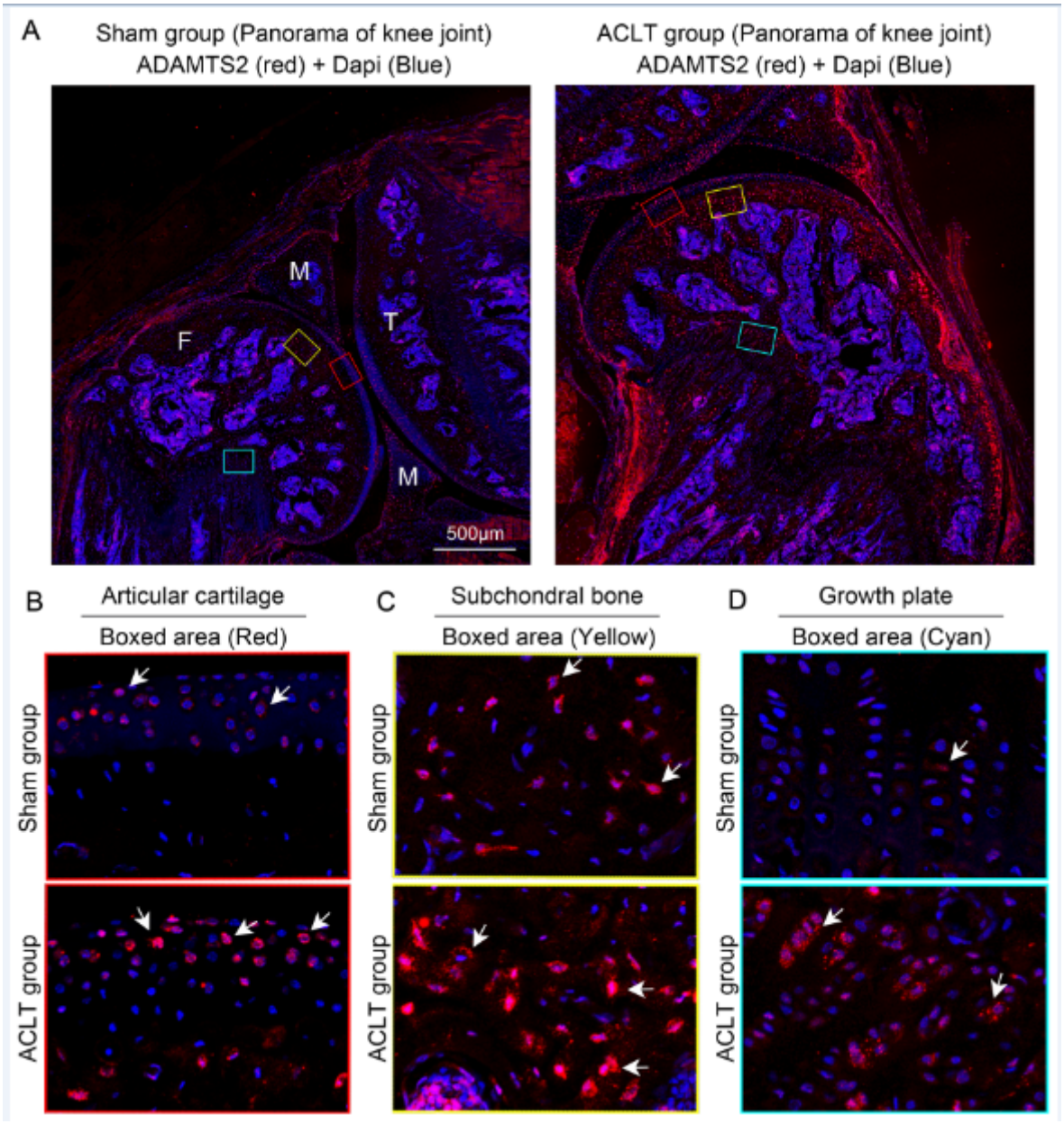

Figure 5

The expression and distribution of ADAMTS2 in the knee joint of mice four weeks post ACLT surgery. (A) Representative panoramic images showing the detailed expression changes of ADAMTS2 by immunofluorescence captured with CLSM. Magnified boxed areas showing the changes of ADAMTS2 in the regions of articular cartilage (B), subchondral bone (C) and growth plate (D).

