

Anti-RANKL antibody treatment mitigates subchondral bone loss and cartilage degeneration without affecting synovial inflammation in a mouse model of rotator cuff tear arthropathy

From Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan

Cite this article:
Bone Joint Res 2026;15(10): 1183–1195.

DOI: 10.1302/2046-3758.1510.BJR-2025-0759.R1

Correspondence should be sent to Shingo Maeda s-maeda@m3.kufm.kagoshima-u.ac.jp

T. Ide,^{1,2} Y. Masuda,² K. Shimanoe,² S. Saho,^{2,3} S. Takada,² K. Uekama,² T. Iuchi,² H. Tawaratsumida,² T. Ijuin,² H. Tominaga,² S. Maeda,⁴ N. Taniguchi^{1,2,3,4}

¹Department of Locomotory Organ Regeneration, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan

²Department of Orthopaedic Surgery, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan

³Department of Medical Joint Materials, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan

⁴Department of Bone and Joint Medicine, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan

Aims

Enhanced osteoclast activity in subchondral bone is implicated in osteoarthritis (OA) progression, but the contribution of osteoclasts versus synovial inflammation in cuff tear arthropathy (CTA) remains unclear. This study investigated whether inhibition of osteoclasts using an anti-receptor activator of nuclear factor κ B ligand (RANKL) antibody can suppress joint degeneration in a mouse CTA model without affecting synovial inflammation.

Methods

A modified CTA (mCTA) model was established in mice by resecting the supraspinatus and infraspinatus tendons, the superior joint capsule, and the long head of the biceps tendon in the right shoulder of C57BL/6J mice. Mice were assigned to four groups: sham or mCTA, each treated with phosphate-buffered saline or anti-RANKL antibody (5 mg/kg subcutaneously every two weeks). Four weeks post-surgery, histological analyses of the humeral head were performed using the Murine Shoulder Arthritis Score (MSAS), immunohistochemistry for osteoclasts, osteoblasts, RANKL/osteoprotegerin (OPG), inflammatory cytokines, and catabolic enzymes, and quantification of synovial M1 macrophages. Subchondral bone volume (BV) was assessed via the bone volume/tissue volume ratio.

Results

The mouse mCTA model reproduced clinically relevant CTA phenotypes, including cartilage degeneration, subchondral bone loss, and joint surface collapse, within four weeks. RANKL expression and osteoclast surface increased in the superior and middle zones, while osteoblasts decreased. Synovial RANKL expression was low and unchanged after mCTA surgery. Anti-RANKL antibody administration significantly suppressed osteoclast surface and preserved subchondral BV, leading to attenuation of cartilage degeneration and reduced expression of inflammatory cytokines and catabolic enzymes in cartilage and subchondral bone. The synovitis score, synovial M1 macrophages, and inflammatory factors were unaffected, indicating that suppression of osteoclasts was sufficient to mitigate joint degeneration.

Conclusion

Inhibition of subchondral osteoclasts via anti-RANKL antibody reduces CTA-associated cartilage and bone degeneration without altering synovitis. These findings suggest that targeting osteoclasts may represent a promising disease-modifying strategy for CTA, and targeting RANKL with denosumab may prevent or slow shoulder OA progression.

Article focus

- We established a modified mouse model of cuff tear arthropathy (CTA) that reproduces key features of shoulder osteoarthritis.
- We determined whether the inhibition of subchondral osteoclasts with an anti-receptor activator of nuclear factor κ B ligand (RANKL) antibody mitigates joint degeneration.
- The relative contribution of synovial inflammation versus subchondral osteoclast activity in CTA pathogenesis was determined.

Key messages

- The modified mouse model of CTA reproduces cartilage degeneration, subchondral bone loss, and joint surface collapse within a clinically relevant timeframe.
- Anti-RANKL antibody treatment preserves subchondral bone and attenuates cartilage degeneration without affecting synovial inflammation.
- Subchondral osteoclasts play a primary role in CTA progression, supporting anti-RANKL therapy as a potential disease-modifying treatment.

Strengths and limitations

- The modified mouse model of CTA enables evaluation of disease-modifying interventions over a time course corresponding to approximately five years of human adulthood.
- The effects of bisphosphonates reported in the rat mCTA model and those of the anti-RANKL antibody observed in the present mouse mCTA model cannot be directly compared.
- Mouse shoulder biomechanics differ from humans; long-term effects and efficacy in late-phase CTA were not assessed.

Introduction

Accumulating evidence indicates that enhanced osteoclast activity in subchondral bone plays a pivotal role in the onset and progression of osteoarthritis (OA). Experimental and clinical studies show that early OA is characterized by increased osteoclast numbers and bone resorption that precede or accompany cartilage degeneration.¹⁻³ Activated osteoclasts release proteolytic enzymes, such as matrix metalloproteinases (MMPs), which accelerate cartilage matrix degradation.⁴ Inhibition of osteoclast activity ameliorates OA progression in animal models,^{1,5} yet randomized controlled trials have shown that bisphosphonates do not improve symptoms or radiological progression in knee OA.^{6,7} Because knee OA typically exhibits late-stage subchondral sclerosis,⁴ most patients likely present after the bone-resorptive phase, limiting the effectiveness of antiresorptive therapy.^{7,8} Although both subchondral bone remodelling and synovial inflammation contribute to OA pathogenesis, their relative

roles may vary depending on the joint and disease context. Degenerative changes in the glenohumeral joint are characterized by complex interactions between cartilage, subchondral bone, and synovial tissue.⁹ Degenerative rotator cuff tears are common, and a substantial proportion of patients fail conservative treatment and progress to more severe joint pathology.¹⁰ For irreparable rotator cuff tears, various surgical options have been reported, including tendon transfer procedures such as latissimus dorsi transfer.^{11,12} Cuff tear arthropathy (CTA), a secondary form of shoulder OA that develops after rotator cuff tears,¹³ is characterized by persistent subchondral bone loss rather than sclerosis, even in advanced stages.¹⁴ This feature suggests a prominent role for osteoclast-mediated bone resorption in CTA progression. In a rat modified CTA (mCTA) model, increased receptor activator of nuclear factor κ B ligand (RANKL) expression and osteoclast numbers are accompanied by reduced bone mass, and treatment with zoledronate markedly suppresses CTA-like changes.^{14,15} However, because zoledronate inhibits both subchondral osteoclasts and synovial inflammation, including M1 macrophages,¹⁴ it remains unclear which component plays the dominant role in disease progression. Therefore, a model that enables selective inhibition of osteoclasts is required to clarify the specific contribution of subchondral bone remodeling in CTA pathogenesis.

To address this question, we focused on RANKL, a specific and essential inducer of osteoclast differentiation, noting that anti-RANKL neutralization inhibits osteoclasts without affecting macrophage activity.^{16,17} Because currently available anti-RANKL antibodies are mouse-specific, the establishment of a mouse model is essential to enable selective inhibition of osteoclasts and to dissect their role independently of other cell types.

Methods

Mouse mCTA model

All animal experiments were approved by the Institutional Animal Care and Use Committee and conducted in accordance with the Animal Experimentation Regulations, and reported in compliance with the ARRIVE guidelines. A total of 54 eleven-week-old male C57BL/6J mice (Japan SLC, Japan) were acclimated to the laboratory environment for one week prior to surgery at 12 weeks of age. Under general anaesthesia, the mCTA model was established in the right shoulder by incising the deltoid muscle and resecting the supraspinatus and infraspinatus tendons at their insertions on the humeral head.¹⁵ The superior half of the joint capsule and the long head of the biceps tendon were also resected. Sham surgery was performed on the left shoulder through a deltoid incision. Mice were euthanized at one, two, or four weeks after surgery via an overdose of isoflurane and subjected to histological and immunohistochemical analyses ($n = 6$ joints per treatment per timepoint). A rotator cuff tenotomy (RCT) model was established in mice by transecting only the supraspinatus and

