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A CCL2/MCP-1 antagonist attenuates fibrosis of the infrapatellar fat pad in a rat model of arthritis

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Abstract

Background Fibrosis of the infrapatellar fat pad (IFP) is a feature of osteoarthritis and contributes substantially to the pain and dysfunction in patients' joints. However, the underlying mechanisms remain unclear. C-C motif chemokine ligand-2 (CCL2) plays a central role in tissue fibrosis. Thus, we aimed to investigate the role of CCL2 in the development of IFP fibrosis in a rat model of arthritis, hypothesizing that a CCL2 antagonist could mitigate fibrotic progression.

Methods We induced arthritis in male Wistar rats using intra-articular injections of carrageenan. Furthermore, to evaluate the effects of a CCL2 antagonist on protein expression and collagen deposition in the IFP of the rats, we transferred an N-terminal-truncated *CCL2* gene into a rat model via electroporation-mediated intramuscular injection. Macrophage infiltration and collagen deposition in the IFP were analyzed in vivo. Groups were compared using the Mann–Whitney U test and Student's *t*-test.

Results We identified infiltrating macrophages as well as increases in CCL2 and TGF-β levels as collagen deposition progressed. Gene transfer of the CCL2-antagonist before arthritis induction attenuated collagen deposition remarkably.

Conclusions We provide initial evidence that anti-CCL2 gene therapy can effectively suppress the development of IFP fibrosis in a rat model. Thus, targeting CCL2 holds promise as a therapeutic strategy for managing tissue fibrosis in osteoarthritis patients.

Keywords Fibrosis, Infrapatellar fat pad, Osteoarthritis, CCL2, Macrophages, Gene therapy

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Background

Fibrosis of the infrapatellar fat pad (IFP) significantly contributes to chronic pain and decreased knee mobility, particularly in patients recovering from knee surgery or those suffering from knee osteoarthritis (KOA) [1, 2]. It is marked by an excessive deposition of extracellular matrix components, which can lead to joint stiffness and impaired mobility. This pathological process is often accompanied by an infiltration of immune cells, particularly macrophages, which are pivotal in mediating inflammatory responses [3–6]. Macrophages play a significant role in tissue fibrosis [7]. Macrophages not only produce several pro-inflammatory cytokines and growth factors but also secrete chemokines such as CCL2 (C-C motif chemokine ligand 2) / Monocyte Chemoattractant Protein-1(MCP-1), which further recruit additional immune cells, thereby exacerbating the inflammatory and fibrotic responses. The chemokine CCL2 is a critical mediator of monocyte and macrophage chemotaxis and activation. Elevated levels of CCL2 have been implicated in various inflammatory conditions, including OA [8, 9]. Previous studies have demonstrated that CCL2 is upregulated in the synovial membrane and infrapatellar fat pads in OA [10]. There is also evidence implicating CCL2 in fibrotic changes of the synovial membrane [11]. This study aimed to investigate the involvement of CCL2 in the pathogenesis of *IFP fibrosis*in vivo. A rodent model of arthritis induced by intraarticular carrageenan injections was utilized to assess macrophage infiltration and CCL2 expression during the transition from acute synovial injury to extracellular matrix formation. Furthermore, we confirmed the functional role of CCL2 in the development of IFP fibrosis using anti-CCL2 gene therapy. We administered the *7ND* gene, which lacks the N-terminal amino acids of human *CCL2* and results in a loss of CCL2 function [12], to a rodent model using in vivo electroporation. We hypothesized that the administration of a CCL2 antagonist could alleviate the development of IFP fibrosis induced in a rat arthritis model.

Methods

Animal studies

This study was conducted following a protocol approved by the Animal Care and Use Committee of Tokyo Medical and Dental University. A total of 123 8-week-old male Wistar rats were purchased from Charles River (Tokyo, Japan). To minimize size and genetic variation between subjects, only male rats were used. The animals were housed under standard conditions with a 12-hour light/dark cycle and a constant temperature of 23 °C, with unrestricted access to food and water. They were acclimatized to the laboratory environment for 1 week prior to the experiments. All rats were randomly allocated to each experimental group. All procedures were performed under general anesthesia by inhalation of 2% isoflurane administered via a nasal cone, and every effort was made to minimize suffering. Rats were humanely euthanized by carbon dioxide inhalation and limbs were harvested for tissue collection. All procedures complied with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Bethesda, MD, USA) and were performed in accordance with ARRIVE guidelines.

Induction of IFP fibrosis

Male Wistar rats received intra-articular injections of 100 µL of normal saline into the right knee and 2% carrageenan (Sigma-Aldrich, St. Louis, MO, USA) into the left knee, as described previously [13]. The day of carrageenan injection was designated as day 0 for all time points. A minimum of five rats were used for each time point or manipulation.

Tissue collection and preparation

Rats were euthanized at 4, 7, 14, and 28 days after the intraarticular injection of carrageenan, and both knees were immediately harvested. The whole knee joints were fixed in 4% paraformaldehyde, decalcified in a solution of 10% formalin and 10% EDTA, dehydrated in a graded ethanol series, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin (HE) as described previously [14–16]. Infrapatellar fat pad sections were examined for synovial hypercellularity, interstitial infiltration of mononuclear cells, and fibrosis. The severity of synovial inflammation from a section stained with HE was semiquantitatively evaluated using the synovial membrane inflammation score, which was scored on a 1– 4 scale, based on the osteoarthritis research society international histopathology recommendations for the rat [17].

Immunohistochemistry

Anti-ED-1 antibodies (1:100 dilution), which specifically targets the CD68 antigen present on the surface of rat macrophages, were purchased from Serotec (Oxford, UK). Sections of the knee joint were incubated with biotin-labeled anti-rat ED-1, and protein expression was detected using the avidin-biotin-peroxidase complex (Dako, Carpinteria, CA, USA) as described previously [14, 15]. The number of ED1-positive cells was counted in 20 randomly chosen high-power fields (×200) in each section, and the average cell number/mm^2 was calculated.

Masson's trichrome staining

The deposition of collagen proteins in IFP was assessed using Masson's trichrome staining. Staining was performed using a Masson's Trichrome Staining Kit (Muto

Pure Chemicals, Tokyo, Japan) according to the manufacturer's protocol.