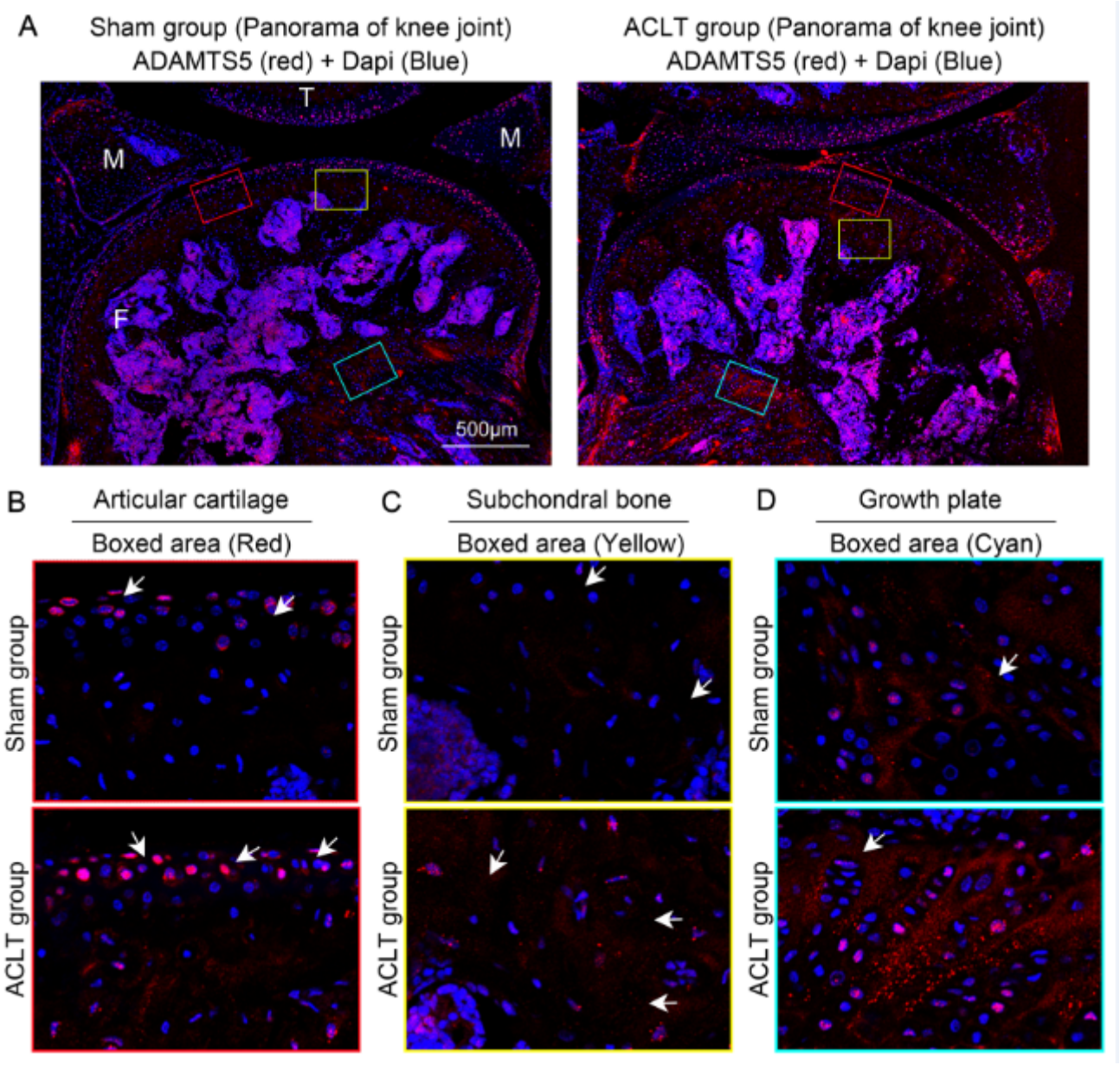

Figure 6

The expression and distribution of ADAMTS5 in the knee joint of mice four weeks post ACLT surgery. (A) Representative panoramic images showing the detailed expression changes of ADAMTS5 by immunofluorescence captured with CLSM. Magnified boxed areas showing the changes of ADAMTS5 in the regions of articular cartilage (B), subchondral bone (C) and growth plate (D).

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