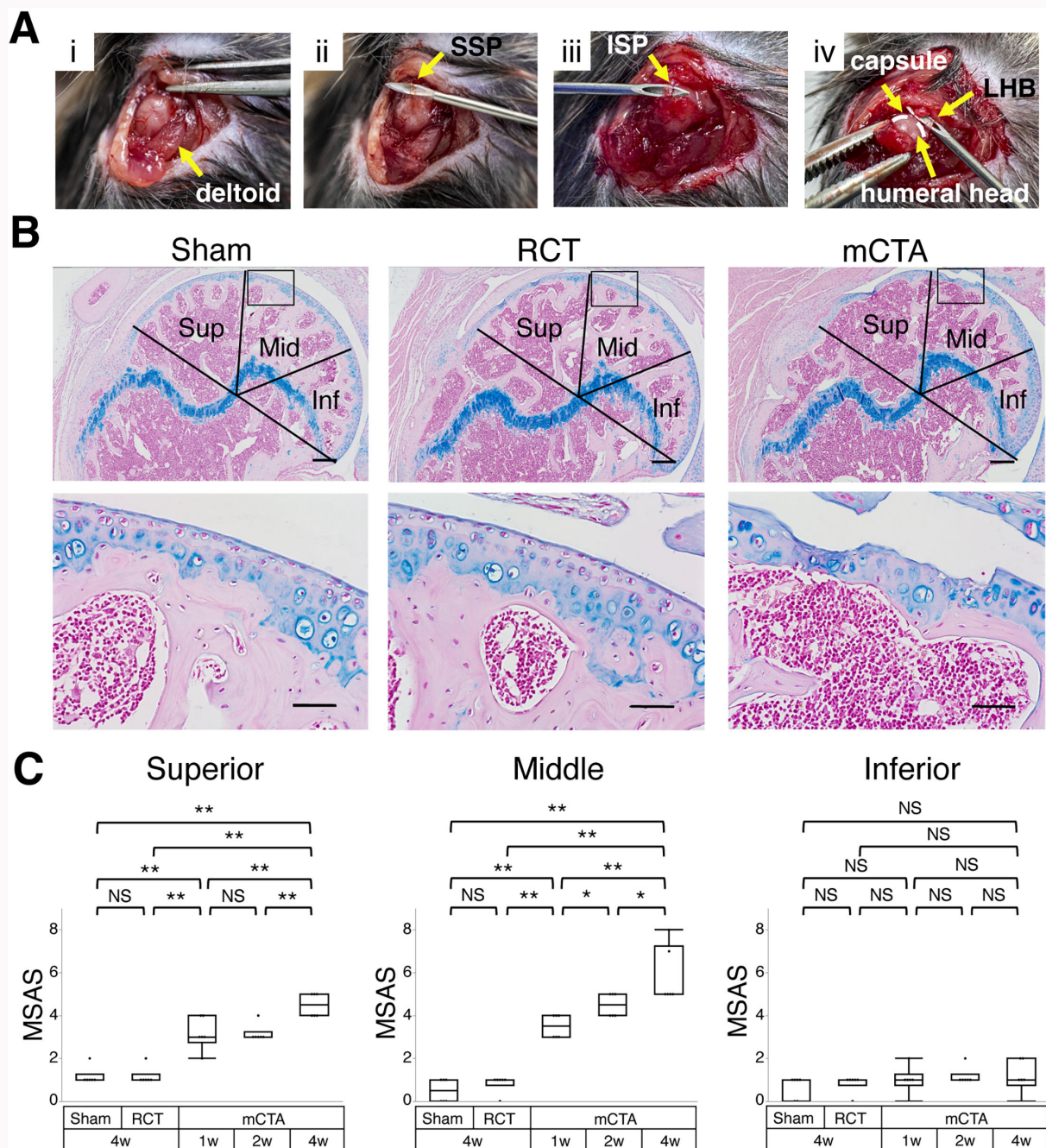


Fig. 1

Establishment of the mouse version of the modified cuff tear arthropathy (mCTA) model and comparison of cartilage degeneration with the rotator cuff tenotomy (RCT) model. a) (i) The deltoid muscle was incised, followed by sequential resection of (ii) the supraspinatus tendon (SSP), (iii) the infraspinatus tendon (ISP), and (iv) the superior half of the joint capsule (dashed line) together with the long head of the biceps tendon (LHB). b) The humeral head stained with Alcian blue was divided into three zones—superior (Sup), middle (Mid), and inferior (Inf)—to measure the Murine Shoulder Arthritis Score (MSAS). The box in the upper panel indicates the area shown in the lower panel. Scale bar = upper panels 200 μ m, lower panels 50 μ m. c) MSAS values for each zone are shown ($n = 6$). Normality was assessed using the Shapiro-Wilk test; and comparisons were performed using the Mann-Whitney U test. * $p < 0.05$, ** $p < 0.01$. NS, not significant; w, weeks.

infraspinatus tendons.¹⁵ This model was used as a comparative control to distinguish the effects of rotator cuff tendon injury alone from those of the mCTA procedure.

Anti-RANKL antibody administration

The dosage of the anti-RANKL neutralizing antibody (OYC1; Oriental Yeast Co., Japan) was set at 5 mg/kg, based on the previous report by Furuya et al.¹⁸ We sought to determine whether its efficacy would last for four weeks in

12-week-old mice. After a single subcutaneous administration of the antibody to 12-week-old male mice, samples were collected at one, two, and four weeks to evaluate the presence of osteoclasts in the primary cancellous bone of the tibia. Osteoclast formation was effectively suppressed up to two weeks post-administration, but reappeared at four weeks (Supplementary Figure a). Based on these findings, a dosing interval of once every two weeks was adopted. The control group ($n = 6$) received phosphate-buffered saline (PBS)

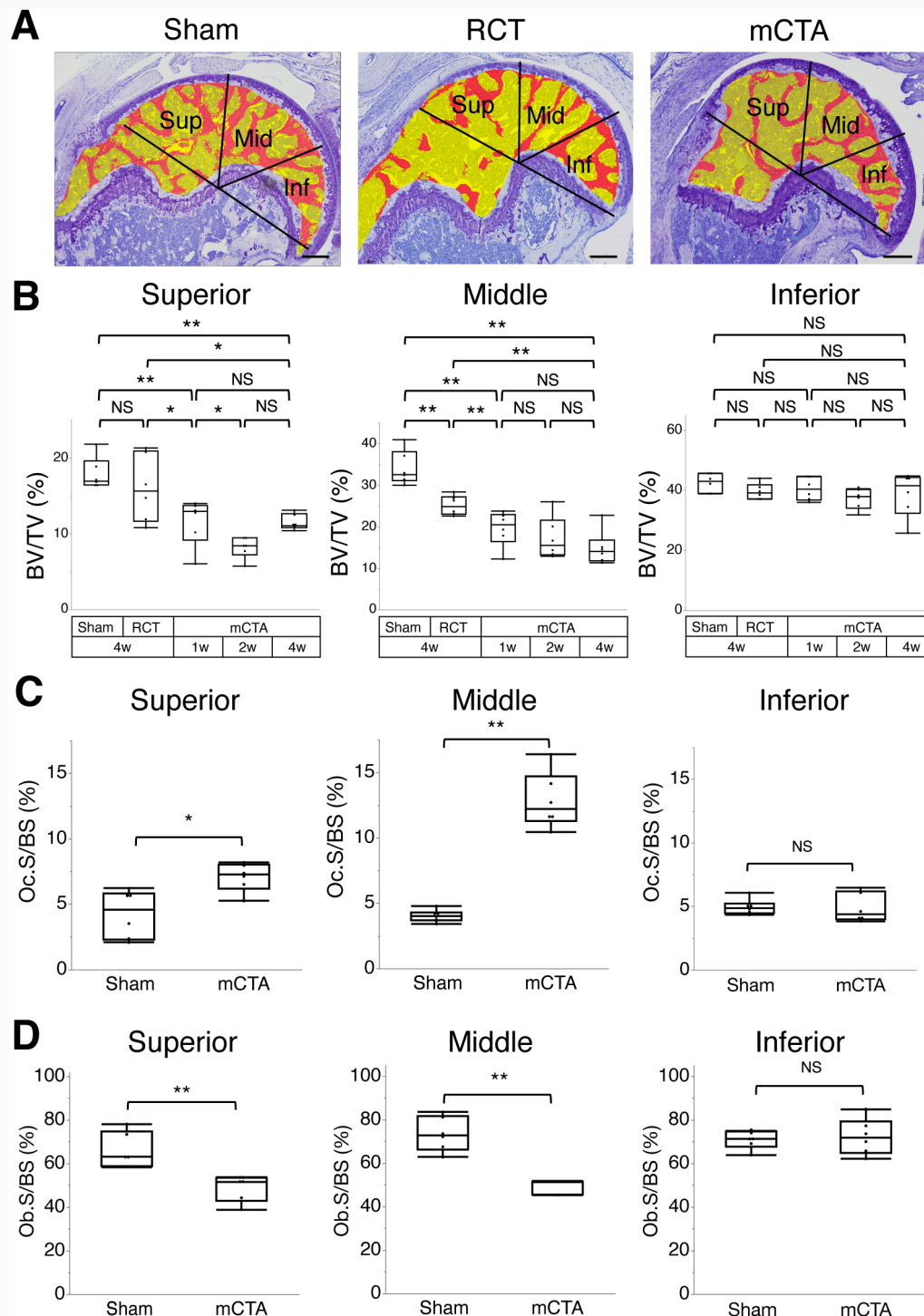


Fig. 2

Analysis of subchondral bone in the humeral head of the modified cuff tear arthropathy (mCTA) and rotator cuff tenotomy (RCT) models. a) Representative toluidine blue-stained images of humeral head subchondral bone. The bone volume (red)/tissue volume (yellow) (BV/TV) ratio was measured for each zone. Scale bar = 200 μ m. b) BV/TV ratios for each zone are shown. c) Tartrate-resistant acid phosphatase 5b (TRAP5b)-positive osteoclasts per bone surface (Oc.S/BS) in each zone at four weeks are shown. d) Alkaline phosphatase (ALP)-positive osteoblasts per bone surface (Ob.S/BS) in each zone at four weeks are shown. Normality was assessed using the Shapiro-Wilk test. Statistical analyses for panel b were performed using the Mann-Whitney U test, while panels c and d were analyzed using paired *t*-tests. **p* < 0.05; ***p* < 0.01. NS, not significant; w, weeks.

injections according to the same schedule. All mice were euthanized after four weeks. Four joint groups were evaluated: Sham/PBS (*n* = 6), Sham/anti-RANKL antibody (*n* = 6), mCTA/PBS (*n* = 6), and mCTA/anti-RANKL antibody (*n* = 6).