Preparation of IPF tissue homogenates

Each rat underwent a joint incision above the patella to harvest the IFP tissue. The homogenates were prepared by mechanical homogenization after adding fresh tissue to 1 mL of T-PER tissue protein extraction reagent (Pierce, Rockford, IL, USA) containing 10 µL of Protease Inhibitor Cocktail (Sigma-Aldrich). Samples were incubated at 4 °C for 1 h and subsequently centrifuged at 10,000 × *g* for 5 min. The supernatant was removed, and the total protein concentrations were determined using the QuantiPro BCA Assay Kit (Sigma-Aldrich). The aliquots were frozen at −80 °C until use.

Cytokine enzyme-linked immunosorbent assay (ELISA) for tissue homogenates

Cytokine levels in the IFP tissue homogenates were determined using commercially available ELISA kits specific for rat CCL2/MCP-1 (BioSource, Carlsbad, CA, USA) and TGF-β1 (R&D Systems, Minneapolis, MN, USA), following the manufacturer's instructions.

In vivo collagen assay

The in vivo collagen assay was performed using the Sircol collagen assay method (Biocolor Ltd., Carrickfergus, UK). IFP specimens were harvested and homogenized. Then, 1000 µL of Sircol dye reagent was added to 100 µL of a test sample and incubated at 25 °C for 30 min. After centrifugation, the supernatants were discarded, and 0.5 N NaOH was added to the collagen-bound dye pellet to release the dye into the solution. The optical density of each sample was measured using a microplate reader (InfiniteF50-R; Tecan, Mannedorf, Switzerland).

Preparation of the 7ND gene and plasmid vectors

An N-terminal deletion mutant of human *CCL2*, termed 7ND, which blocks the CCL2 signaling pathway [12], was utilized. The pCAGGS-7ND plasmid was constructed by inserting human 7ND cDNA into a unique *Eco*RI site in the pCAGGS expression vector [18]. An empty pCAGGS plasmid served as a control. Plasmids were grown in *Escherichia coli* XL-1 blue strain cells (Takara Bio Inc., Shiga, Japan) and isolated using a Mega kit (Qiagen, Tokyo, Japan). Plasmid quantity and quality were evaluated by measuring optical density values at 260 and 280 nm. Prior to injection, the DNA was diluted to a concentration of 2.0 µg/µL in phosphate-buffered saline.

Intramuscular DNA injection and electroporation

Muscle-targeted gene transfer through in vivo electroporation was performed as described previously [19–21]. The plasmid encoding *7ND* or the empty vector was delivered into the hind limb muscles of rats 7 days before the induction of arthritis using an in vivo electroporation method with a CUY 21 pulse generator (NepaGene, Chiba, Japan). Briefly, 400 µg of the plasmid vector was injected into the bilateral quadriceps muscles of the rats using a 27-gauge needle. To enhance transgene expression, immediately after the femoral muscle injections, electroporation was performed by inserting electrode needles (Tokiwa Science Ltd., Fukuoka, Japan) into the muscle within 5 mm of the injection site by delivering electric pulses. Three pulses of 200 V each were delivered to the injection site at a rate of 1 pulse/s, followed by three pulses of opposite polarity.

Measurement of serum 7ND levels in treated animals

To evaluate the amount of 7ND released from the transfected skeletal muscle, serum 7ND concentrations were measured using a human CCL2/MCP-1 ELISA kit (BioSource International Inc., Camarillo, CA, USA). As this human CCL2 ELISA kit does not cross-react with rat CCL2, it is suitable for measuring serum 7ND concentrations [21]. Serum samples were collected 4, 7, and 14 days after the introduction of the *7ND* gene to determine 7ND levels in the treated rats. Equal amounts of serum from each animal were analyzed, following the manufacturer's instructions. The cross-reactivity with rat CCL2 was less than 5% (data not shown).

Statistical analysis

All statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, N.Y., USA). Data are presented as mean value±standard deviation. Differences between experimental groups were analyzed using the Mann–Whitney U test and Student's *t*-test. A P-value of less than 0.05 was considered statistically significant.

Results

Histological and biochemical analysis of the fibrosis of the infrapatellar fat pad (IFP)

To assess the suitability of a rodent arthritis model for IFP fibrosis, we injected 100 µL of normal saline or 2% carrageenan into the knee joints of male Wistar rats. Histological analysis revealed that inflammatory cell infiltration was observed in the superficial layer of IFP on day 4. This infiltration gradually extended into the stroma by day 14 and was maintained until day 28. We detected the pleiomorphic infiltration of inflammatory cells and proliferation of interstitial cells in the affected joints during arthritis progression. (Fig. 1, representative images of each time point are shown.) The number of ED-1-positive cells, representing infiltrating macrophages, peaked on day 7 after arthritis induction and remained elevated for 4 weeks (Fig. 2A, B). Then, we explored the profiles of cytokines involved in the IFP fibrosis pathology. To

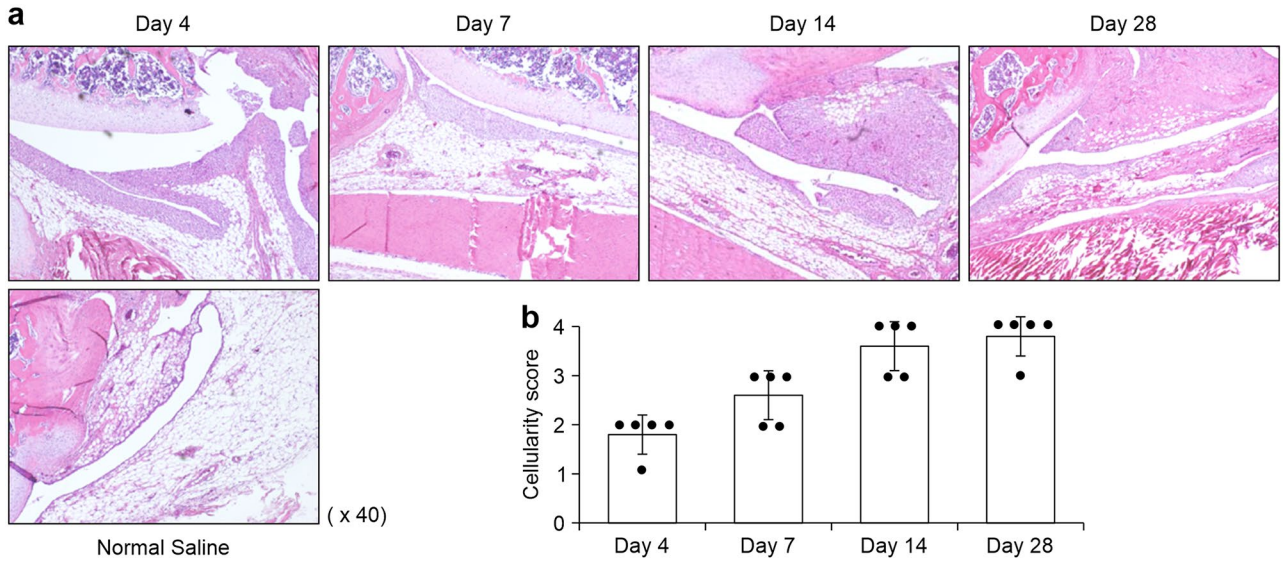

Fig. 1 Time course of carrageenan-induced arthritis. **A**) Representative images of sagittal sections of IFP stained with HE. **B**) Semiquantitative evaluation of IFP inflammation. Each dot indicated the mean value at each time point. Data were also indicated as mean and SD value