Histological analysis

The glenohumeral joint and surrounding tissues were harvested and fixed in 10% neutral-buffered formalin for 24 hours at room temperature, followed by decalcification in K-CX AT solution (CS-6101; Falma, Japan) at 4°C for four days. Paraffin-embedded sections were prepared, and Alcian

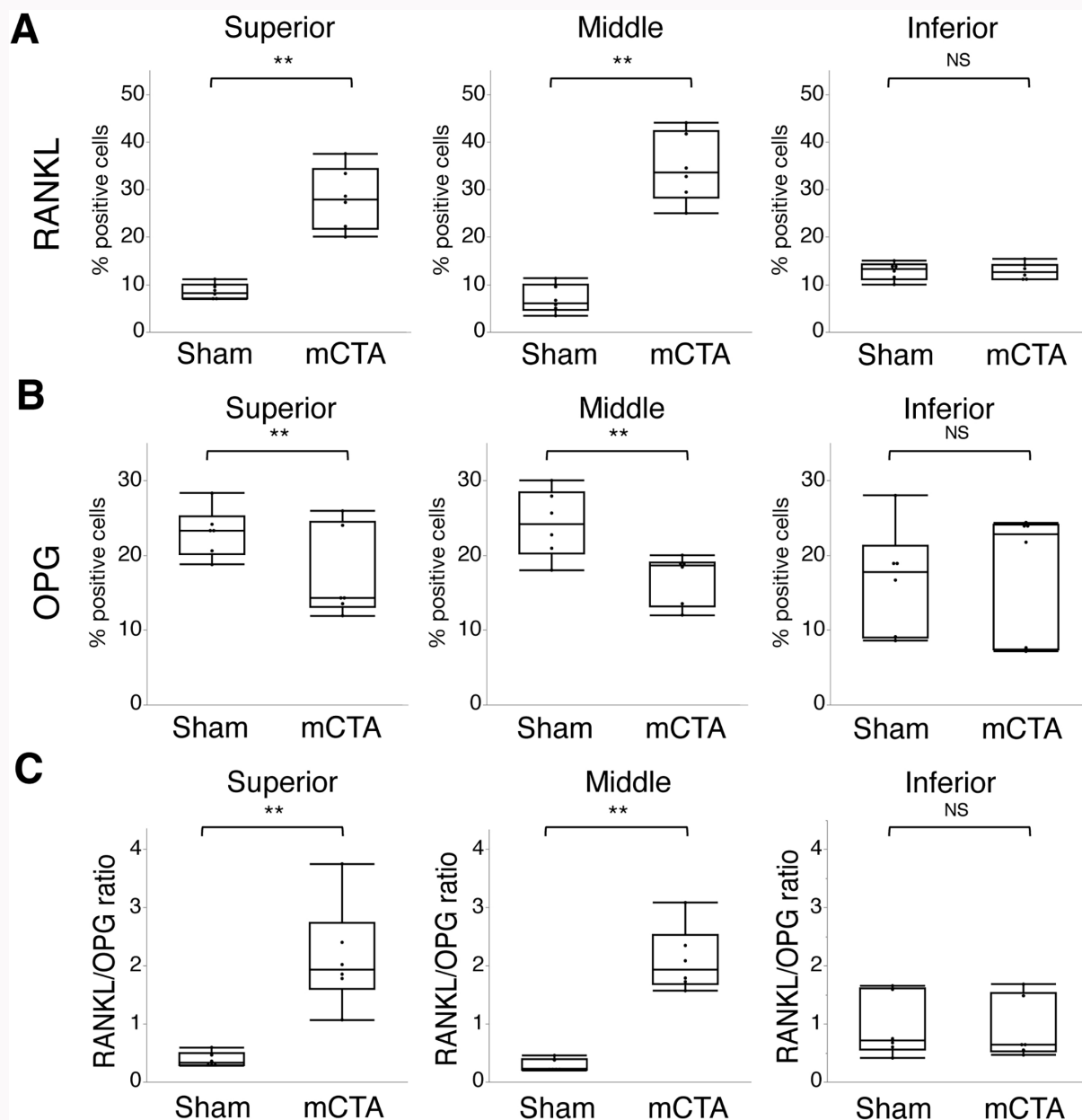


Fig. 3

Immunohistochemical analysis of receptor activator of nuclear factor κ B ligand (RANKL) and osteoprotegerin (OPG) expression in the subchondral bone of the modified cuff tear arthropathy (mCTA) humeral head at four weeks. Zone-specific a) percentages of RANKL-positive cells, b) percentages of OPG-positive cells, and c) the ratio of RANKL-positive to OPG-positive cell numbers are shown. Normality was assessed using the Shapiro-Wilk test. Statistical analyses were performed using paired *t*-tests. ***p* < 0.01. NS, not significant.

blue (pH 1.0) staining was performed to evaluate osteoarthritic changes using the Murine Shoulder Arthritis Score (MSAS),¹⁹ a scoring system specifically developed for murine shoulder OA. The synovial tissue analyzed was located over the superior to middle zones of the humeral head, corresponding to the subacromial bursa. The synovitis score was determined based on histological features observed in haematoxylin and eosin-stained sections as described.²⁰

Immunohistochemistry and immunofluorescence

Antigen retrieval was performed by incubating sections in either Liberate Antibody Binding (LAB) solution (24310; Polysciences, USA) or ethylenediaminetetraacetic acid buffer (pH 9.0) at 95°C for five minutes. For immunohistochemistry,

endogenous peroxidase activity was quenched with 3% hydrogen peroxide, and non-specific binding was blocked using CAS Block (8120; Thermo Fisher Scientific, USA). The primary and secondary antibodies used in this study are listed in the Supplementary Material. Signals were visualized with diaminobenzidine solution (415171; Nichirei Biosciences, Japan). The cell nuclei were counterstained with haematoxylin (131-09665; Wako, Japan). For immunofluorescence, cell nuclei were counterstained with Hoechst dye (1:1,000, H21492; Thermo Fisher Scientific). Images were captured using a BZ-X710/BZ-X700 microscope system (Keyence, Japan). For quantification of positive staining, the number of signal-positive cells was divided by the total number of cells. The middle zone of the articular cartilage, which exhibited the most severe