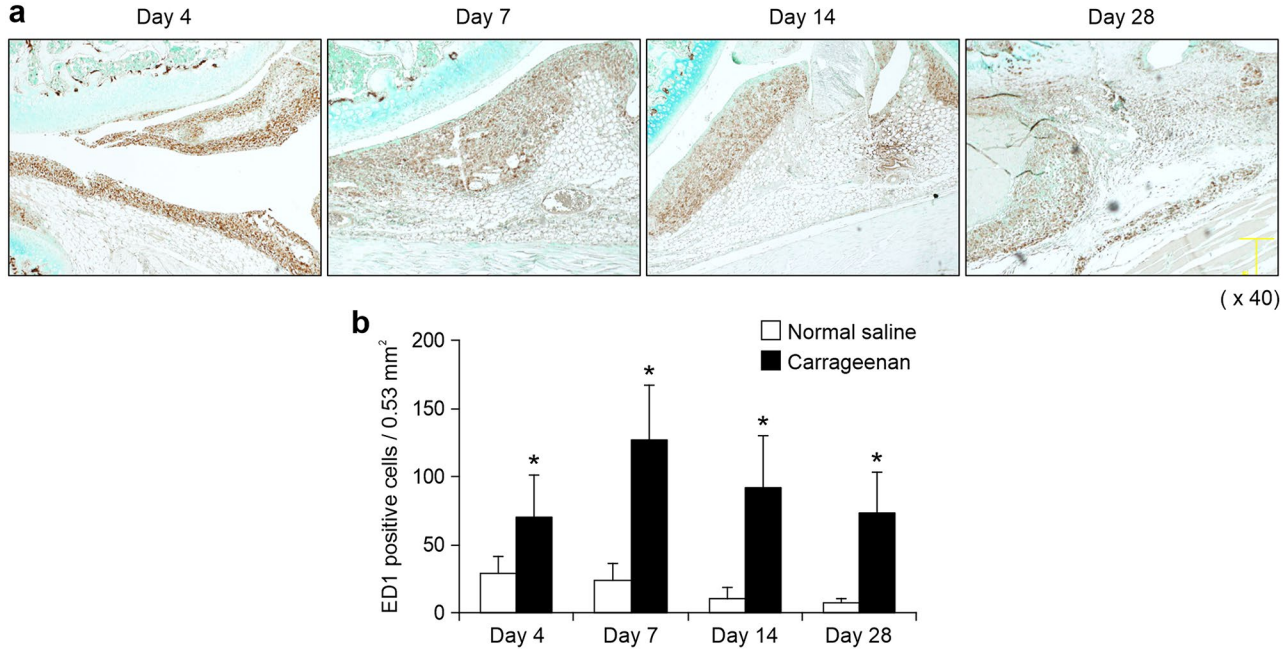

Fig. 2 Time course of macrophage infiltration in IFP. **A**) Immunohistochemical analysis of ED1-positive macrophages. Representative images of sagittal sections of IFP are shown. a) Analysis of ED1-positive cells in the IFP. The density of macrophages is highest 7 days after the onset of arthritis and remains elevated for 4 weeks. * indicates that the value was significantly different ($P < 0.05$) from the control

investigate the role of CCL2 and TGF-β in the rat model, we examined the expression of these molecules in the established fibrotic IFP. ELISA results showed increased production of CCL2 several days after arthritis induction, which persisted for over 4 weeks (Fig. 3A). Additionally, TGF-β expression levels were significantly higher at each time point in the carrageenan-induced arthritic tissues than in non-arthritic tissues (Fig. 3B).

Collagen deposition in the IFP

Masson's trichrome staining revealed a wide distribution of collagen fibers along the synovial lining in rats with carrageenan-induced arthritis. Interstitial collagen deposition increased gradually until day 14 after arthritis induction and continued progressing until day 28 (Fig. 4a). In IFP tissues, there was a significant increase in collagen deposition in the carrageenan-induced arthritic tissue on day 28 (Fig. 4b).

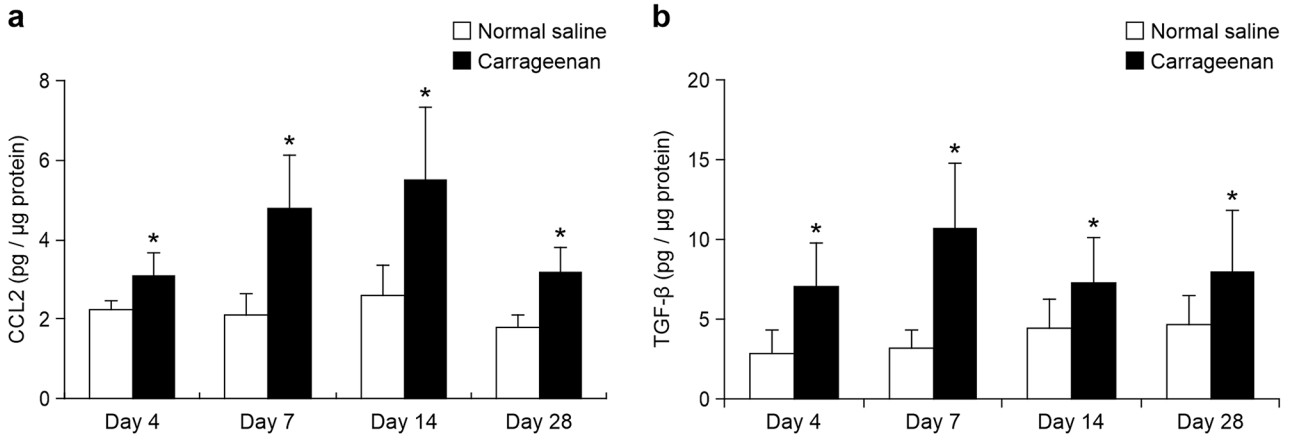

Fig. 3 Enzyme-linked immunosorbent assay (ELISA) of knee joints in carrageenan-treated rats. **a**) ELISA of CCL2 protein levels from synovial tissue homogenates. CCL2 production peaks on day 14 and remains high on day 28. **b**) TGF-β protein levels in synovial tissues measured by ELISA are significantly higher in carrageenan-induced arthritis at each time point compared with levels in non-arthritic tissues. There were 10 rats per group at each time point in all experiments. Values are presented as the mean and standard deviation ($n=10$). *$P<0.05$ versus control