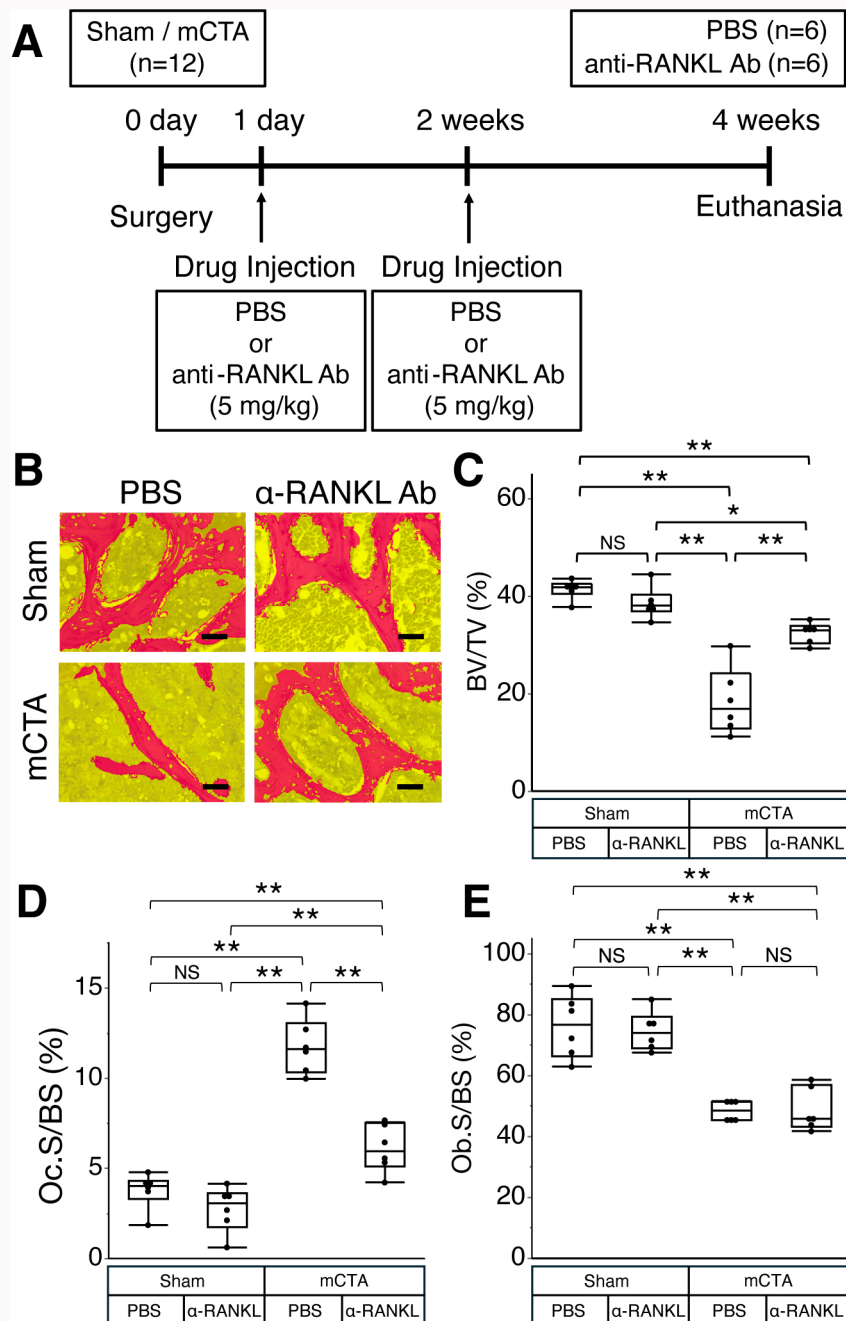


Fig. 4

Effects of anti-receptor activator of nuclear factor κ B ligand (RANKL) antibody (Ab) administration on subchondral bone in the mouse modified cuff tear arthropathy (mCTA) model at four weeks. a) Schematic of the subcutaneous anti-RANKL antibody injection protocol and the analysis timeline. b) Representative toluidine blue-stained images of subchondral bone in the middle zone. The bone volume (red)/tissue volume (yellow) (BV/TV) ratio was measured. Scale bar = 50 μ m. c) BV/TV ratio, d) tartrate-resistant acid phosphatase 5b (TRAP5b)-positive osteoclasts per bone surface (Oc.S/BS), and e) alkaline phosphatase (ALP)-positive osteoblasts per bone surface (Ob.S/BS) are shown. Normality was assessed using the Shapiro-Wilk test. For comparisons between sham and mCTA groups within the same treatment condition (PBS or anti-RANKL antibody), paired *t*-tests were performed. For other two-group comparisons, the Mann-Whitney U test was used for non-normally distributed data. **p* < 0.05; ***p* < 0.01. NS, not significant; PBS, phosphate-buffered saline.

degeneration, was evaluated. Positive cells were counted in three randomly selected fields per section and expressed as the number of positive cells per field. Cell counts were independently performed by two blinded observers (TI, YM) and the mean values were used for analysis.

Bone volume/tissue volume

The BV/TV ratio was quantified from toluidine blue-stained sections as previously described by Nagira et al.²¹ The humeral

head was imaged using a BZ-X710/BZ-X700 microscope system (Keyence). The subchondral trabecular bone area was defined using BZ-X Analyzer software (Keyence) and measured as the tissue volume (TV). The internal bone area was measured as the BV. The BV/TV ratio was then calculated using one section per sample.

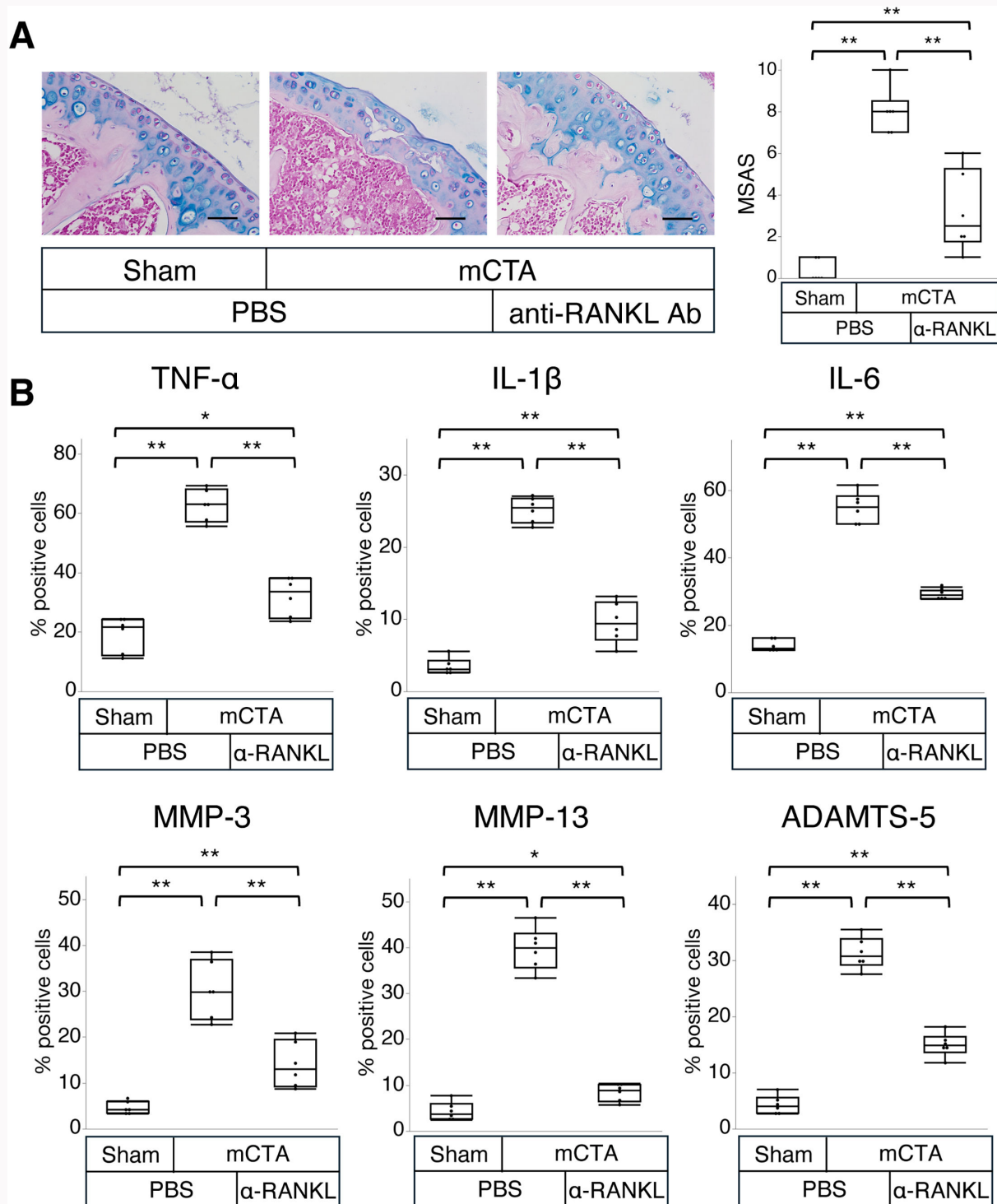


Fig. 5

Effects of anti-receptor activator of nuclear factor κ B ligand (RANKL) antibody (Ab) administration on articular cartilage in the mouse modified cuff tear arthropathy (mCTA) model at 4 weeks. a) Alcian blue-stained images of middle zone articular cartilage (left panel) and the corresponding Murine Shoulder Arthritis Score (MSAS) values (right panel). Scale bar = 50 μ m. b) Percentages of articular chondrocytes positive for inflammatory cytokines and catabolic enzymes, as determined by immunohistochemistry. Normality was assessed using the Shapiro-Wilk test. For comparisons between sham and mCTA groups in the PBS-treated mice, paired *t*-tests were performed. For other two-group comparisons, the Mann-Whitney U test was used for non-normally distributed data. **p* < 0.05; ***p* < 0.01. IL-1 β , interleukin-1 beta; IL-6, interleukin-6; MMP, matrix metalloproteinase; NS, not significant; PBS, phosphate-buffered saline; TNF- α , tumour necrosis factor alpha.

Statistical analysis

All statistical analyses were performed using JMP Pro software (v. 18.0.2; SAS Institute, USA). Because sham and mCTA groups comprised the same individuals within each treatment

condition, paired *t*-tests were used for these comparisons after confirming normality with the Shapiro-Wilk test. For other comparisons, data normality was assessed using the Shapiro-Wilk test; normally distributed data were analyzed

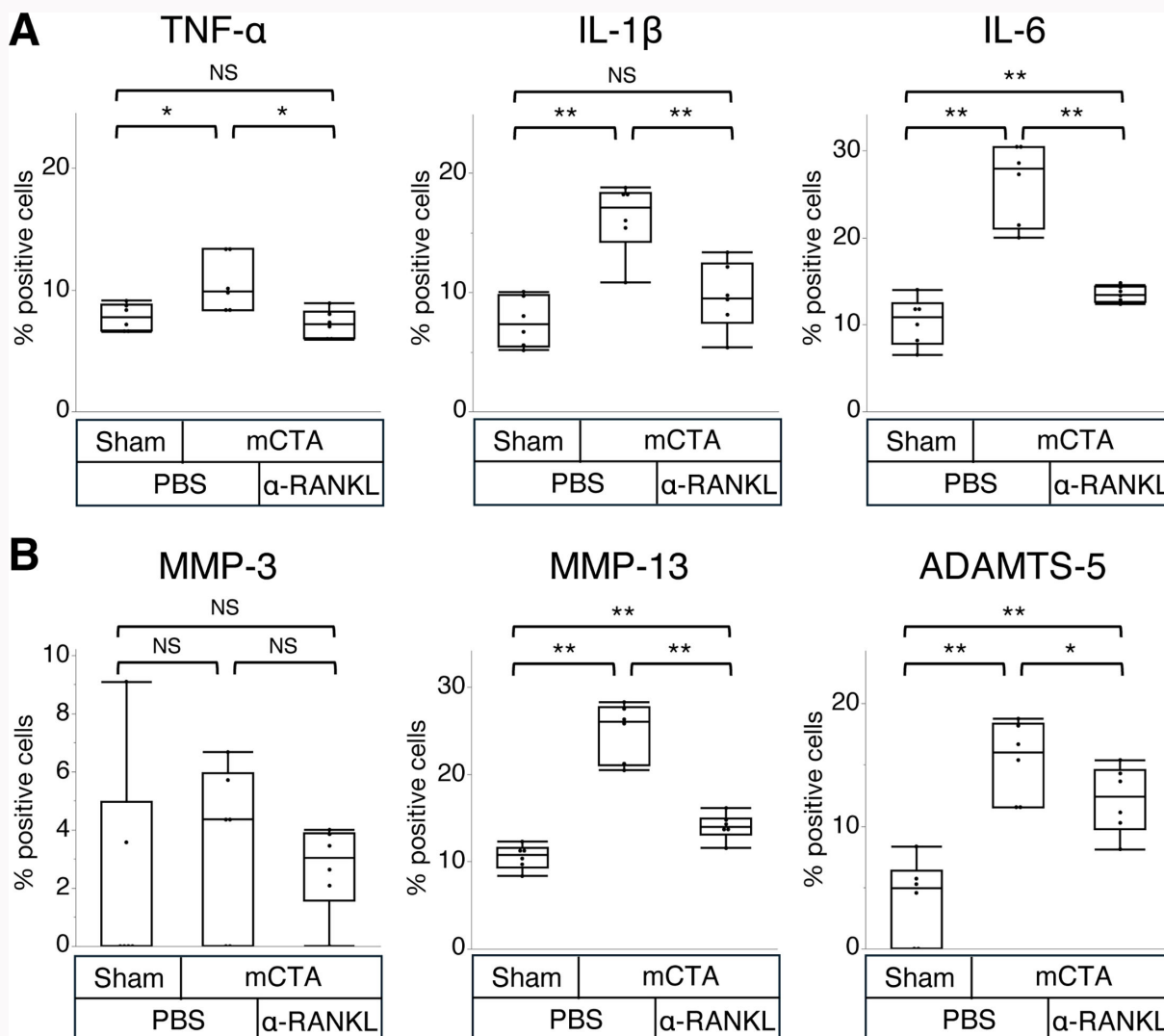


Fig. 6

Effects of anti-receptor activator of nuclear factor κ B ligand (RANKL) antibody administration on subchondral bone in the mouse modified cuff tear arthropathy (mCTA) model at four weeks. The percentages of cells expressing a) inflammatory cytokines and b) catabolic enzymes in subchondral bone were quantified by immunohistochemical staining. Normality was assessed using the Shapiro-Wilk test. For comparisons between sham and mCTA groups in the PBS-treated mice, paired *t*-tests were performed. For other two-group comparisons, the Mann-Whitney U test was used for non-normally distributed data. **p* < 0.05; ***p* < 0.01. IL-1 β , interleukin-1 beta; IL-6, interleukin-6; MMP, matrix metalloproteinase; NS, not significant; PBS, phosphate-buffered saline; TNF- α , tumour necrosis factor alpha.

using pooled *t*-tests, whereas non-normally distributed data were analyzed using the Mann-Whitney U test. Statistical significance was set at *p* < 0.05.

Results

Cartilage degeneration in the mouse version of the mCTA model

In establishing the mouse version of the mCTA model, no additional technical difficulties were encountered compared with the rat version (Figure 1a). In a rat mCTA model developed by Ijuin et al.¹⁵ the articular surface of the humeral head was divided into three zones (superior, middle, and inferior) according to the method described by Kramer et al.²² and the same zoning was applied in the mouse model (Figure 1b). When articular cartilage degeneration was quantitatively evaluated using the MSAS, no changes were observed at four weeks following rotator cuff resection alone (RCT; Figure 1c), consistent with previously reported mouse models and the rat

RCT model used in Ijuin et al.^{15,19} In contrast, in the superior and middle zones of the mCTA model, cartilage degeneration was apparent as early as one week postoperatively and further progressed by four weeks, which corresponds to approximately five years of ageing in human adulthood,²³ thereby reflecting a clinically relevant time course of disease progression,²⁴ whereas MSAS was not elevated in the inferior zone (Figure 1c).

Subchondral bone changes in the humeral head of the mouse mCTA model

The BV of the subchondral bone was analyzed using the same zonal classification (Figure 2a). In the inferior zone, the subchondral BV/TV ratio remained unchanged following either RCT or mCTA procedures (Figure 2b). In the RCT group, only a mild reduction was observed in the middle zone (Figure 2b). In contrast, in the mCTA group, BV/TV was markedly reduced in the superior and middle zones at one week