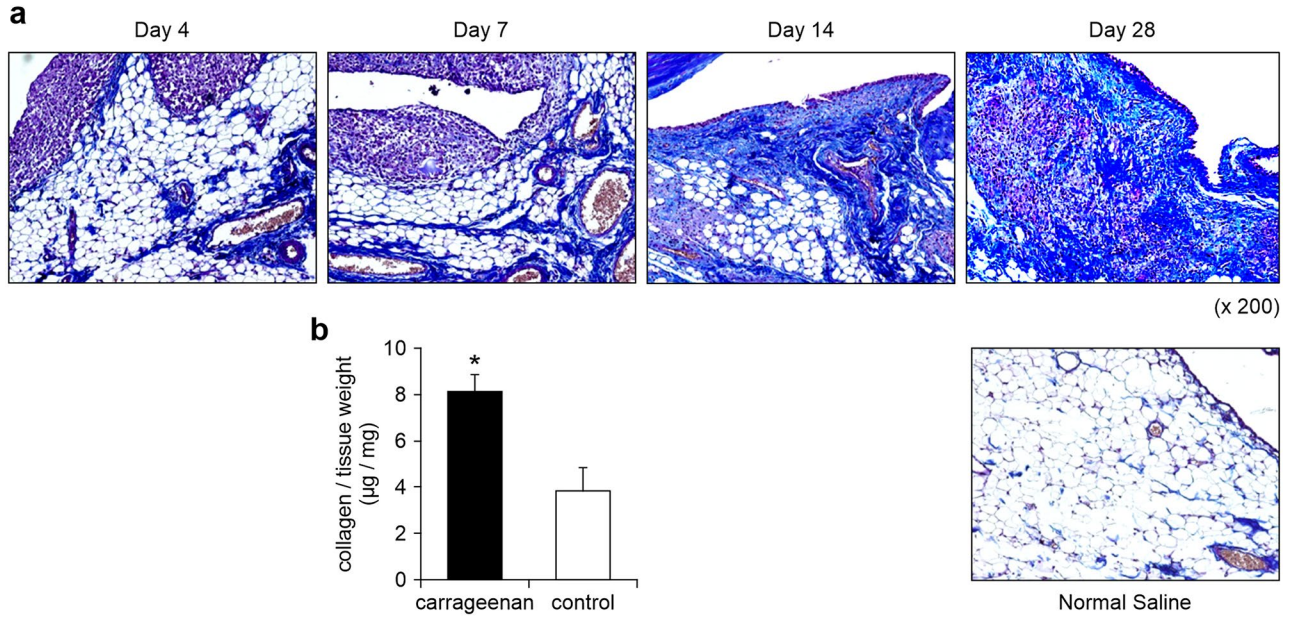

Fig. 4 Collagen distribution in the synovial tissue area of the IFP fibrosis rat model. (**a**) As determined by Masson's trichrome staining, the carrageenan-induced arthritis shows a wide deposition of collagen fibers along the IFP and synovial membrane on day 28. Representative joint sections from five rats are shown (Original magnification ×200). (**b**) Quantitative analysis of collagen deposition in carrageenan-induced arthritis. Collagen in IFP was measured using a collagen detection kit. A significant increase in collagen deposition in carrageenan-induced arthritis is seen on day 28. Values are presented as the mean and standard deviation of experiments involving 10 rats per group. *$P<0.05$ versus control

Efficacy of gene transfer; 7ND expression in the sera

To establish the role of CCL2 in the development of IFP fibrosis, we administered the *7ND* gene, an antagonist of CCL2, into a rat model via electroporation. To confirm the transfer efficacy, we performed preliminary experiments to measure plasma 7ND concentrations in rats ($n=7$ per group) after electroporation using a human CCL2/MCP-1 ELISA kit. A considerable amount of 7ND protein was detected in the serum during the first week following transfection, and serum 7ND levels remained detectable throughout the 2-week follow-up. In contrast, rats injected with the empty vector control showed no detectable 7ND protein in the serum 7 days after induction (Fig. 5).

Effects of 7ND on macrophage infiltration and CCL2 expression

Rats were injected with either 7ND or an empty plasmid before the induction of arthritis to determine the effects of *7ND* gene transfer on macrophage recruitment.

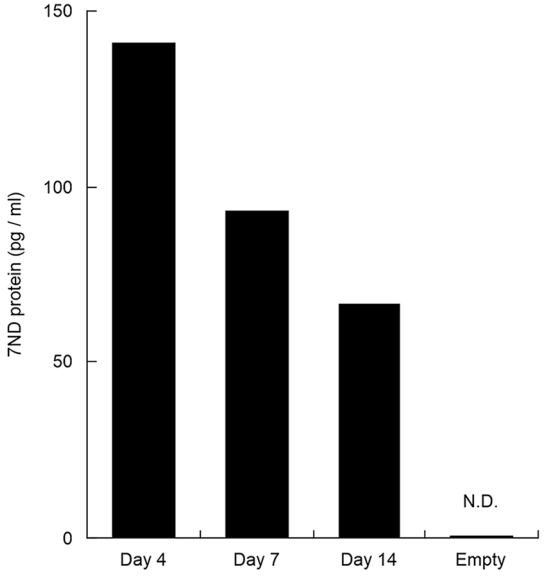

Fig. 5 Serum concentrations of 7ND after injection of the plasmid vector. Rats were treated with pCAGGS-7ND or an empty vector ($n=7$ in each group). Equal amounts of serum were obtained from pCAGGS-7ND-treated animals at the indicated times, followed by measurement of the 7ND concentration using an ELISA. A significant amount of 7ND is detected in the serum from pCAGGS-7ND-treated animals within the second week after injection. Human CCL2 protein is not detected in empty vector-treated rats. The mean values from seven rats in each experiment are shown. ND = not detected

Immunohistochemical analysis revealed significantly lower ED-1-positive macrophage infiltration in the group treated with 7ND than in the group treated with the empty plasmid on day 28 (Fig. 6A). The rats that were administered 7ND showed significantly lower levels of synovial inflammation, synovial lining hyperplasia, and cellular infiltration than those in the control group in a histological analysis on day 28 (Fig. 6B). The 7ND-treated rats also exhibited markedly attenuated endogenous CCL2 expression compared with that in the empty plasmid-treated rats on day 28 (Fig. 6C).