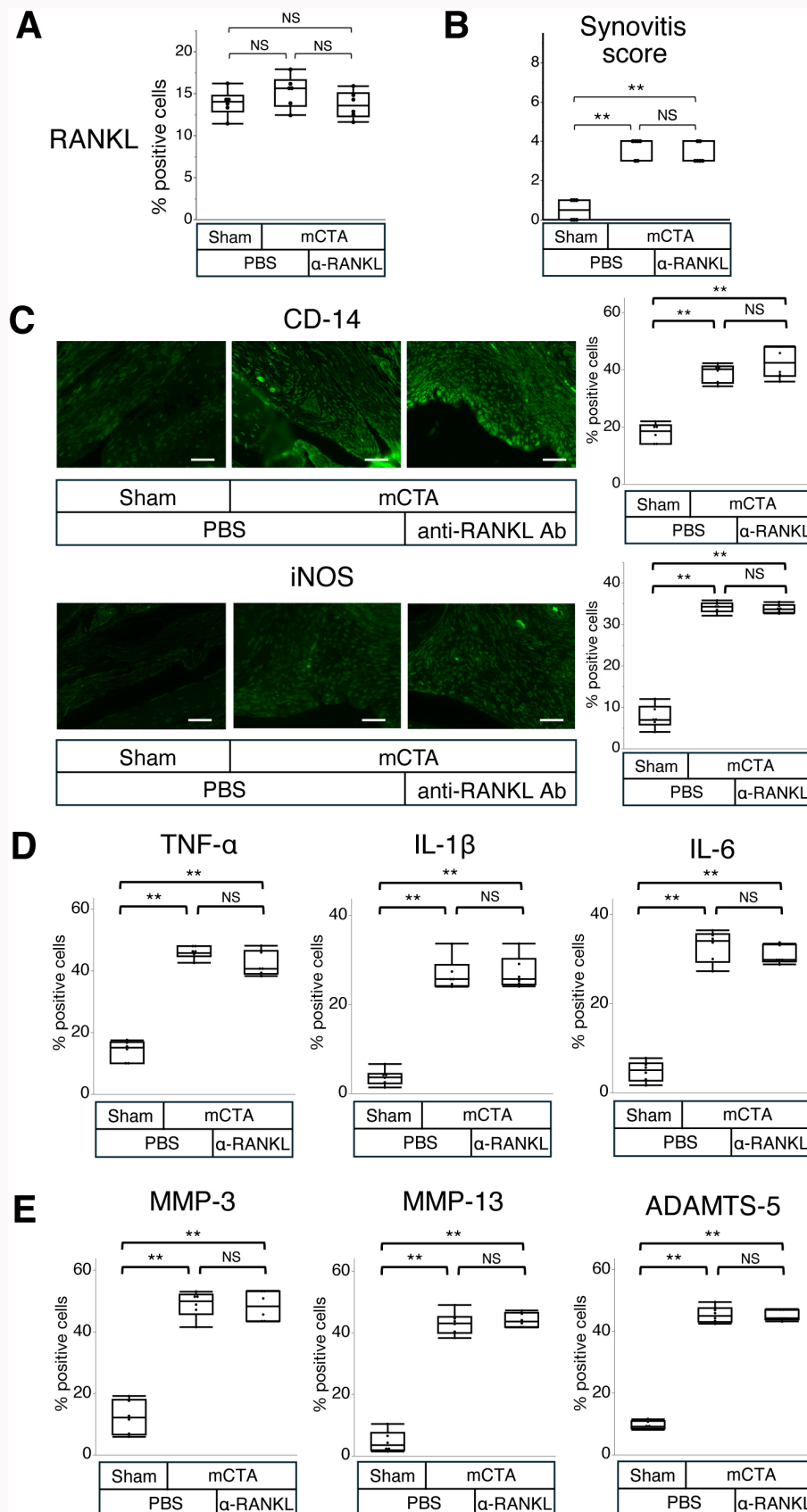


Fig. 7

Effects of anti-receptor activator of nuclear factor κ B ligand (RANKL) antibody administration on the synovium in the mouse modified cuff tear arthropathy (mCTA) model at four weeks. a) The percentage of cells expressing RANKL in the synovium was quantified by immunohistochemical staining. b) The synovitis score was determined based on histological features observed in haematoxylin and eosin (H&E)-stained sections. c) The number of M1 macrophages in the synovium was evaluated by the percentage of cells positive for the specific markers CD14 and inducible nitric oxide synthase (iNOS) using immunofluorescence (left panels) and quantified (right panels). Scale bar = 50 μ m. The percentages of cells positive for d) inflammatory cytokines and e) catabolic enzymes in the synovium were quantified by immunohistochemical staining. Normality was assessed using the Shapiro-Wilk test. For comparisons between sham and mCTA groups in the PBS-treated mice, paired *t*-tests were performed. For other two-group comparisons, the Mann-Whitney U test was used for non-normally distributed data. **p* < 0.05; ***p* < 0.01. IL-1 β , interleukin-1 beta; IL-6, interleukin-6; MMP, matrix metalloproteinase; NS, not significant; PBS, phosphate-buffered saline; TNF- α , tumour necrosis factor alpha.

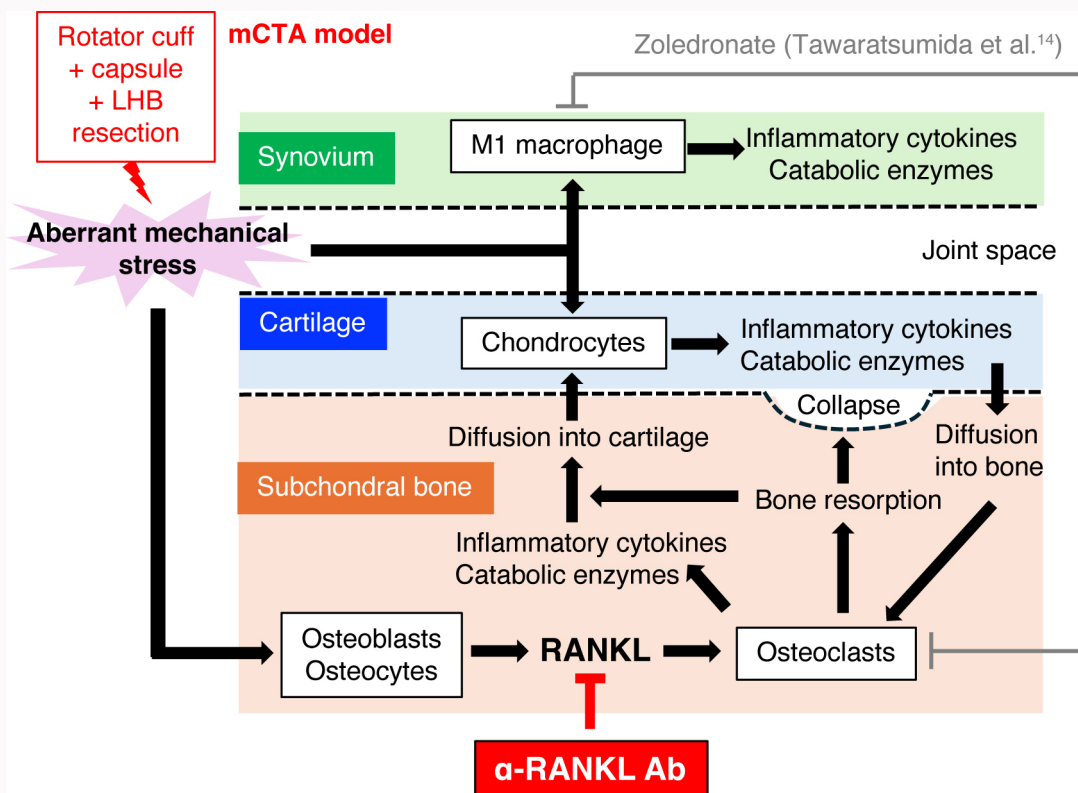


Fig. 8

Schematic of degenerative mechanisms in the mouse modified cuff tear arthropathy (mCTA) model. mCTA surgery induces abnormal mechanical stress in the humeral head, increasing receptor activator of nuclear factor κ B ligand (RANKL) expression in osteoblasts and osteocytes and activating osteoclasts. This leads to subchondral bone resorption, bone loss, and collapse. Activated osteoclasts release inflammatory cytokines and catabolic enzymes,¹⁴ promoting cartilage degeneration. While zoledronate suppresses both osteoclasts and synovial M1 macrophages in the rat mCTA model, the anti-RANKL antibody (α -RANKL Ab) inhibits osteoclasts, protecting subchondral bone and cartilage without affecting synovial inflammation in the present mouse mCTA model. α -RANKL Ab, anti-RANKL antibody; LHB, long head of the biceps tendon.

postoperatively, and this decrease was maintained at four weeks without further reduction (Figure 2b).

Immunohistochemical staining was performed to assess tartrate-resistant acid phosphatase 5b (TRAP5b)-positive osteoclast surface and alkaline phosphatase (ALP)-positive osteoblast surface (Supplementary Figure b). In the superior and middle zones at four weeks after mCTA surgery, osteoclast surface in the subchondral bone was increased (Figure 2c), whereas osteoblast surface was decreased (Figure 2d).

RANKL expression was increased in the subchondral bone of the mouse mCTA model

As a basis for administering anti-RANKL in the mCTA model, we determined whether RANKL expression was increased in the subchondral bone of the mouse mCTA model by immunohistochemical staining (Supplementary Figure c). No change was observed in the inferior zone; however, in the superior and middle zones, a marked increase in RANKL expression was observed at four weeks (Figure 3a). Expression of osteoprotegerin (OPG), an antagonist of RANKL produced by osteoblasts,¹⁶ was decreased in the superior and middle zones (Figure 3b, Supplementary Figure c). Consequently, the RANKL/OPG ratio, an indicator of osteoclastogenic activation, remained unchanged in the inferior zone, whereas it was increased in the superior and middle zones (Figure 3c).

Administration of an anti-RANKL antibody mitigates subchondral bone loss in the mouse mCTA model

The effect of administering an anti-RANKL neutralizing antibody was evaluated at four weeks (Figure 4a). The analysis was focused on the middle zone, where the most pronounced changes were observed in this mouse mCTA model. Bone histomorphometry was performed to measure BV/TV (Figure 4b), and the results indicate that administration of anti-RANKL antibody prevented or reversed the bone loss induced by mCTA surgery (Figure 4c). The anti-RANKL antibody showed no effect in the inferior zone of subchondral bone, where osteoclasts were not increased after mCTA, but increased bone volume in the metaphyseal bone, where osteoclasts were abundant, confirming its biological efficacy (Supplementary Figure d). TRAP5b-positive osteoclast surface in the anti-RANKL antibody-treated group after mCTA was reduced (Figure 4d, Supplementary Figure e). In contrast, ALP-positive osteoblast surface remained unchanged after anti-RANKL antibody administration (Figure 4e, Supplementary Figure e).

Anti-RANKL antibody treatment attenuates cartilage degeneration in the mouse mCTA model

The potential effect of anti-RANKL antibody on articular cartilage degeneration was evaluated using the MSAS and immunohistochemical analysis of inflammatory cytokines and catabolic enzymes. Although the MSAS values did not fully return to sham levels, a marked improvement

was observed (Figure 5a). The expression of inflammatory cytokines, including tumour necrosis factor alpha (TNF- α), interleukin-1 beta (IL-1 β), and interleukin-6 (IL-6), as well as catabolic enzymes, including MMP-3, MMP-13, and ADAMTS-5, was reduced in the anti-RANKL antibody-treated group (Supplementary Figure f, Figure 5b), in parallel with the MSAS findings.