Effects of 7ND on carrageenan-induced collagen production

We assessed the preventive effects of CCL2 inhibition on the progression of IFP fibrosis. Collagen deposition levels following in vivo electroporation with pCAGGS-7ND were significantly lower than those in rats treated with the empty vector (Fig. 7A, B). These results indicated that delivery of the *7ND* gene before IFP fibrosis development significantly attenuated collagen deposition in the rat model.

Discussion

In this study, we utilized a carrageenan induced arthritis model to analyze macrophage infiltration and CCL2 expression during the progression of collagen deposition. Our findings demonstrate that anti-CCL2 gene therapy using pCAGGS-7ND significantly attenuates collagen deposition in the IFP of rat model.

The IFP is an interstitial adipose tissue with critical functions for joint motion and serves as a potential source of nociceptive innervation. Addressing fibrotic changes in the IFP is critical due to its role in pain generation in knee osteoarthritis (KOA) [2, 10]. Our previous studies have shown that fibrosis in the IFP contributes to the pathogenesis of KOA by inducing macrophage infiltration, new vessel formation, and sensory nerve fiber endings, leading to prolonged pain [15, 16]. Inflammation-induced pathological fibrosis is widely observed in various diseases and is driven by macrophage activation [7]. These cells play an important role in tissue repair and induce the production and accumulation of extracellular matrix through the recruitment of inflammatory cells. Thus, macrophages may play a central role in the inflammatory and fibrotic processes that occur in IFP.

CCL2/MCP-1 serves as a potent chemoattractant for macrophages [22]. The production of CCL2 appears to be variable and dependent on the cell type [8]. Upregulated CCL2 production has been observed in various fibrotic diseases, including liver disease [23], renal diseases [24], and systemic sclerosis skin lesions [25]. These data suggest that CCL2 contributes to the initiation of inflammatory infiltration in fibrotic diseases by recruiting macrophages and potentially enhancing collagen production via the upregulation of profibrotic mediators (e.g., endogenous TGF-β). In this study, we demonstrated the relationship between IFP fibrosis, macrophage infiltration, and CCL2 expression using rat arthritis model. These results indicated that macrophages play a central role in the pathology of infrapatellar fat pad fibrosis. They are key mediators of inflammation and contribute to fibrosis by secreting pro-inflammatory and profibrotic cytokines such as TGF-β.

The CCL2/CCR2 axis is the most widely reported mechanism underlying circulating monocyte recruitment in OA [9, 26]. CCL2 levels are significantly increased in the infrapatellar fat pad, the synovium [10, 11] and serum of patients with OA [27]. Furthermore, Raghu et al. demonstrated that monocytes recruited via the CCL2/CCR2 pathway promote synovitis in OA [28]. Zarebska et al. reported that the CCL2/CCR2 axis plays an important role in the development of pain in murine OA [29]. Thus, therapeutic targeting of CCL2 signaling may help slow or prevent tissue damage in patients with OA.

NH2-terminal truncated or modified CCL2 analogs have been shown to function as receptor antagonists. Previous studies have demonstrated the inhibitory effects of 7ND in animal models of atherosclerosis [30] and renal fibrosis [21] in vivo. Recent studies have also highlighted the effects of 7ND in musculoskeletal diseases. It