Inhibitory effect of the anti-RANKL antibody on the upregulation of inflammatory cytokines and catabolic enzymes in the subchondral bone of the mCTA model

Similar to articular cartilage, the expression of TNF- α , IL-1 β , IL-6 (Figure 6a), and MMP-13 (Figure 6b) was strongly suppressed by the anti-RANKL neutralizing antibody in the subchondral bone (Supplementary Figure g). However, the reduction in ADAMTS-5 expression was only mild (Figure 6b). No increase in MMP-3 was observed in this mouse mCTA model, and the anti-RANKL antibody had no effect on its expression (Figure 6b).

Effect of anti-RANKL antibody treatment on synovial changes in the mCTA model

As the effect of the anti-RANKL antibody on the synovium depends on RANKL expression levels, this was assessed by immunohistochemistry. In sham synovium, the proportion of RANKL-positive cells was low (~15%) and remained unchanged after mCTA and following anti-RANKL antibody treatment (Figure 7a, Supplementary Figure h). Consistent with this, the mCTA-induced increase in synovitis score was not affected by anti-RANKL antibody treatment (Figure 7b). The number of M1 macrophages, which was reported to increase in the synovium of knee OA models and the rat mCTA model,¹⁴ was assessed by counting CD14- and inducible nitric oxide synthase (iNOS)-positive immunofluorescent cells (Figure 7c). M1 macrophages were significantly increased in the synovium of the mouse mCTA model, and this increase was not affected by anti-RANKL antibody treatment (Figure 7c). The inflammatory cytokines, including TNF- α , IL-1 β , and IL-6, and catabolic enzymes, including MMP-3, MMP-13, and ADAMTS-5, were also increased in the synovium of the mouse mCTA model; however, the anti-RANKL antibody had no effect on the expression of these factors (Figures 7d and 7e, Supplementary Figure h).

Discussion

Based on our present findings, a working model of the degenerative mechanisms underlying the mCTA model is presented in Figure 8. Following mCTA surgery, the humeral head is subjected to abnormal mechanical stress. In the subchondral bone, this aberrant mechanical environment enhances RANKL secretion by osteoblasts and osteocytes, thereby promoting osteoclast differentiation and activation. As a consequence, subchondral bone resorption becomes predominant, leading to a reduction in bone mass and joint surface collapse. In parallel, activated osteoclasts release inflammatory cytokines and catabolic enzymes,⁴ which infiltrate the articular cartilage through breaches in the disrupted subchondral plate and induce cartilage inflammation and degeneration. Concurrently, abnormal mechanical stress stimulates chondrocytes, synoviocytes, and macrophages adjacent to the joint space. This enhances the

production of inflammatory cytokines and catabolic enzymes, exacerbating inflammation and degeneration within both the cartilage and synovium. A recent study has reported that, in murine knee OA models, fibroblast-like synoviocytes express RANKL and exacerbate synovitis and OA via the RANK/TRAF6/FSTL1 pathway, effects that are attenuated by anti-RANKL antibody treatment.²⁰ In contrast, we did not observe increased RANKL expression in the synovium of our mCTA model. In the rat mCTA model, zoledronate (a bisphosphonate) suppresses both synovial M1 macrophages and subchondral osteoclasts,¹⁴ whereas the anti-RANKL antibody treatment in the present mouse mCTA model inhibited osteoclasts and achieved comparable protection against mCTA-induced changes in the articular cartilage and subchondral bone. This protection was observed without suppression of synovial inflammation, likely due to unchanged RANKL expression in the mCTA synovium. Accordingly, the apparent 'selectivity' of anti-RANKL antibody observed in this study should be interpreted within the specific pathological context of the mouse mCTA model, rather than as a universal property of RANKL inhibition. Previous studies of OA have predominantly focused on the knee joint, where synovial inflammation and the release of cytokines into the synovial fluid have been implicated in both the initiation and progression of OA.²⁵ However, our results suggest that, in the shoulder, the contribution of synovial inflammation to articular cartilage degeneration may be limited. This notion is supported by the clinical observation that, even after episodes of severe synovitis, such as that seen in frozen shoulder, degenerative OA changes in the articular cartilage rarely develop. Interestingly, Kramer et al²² developed a suprascapular nerve denervation model without tenotomy and showed that it induced CTA-like changes comparable to those of a conventional tenotomy model. Although synovitis is unlikely to be directly induced in the denervation model, the occurrence of CTA-like changes suggests that synovitis may play only a minor role in the pathogenesis of CTA. In addition, although the synovitis score was increased in the mouse mCTA model (Figure 7b), the magnitude of this increase was modest (score < 4) compared with that reported in the mouse knee OA model (~5),²⁰ indicating a relatively mild degree of synovial inflammation. This may be related to the absence of increased RANKL expression.

Unlike knee OA, in which the subchondral bone typically becomes sclerotic during the late phase,⁴ CTA cases appear to maintain reduced bone volume even in the progressive stage.¹⁴ Therefore, in addition to bisphosphonates,¹⁴ anti-RANKL neutralizing antibodies such as denosumab targeting subchondral osteoclasts may hold promise as disease-modifying agents for CTA. However, the anti-RANKL antibody did not ameliorate synovial inflammation, suggesting that synovium-derived pain and the resulting restriction of joint motion would likely persist despite preventing cartilage and bony deformity. Thus, even if denosumab prevents CTA-related deformation clinically, patient satisfaction may be limited. In this context, bisphosphonates, which can suppress both subchondral bone loss and synovial inflammation, may offer a therapeutic advantage over denosumab as disease-modifying agents for CTA. However, long-term systemic administration of anti-RANKL antibody or bisphosphonates may suppress osteoclast activity not only at the target

site but also in healthy bone, raising concerns about potential adverse effects such as osteopetrosis-like changes and atypical fractures, particularly in patients without osteoporosis. Therefore, the use of antiresorptive agents for the prevention or treatment of CTA should be limited to patients with concomitant osteoporosis, in whom the benefits of antiresorptive therapy may outweigh the potential risks.

This study has several limitations. First, mice are quadrupedal animals, and their shoulder joints are not completely unloaded, do not perform overhead movements like humans, and have a relatively limited range of motion. However, among commonly used experimental animals, murine species are reported to possess shoulder joint structures whose similarity to those of humans is second only to non-human primates. Experiments using non-human primates are challenging because of ethical considerations, limited availability, and high costs. A second limitation is that the degrees of collapse and MSAS changes in this mouse model were milder than those observed in the rat mCTA model,^{14,15} raising the question of whether it fully reproduces the clinical phenotype of CTA and is thus a valid model for the arthropathy. Nevertheless, this mouse version of the mCTA model reproduces key CTA phenotypes, including articular cartilage degeneration, subchondral bone loss, and joint surface collapse, within a four-week period, corresponding to approximately five years of human adulthood.²³ This clinically relevant time course offers greater reproducibility compared with existing mouse CTA models,¹⁹ in which a significant increase in MSAS requires up to 45 weeks. In addition, the rat mCTA model is known to exhibit pronounced joint surface collapse that may exceed what is typically observed clinically,^{14,15} suggesting that it may represent a somewhat exaggerated phenotype. In this regard, the mouse mCTA model may more closely reflect the clinical condition. We believe that the lack of effect of anti-RANKL antibody on synovial inflammation in the mouse model is unlikely to be due to a milder phenotype, but rather may be attributable to the absence of increased RANKL expression in the synovium. Third, the effects of zoledronate have not been evaluated in the present mouse mCTA model, and therefore direct comparison within the same species remains an important subject for future investigation. Finally, in this study, the anti-RANKL antibody was administered starting the day after mCTA surgery, and follow-up was limited to four weeks. Therefore, the long-term effects and efficacy of anti-RANKL treatment in already progressed, late-phase CTA remain uninvestigated, and represent important questions for future studies.

In conclusion, we established a mouse mCTA model and demonstrated that administration of an anti-RANKL antibody suppresses joint degeneration. This effect appears to result from inhibition of osteoclasts without affecting synovial inflammation, suggesting that synovial inflammation has a limited direct impact on CTA progression. By targeting subchondral osteoclasts, the fragility of the subchondral bone and articular cartilage degeneration can both be mitigated. These findings imply that denosumab, which is widely used in the treatment of osteoporosis, may function as a disease-modifying agent capable of preventing the onset or slowing the progression of shoulder OA.

Supplementary material

Sample size determination, information on antibodies used in this study, and representative immunohistochemistry images corresponding to the quantified data; the ARRIVE checklist is also included.