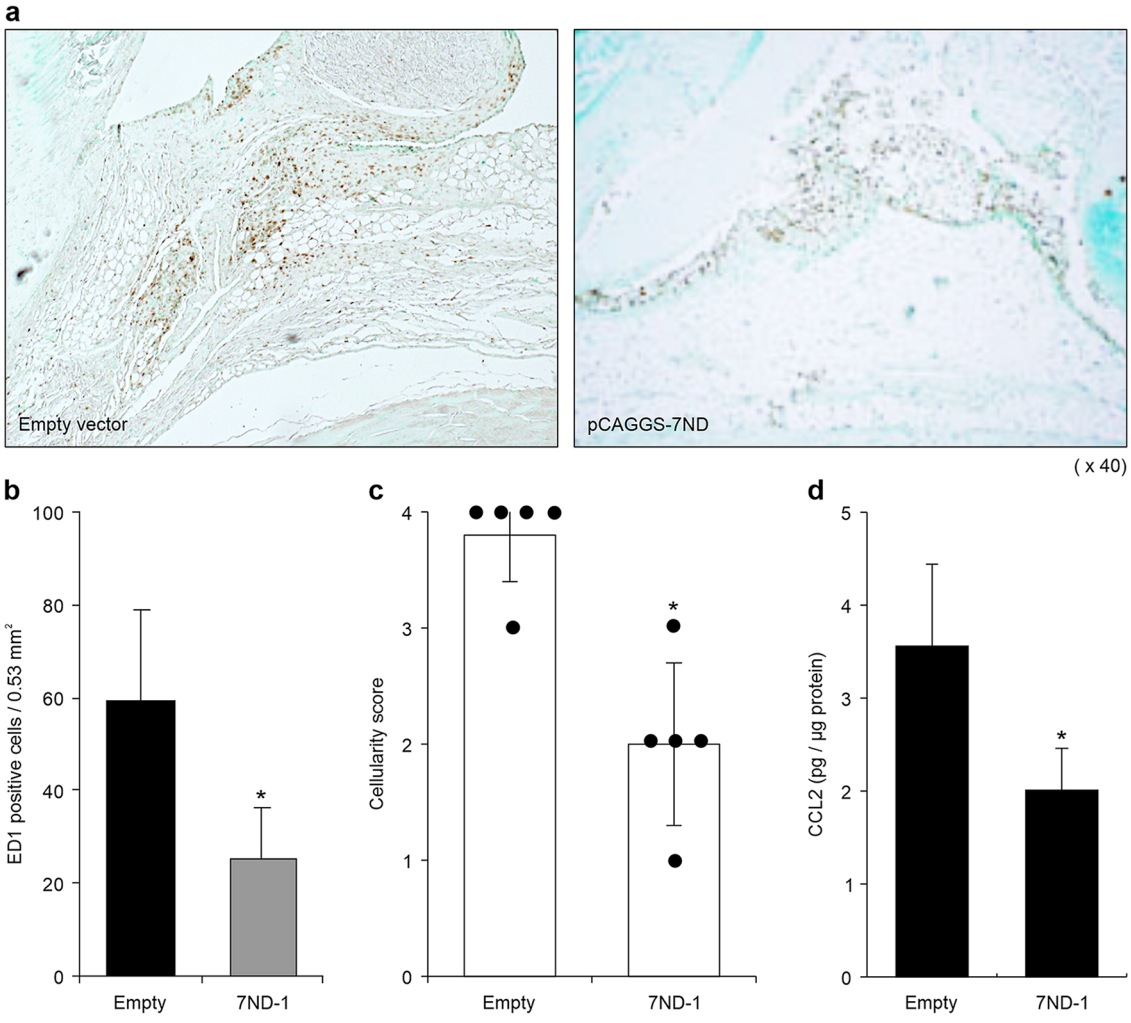

Fig. 6 Amelioration of morphologic lesions and quantitative analysis of CCL2 in 7ND-treated animals. **a**) Rats were treated with 7ND or an empty vector 7 days prior to the intraarticular injection of carrageenan and sacrificed on day 28. Representative images on day28 from 7ND- or empty vector-treated rats are shown. **b**) The graph shows the density of ED-1-positive macrophages in the IFP and synovial tissues. **c**) Cellularity score of IFP from 7ND- or empty vector-treated rats on day28. **d**) ELISA of CCL2 protein levels from IFP in 7ND- or empty vector-treated rats. There were 15 rats per group in each experiment, and the values are presented as the mean and standard deviation. *$P < 0.05$ vs. control

has been suggested that CCL2 is a viable clinical target for blocking osteoclast differentiation, as 7ND prevents human osteoclast differentiation [31]. Furthermore, 7ND treatment significantly reduced wear particle-induced osteolysis and the number of recruited inflammatory cells and osteoclasts [32]. In the present study, we demonstrated that *7ND* gene delivery effectively inhibited the histopathological manifestations of IFP fibrosis. The number of infiltrating macrophages in synovial tissues was significantly reduced in the 7ND-treated group. Moreover, 7ND-treated rats exhibited a significant reduction in endogenous CCL2 levels, likely produced by infiltrating macrophages, thereby resulting in an amplification loop. Therefore, inhibiting macrophage migration by blocking CCL2 reduces the number of inflammatory cells and the production of additional chemokines in the IFP. CCL2 inhibition appears to be the primary factor in attenuating IFP fibrosis. However, the exact mechanisms underlying the antagonistic effects of 7ND in vivo remain unclear. Further studies are necessary to elucidate the exact mechanisms underlying the effects of 7ND.

This is the first in vivo study targeting CCL2 in IFP fibrosis using a rat arthritis model. However, there are some limitations to consider. First, the small sample size may affect the generalizability of the results. Also, positive and negative control groups were not included to minimize the number of animals used. Second, we focused solely on IFP fibrosis without examining changes in cartilage, ligaments, capsules, or extra-articular tissues. Our aim was to specifically induce IFP-related inflammation while excluding other factors contributing to OA pathology such as cartilage degradation, meniscal

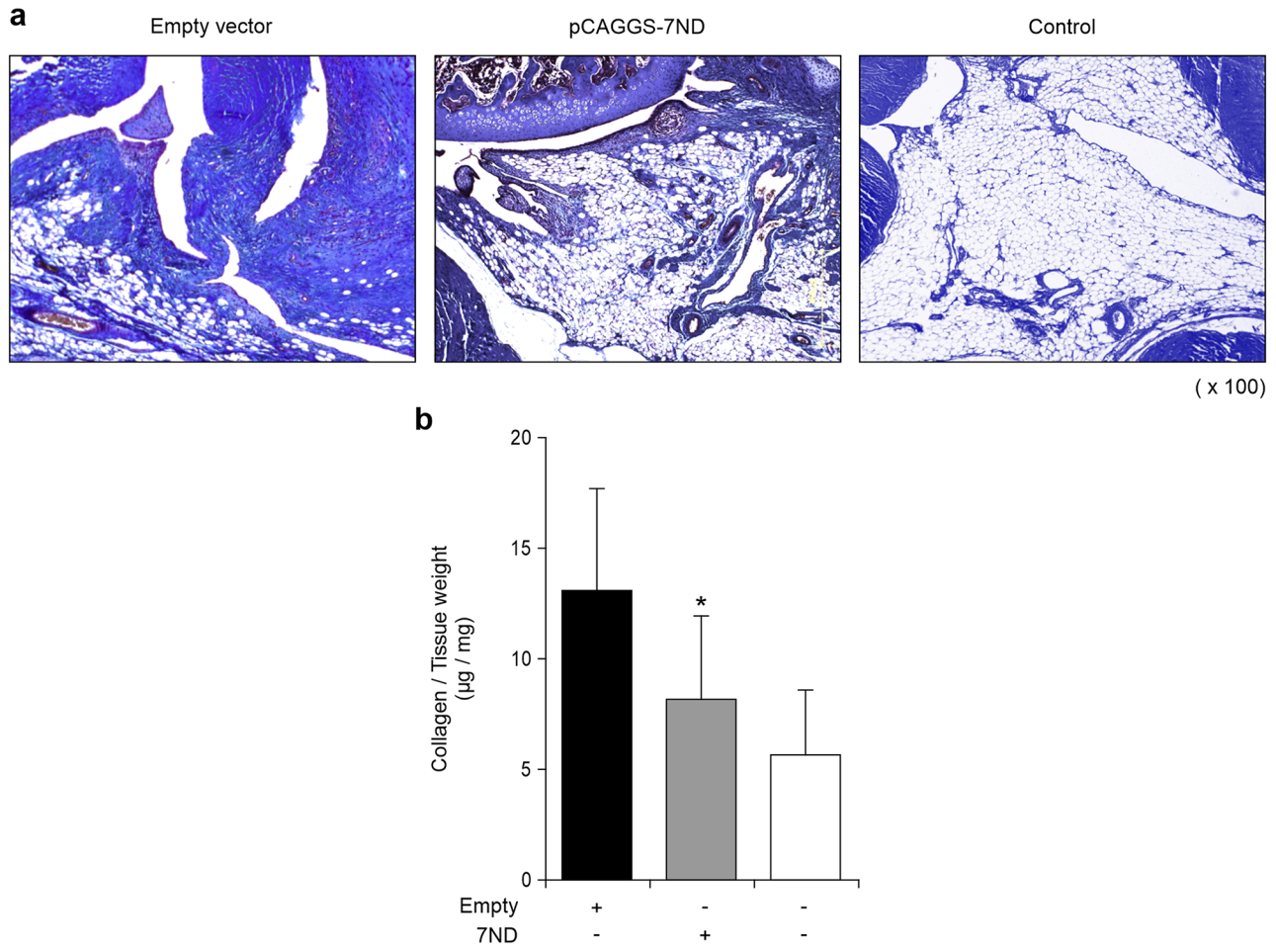

Fig. 7 Amelioration of collagen deposition in 7ND-treated animals. **a**) Masson's trichrome-stained sections demonstrating the distribution of collagen proteins. Representative photographs are shown, and there were 5 rats per group in each experiment. **b**) Rats were treated with 7ND 7 days before the administration of carrageenan. Collagen production was determined by the Sircol collagen assay, and there were 15 rats per group in each experiment. Values are presented as the mean and standard deviation ($n = 15$). *$P < 0.05$ versus empty vector-treated rats

damage, or joint instability. Carrageenan is commonly used to study osteoarthritis (OA) and other inflammatory conditions [33], but it does not lead to cartilage destruction [13]. Thus, we considered this model to be appropriate for this study. However, future studies should employ other experimental models, such as the monosodium iodoacetate (MIA)-induced or surgical OA models, to further investigate these aspects. Additionally, this study did not assess the impact of CCL2 on joint function or pain, Future research should include assessments of joint range of motion, swelling, and pain behavioral tests to comprehensively evaluate these effects. Third, no fatal complications were observed in the present study, but a comprehensive systemic examination for toxicity and safety was not conducted. While 7ND gene transfer has shown promise in various studies [12, 21, 30, 34], potential toxicity and adverse effects of systemic CCL2 suppression remain unclear. Further investigation is needed for clinical application in humans. Lastly, although we focused on CCL2, other mediators should be considered to fully understand the complex mechanisms involved. Future research should explore additional pathways to better understand intra-articular fibrosis development.

Conclusions

Our study demonstrates the efficacy of anti-CCL2 gene therapy, specifically using the *7ND* gene, in attenuating IFP fibrosis in a rat model. By inhibiting macrophage migration and reducing endogenous CCL2 levels, *7ND* gene delivery significantly mitigated histopathological manifestations associated with IFP fibrosis. However, further research is needed to elucidate the exact mechanisms underlying the antagonistic effects of 7ND and evaluate its safety and efficacy for clinical application in humans.

Abbreviations

CCL2 CC chemokine ligand 2
ELISA Enzyme-linked immunosorbent assay

MCP-1 Monocyte chemoattractant protein
OA Osteoarthritis

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Author contributions
HY: Designed the experiments, Conducted the experiments, Analyzed the data, Drafted the manuscript. YN: Analyzed and interpreted the data, Edited the manuscript. TM: Conceived and designed the experiments, Interpreted the data. HK: Analyzed and interpreted the data. All authors read and approved the final manuscript.

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Data availability
The data that support the findings of this study are available from the corresponding author, [H Y], upon reasonable request.