References

1. Liang W, Feng R, Li X, et al. A RANKL-UCHL1-sCD13 negative feedback loop limits osteoclastogenesis in subchondral bone to prevent osteoarthritis progression. *Nat Commun*. 2024;15(1):8792.
2. Funck-Brentano T, Cohen-Solal M. Crosstalk between cartilage and bone: when bone cytokines matter. *Cytokine Growth Factor Rev*. 2011; 22(2):91–97.
3. Hu Y, Chen X, Wang S, Jing Y, Su J. Subchondral bone microenvironment in osteoarthritis and pain. *Bone Res*. 2021;9(1):20.
4. Hu W, Chen Y, Dou C, Dong S. Microenvironment in subchondral bone: predominant regulator for the treatment of osteoarthritis. *Ann Rheum Dis*. 2021;80(4):413–422.
5. Chen W, Wang Q, Tao H, et al. Subchondral osteoclasts and osteoarthritis: new insights and potential therapeutic avenues. *Acta Biochim Biophys Sin (Shanghai)*. 2024;56(4):499–512.
6. Xing RL, Zhao LR, Wang PM. Bisphosphonates therapy for osteoarthritis: a meta-analysis of randomized controlled trials. *Springerplus*. 2016; 5(1):1704.
7. Vaysbrot EE, Osani MC, Musetti MC, McAlindon TE, Bannuru RR. Are bisphosphonates efficacious in knee osteoarthritis? A meta-analysis of randomized controlled trials. *Osteoarthritis Cartilage*. 2018;26(2):154–164.
8. Fernández-Martín S, López-Peña M, Muñoz F, Permuy M, González-Cantalapiedra A. Bisphosphonates as disease-modifying drugs in osteoarthritis preclinical studies: a systematic review from 2000 to 2020. *Arthritis Res Ther*. 2021;23(1):60.
9. Toegel S, Martelanz L, Alphonsus J, et al. The degenerated glenohumeral joint. *Bone Joint Res*. 2024;13(10):596–610.
10. Martínez-Catalan N, Gabardo-Calvo S, Valencia M, et al. Factors associated with failed conservative treatment in patients with degenerative full-thickness supraspinatus tears: a prospective cohort study. *Bone Joint J*. 2025;107-B(12):1295–1300.
11. Baek CH, Kim JG, Kim BT. Long-term outcomes of anterior latissimus dorsi tendon transfer for irreparable subscapularis tears. *Bone Joint J*. 2024;106-B(9):957–963.
12. Baek CH, Kim JG, Kim BT, Lim C, Kim SJ. Mid-term outcomes of combined anterior latissimus dorsi and teres major tendon transfer for irreparable anterosuperior rotator cuff tears: a minimum five-year follow-up study. *Bone Joint J*. 2025;107-B(12):1309–1316.
13. Neer CS, Craig EV, Fukuda H. Cuff-tear arthropathy. *J Bone Joint Surg Am*. 1983;65-A(9):1232–1244.
14. Tawaratsumida H, Iuchi T, Masuda Y, et al. Zoledronate alleviates subchondral bone collapse and articular cartilage degeneration in a rat model of rotator cuff tear arthropathy. *Osteoarthritis Cartilage*. 2025;33(1): 101–115.
15. Ijuin T, Iuchi T, Tawaratsumida H, et al. Development of a novel animal model of rotator cuff tear arthropathy replicating clinical features of progressive osteoarthritis with subchondral bone collapse. *Osteoarthritis Cartil Open*. 2023;5(3):100389.
16. Yasuda H. Discovery of the RANKL/RANK/OPG system. *J Bone Miner Metab*. 2021;39(1):2–11.
17. Xiong J, Onal M, Jilka RL, Weinstein RS, Manolagas SC, O'Brien CA. Matrix-embedded cells control osteoclast formation. *Nat Med*. 2011; 17(10):1235–1241.
18. Furuya Y, Mori K, Ninomiya T, et al. Increased bone mass in mice after single injection of anti-receptor activator of nuclear factor-kappaB ligand-neutralizing antibody: evidence for bone anabolic effect of parathyroid hormone in mice with few osteoclasts. *J Biol Chem*. 2011; 286(42):37023–37031.
19. Zingman A, Li H, Sundem L, et al. Shoulder arthritis secondary to rotator cuff tear: a reproducible murine model and histopathologic scoring system. *J Orthop Res*. 2017;35(3):506–514.

20. Hu Y, Chen W, Lan S, et al. Denosumab attenuates knee osteoarthritis progression by inhibiting synovial inflammation via the RANK/TRAF6/FSTL1 signalling. *Nat Commun.* 2025;16(1):11394.
21. Nagira K, Ikuta Y, Shinohara M, et al. Histological scoring system for subchondral bone changes in murine models of joint aging and osteoarthritis. *Sci Rep.* 2020;10(1):10077.
22. Kramer EJ, Bodendorfer BM, Laron D, et al. Evaluation of cartilage degeneration in a rat model of rotator cuff tear arthropathy. *J Shoulder Elbow Surg.* 2013;22(12):1702–1709.
23. Dutta S, Sengupta P. Men and mice: relating their ages. *Life Sci.* 2016;152:244–248.
24. Chalmers PN, Salazar DH, Steger-May K, et al. Radiographic progression of arthritic changes in shoulders with degenerative rotator cuff tears. *J Shoulder Elbow Surg.* 2016;25(11):1749–1755.
25. De Roover A, Escribano-Núñez A, Monteagudo S, Lories R. Fundamentals of osteoarthritis: inflammatory mediators in osteoarthritis. *Osteoarthritis Cartilage.* 2023;31(10):1303–1311.

Author information

T. Ide, MD, Orthopaedic Surgeon, Department of Locomotory Organ Regeneration, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan; Department of Orthopaedic Surgery, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan.

Y. Masuda, MD, Orthopaedic Surgeon

K. Shimanoe, MD, Orthopaedic Surgeon

S. Takada, PT, PhD, Physical Therapist

K. Uekama, MD, Orthopaedic Surgeon

T. Iuchi, MD, Orthopaedic Surgeon

H. Tawaratsumida, MD, PhD, Orthopaedic Surgeon

T. Ijuin, MD, PhD, Orthopaedic Surgeon, Assistant Professor

H. Tominaga, MD, PhD, Orthopaedic Surgeon, Associate Professor
Department of Orthopaedic Surgery, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan.

S. Saho, MD, Orthopaedic Surgeon, Department of Orthopaedic Surgery, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan; Department of Medical Joint Materials, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan.

S. Maeda, MD, PhD, Specially Appointed Professor, Department of Bone and Joint Medicine, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan.

N. Taniguchi, MD, PhD, Orthopaedic Surgeon, Professor, Department of Locomotory Organ Regeneration, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan; Department of Orthopaedic Surgery, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan; Department of Medical Joint Materials, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan; Department of Bone and Joint Medicine, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan.

Author contributions

T. Ide: Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing.

Y. Masuda: Conceptualization, Data curation, Investigation, Writing – original draft, Writing – review & editing.

K. Shimanoe: Investigation, Validation, Writing – review & editing.

S. Saho: Investigation, Validation, Writing – review & editing.

S. Takada: Investigation, Validation, Writing – review & editing.

K. Uekama: Investigation, Validation, Writing – review & editing.

T. Iuchi: Investigation, Validation, Writing – review & editing.

H. Tawaratsumida: Resources, Writing – review & editing.

T. Ijuin: Resources, Writing – review & editing.

H. Tominaga: Funding acquisition, Supervision, Writing – review & editing.

S. Maeda: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing.

N. Taniguchi: Funding acquisition, Project administration, Supervision, Writing – review & editing.

T. Ide and Y. Masuda contributed equally to this work.

Funding statement

The authors disclose receipt of the following financial or material support for the research, authorship, and/or publication of this article: funding from the Japan Society for the Promotion of Science Grant-in-Aid for Scientific Research (JSPS KAKENHI) funding category (C) (grant no. 22K09309, 24K12333, and 25K12462), as reported by S. Maeda and N. Taniguchi; and (grant no. 22K09336), as reported by H. Tominaga, S. Maeda, and N. Taniguchi.

ICMJE COI statement

The authors declare that they have no conflicts of interest related to this work. S. Maeda and N. Taniguchi report funding from Japan Society for the Promotion of Science Grant-in-Aid for Scientific Research (JSPS KAKENHI) (grant numbers 22K09309, 24K12333, and 25K12462). H. Tominaga, S. Maeda, and N. Taniguchi also report funding from JSPS KAKENHI (grant number 22K09336). All grants were related to this study.

Data sharing

The datasets generated and analyzed in the current study are not publicly available due to data protection regulations. Access to data is limited to the researchers who have obtained permission for data processing. Further inquiries can be made to the corresponding author.

Acknowledgements

The authors would like to thank Hui Gao and Miki Sakoda for providing technical assistance. ChatGPT (OpenAI, USA) was used only for translation from Japanese to English. All content was reviewed and verified by the authors and the text was further edited by a native English speaker. We thank Matthew Grimshaw, PhD, from Edanz (<https://jp.edanz.com/ac>) for editing a draft of this manuscript.

Ethical review statement

All animal experimental procedures were approved by the Animal Experiment Committee of Kagoshima University (MD24054) and conducted according to institutional guidelines.

Open access funding

The authors report that they received open access funding for their manuscript from Kagoshima University.

© 2026 Ide et al. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (CC BY-NC-ND 4.0) licence, which permits the copying and redistribution of the work only, and provided the original author and source are credited. See <https://creativecommons.org/licenses/by-nc-nd/4.0/>