Declarations

Ethics approval and consent to participate
This study was conducted following a protocol approved by the Animal Care and Use Committee of Tokyo Medical and Dental University. All procedures followed the Animal Welfare Act and Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Bethesda, MD, USA).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Nakagawa Y, Tsuji K, Nakamura T, Katagiri H, Ozeki N, Shioda M, An JS, Yoshida R, Sekiya I, Koga H. Association of Infrapatellar Fat Pad Fibrosis at 3 months after ACL Reconstruction with short-term clinical outcomes and inflammatory cytokine levels in the synovial fluid. Orthop J Sports Med. 2023;11(4).
2. Belluzzi E, Stocco E, Pozzuoli A, Granzotto M, Porzionato A, Vettor R, De Caro R, Ruggieri P, Ramonda R, Rossato M et al. Contribution of infrapatellar fat pad and synovial membrane to knee osteoarthritis pain. Biomed Res Int 2019: 6390182, 2019.
3. Clockaerts S, Bastiaansen-Jenniskens YM, Runhaar J, Van Osch GJVM, Verhaar JAN, De Clerck LS, et al. The infrapatellar fat pad should be considered as an active osteoarthritic joint tissue: a narrative review. Osteoarthritis Cartilage. 2010;18:876–82.
4. Sellam J, Berenbaum F. The role of synovitis in pathophysiology and clinical symptoms of osteoarthritis. Nat Rev Rheumatol. 2010;6:625–35.
5. Eymard F, Pigenet A, Citadelle D, Flouzat-Lachaniette CH, Poignard A, Benelli C, et al. Induction of an inflammatory and prodegradative phenotype in autologous fibroblast-like synoviocytes by the infrapatellar fat pad from patients with knee osteoarthritis. Arthritis Rheumatol. 2014;66:2165–74.
6. Sanchez-Lopez E, Coras R, Torres A, Lane NE, Guma M. Synovial inflammation in osteoarthritis progression. Nat Rev Rheumatol. 2022;18:258–75. https://doi.org/10.1038/s41584-022-00749-9.
7. Wynn TA, Ramalingam TR. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. Nat Med. 2012;18:1028–40.
8. Gerard C, Rollins BJ. Chemokines and disease. Nat Immunol. 2001;2:108–15.
9. Yuan GH, Masuko-Hongo K, Sakata M, Tsuruha J, Onuma H, Nakamura H, et al. The role of C-C chemokines and their receptors in osteoarthritis. Arthritis Rheum. 2001;44:1056–70.
10. Bastiaansen-Jenniskens YM, Wei W, Feijt C, Waarsing JH, Verhaar JA, Zuurmond AM, et al. Stimulation of fibrotic processes by the infrapatellar fat pad in cultured synoviocytes from patients with osteoarthritis: a possible role for prostaglandin f2α. Arthritis Rheum. 2013;65:2070–80.
11. Favero M, El-Hadi H, Belluzzi E, Granzotto M, Porzionato A, Sarasin G, et al. Infrapatellar fat pad features in osteoarthritis: a histopathological and molecular study. Rheumatology (Oxford). 2017;56:1784–93.
12. Egashira K, Koyanagi M, Kitamoto S, Ni W, Kataoka C, Morishita R, et al. Anti-monocyte chemoattractant protein-1 gene therapy inhibits vascular remodeling in rats: blockade of MCP-1 activity after intramuscular transfer of a mutant gene inhibits vascular remodeling induced by chronic blockade of NO synthesis. FASEB J. 2000;14:1974–8.
13. Ashraf S, Mapp PI, Shahtaheri SM, Walsh DA. Effects of carrageenan induced synovitis on joint damage and pain in a rat model of knee osteoarthritis. Osteoarthr Cartil. 2018;26:1369–78.
14. Okuno M, Muneta T, Koga H, Ozeki N, Nakagawa Y, Tsuji K, et al. Meniscus regeneration by syngeneic, minor mismatched, and major mismatched transplantation of synovial mesenchymal stem cells in a rat model. J Orthop Res. 2014;32(7):928–36.
15. Inomata K, Tsuji K, Onuma H, Hoshino T, Udo M, Akiyama M, et al. Time course analyses of structural changes in the infrapatellar fat pad and synovial membrane during inflammation-induced persistent pain development in rat knee joint. BMC Musculoskelet Disord. 2019;20:8.
16. Onuma H, Tsuji K, Hoshino T, Inomata K, Udo M, Nakagawa Y, et al. Fibrotic changes in the infrapatellar fat pad induce new vessel formation and sensory nerve fiber endings that associate prolonged pain. J Orthop Res. 2020;38:1296e306.
17. Gerwin N, Bendele AM, Glasson S, Carlson CS. The OARSI histopathology initiative - recommendations for histological assessments of osteoarthritis in the rat. Osteoarthritis Cartilage. 2010;18 Suppl 3:S24-34. https://doi.org/10.1016/j.joca.2010.05.030. PMID: 20864021.
18. Niwa H, Yamamura K, Miyazaki J. Efficient selection for high-expression transfectants with a novel eukaryotic vector. Gene. 1991;108:193–9.
19. Aihara H, Miyazaki J. Gene transfer into muscle by electroporation in vivo. Nat Biotechnol. 1998;16:867–70.
20. Maruyama H, Sugawa M, Moriguchi Y, Imazeki I, Ishikawa Y, Ataka K, et al. Continuous erythropoietin delivery by muscle-targeted gene transfer using in vivo electroporation. Hum Gene Ther. 2000;11:429–37.
21. Shimizu H, Maruyama S, Yuzawa Y, Kato T, Miki Y, Suzuki S, et al. Anti-monocyte chemoattractant protein-1 gene therapy attenuates renal injury induced by protein-overload proteinuria. J Am Soc Nephrol. 2003;14:1496–505.
22. Yoshimura T, Robinson EA, Tanaka S, Appella E, Leonard EJ. Purification and amino acid analysis of two human monocyte chemoattractants produced by phytohemagglutinin-stimulated human blood mononuclear leukocytes. J Immunol. 1989;142:1956–62.
23. Tacke F. Targeting hepatic macrophages to treat liver diseases. J Hepatol. 2017;66:1300–12.
24. Mansour SG, Puthumana J, Coca SG, Gentry M, Parikh CR. Biomarkers for the detection of renal fibrosis and prediction of renal outcomes: a systematic review. BMC Nephrol. 2017;18:72.
25. Distler JH, Akhmetshina A, Schett G, Distler O. Monocyte chemoattractant proteins in the pathogenesis of systemic sclerosis. Rheumatology (Oxford). 2009;48:98–103.
26. Miller RE, Malfait AM. Can we target CCR2 to treat osteoarthritis? The trick is in the timing! Osteoarthr Cartil. 2017;25:799–801.
27. Longobardi L, Jordan JM, Shi XA, Renner JB, Schwartz TA, Nelson AE, et al. Associations between the chemokine biomarker CCL2 and knee osteoarthritis outcomes: the Johnston County Osteoarthritis Project. Osteoarthr Cartil. 2018;26:1257–61.
28. Raghu H, Lepus CM, Wang Q, Wong HH, Lingampalli N, Oliviero F, et al. CCL2/CCR2, but not CCL5/CCR5, mediates monocyte recruitment, inflammation and cartilage destruction in osteoarthritis. Ann Rheum Dis. 2017;76:914–22.
29. Miotla Zarebska J, Chanalaris A, Driscoll C, Burleigh A, Miller RE, Malfait AM, et al. CCL2 and CCR2 regulate pain-related behaviour and early gene expression in post-traumatic murine osteoarthritis but contribute little to chondropathy. Osteoarthritis Cartilage. 2017;25(3):406–12. https://doi.org/10.1016/j.joca.2016.10.008.
30. Usui M, Egashira K, Ohtani K, Kataoka C, Ishibashi M, Hiasa K, et al. Anti-monocyte chemoattractant protein-1 gene therapy inhibits restenotic changes (neointimal hyperplasia) after balloon injury in rats and monkeys. FASEB J. 2002;16:1838–40.

31. Morrison NA, Day CJ, Nicholson GC. Dominant negative MCP-1 blocks human osteoclast differentiation. J Cell Biochem. 2014;115:303–12.
32. Yao Z, Keeney M, Lin TH, Pajarinen J, Barcay K, Waters H, et al. Mutant monocyte chemoattractant protein 1 protein attenuates migration of and inflammatory cytokine release by macrophages exposed to orthopedic implant wear particles. J Biomed Mater Res A. 2014;102:3291–7.
33. Achari Y, Reno CR, Frank CB, Hart DA. Carrageenan-induced transient inflammation in a rabbit knee model: molecular changes consistent with an early osteoarthritis phenotype. Inflamm Res. 2012;61(8):907–14. https://doi.org/10.1007/s00011-012-0483-1.
34. Egashira K, Nakano K, Ohtani K, Funakoshi K, Zhao G, Ihara Y, et al. Local delivery of anti-monocyte chemoattractant protein-1 by gene-eluting stents attenuates in-stent stenosis in rabbits and monkeys. Arterioscler Thromb Vasc Biol. 2007;27(12):2563–8. https://doi.org/10.1161/ATVBAHA.107.154609.

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