Tenascin-C Prevents Articular Cartilage Degeneration in Murine Osteoarthritis Models

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Abstract

Objective. The objective of this study was to determine whether intra-articular injections of tenascin-C (TNC) could prevent cartilage damage in murine models of osteoarthritis (OA). **Design.** Fluorescently labeled TNC was injected into knee joints and its distribution was examined at 1 day, 4 days, 1 week, 2 weeks, and 4 weeks postinjection. To investigate the effects of TNC on cartilage degeneration after surgery to knee joints, articular spaces were filled with 100 µg/mL (group I), 10 µg/mL (group II) of TNC solution, or control (group III). TNC solution of 10 µg/mL was additionally injected twice after 3 weeks (group IV) or weekly after 1 week, 2 weeks, and 3 weeks (group V). Joint tissues were histologically assessed using the Mankin score and the modified Chambers system at 2 to 8 weeks after surgery. **Results.** Exogenous TNC was maintained in the cartilage and synovium for 1 week after administration. Histological scores in groups I and II were better than scores in group III at 4 and 6 weeks, but progressive cartilage damage was seen in all groups 8 weeks postoperatively. Sequential TNC injections (groups IV and V) showed significantly better Mankin score than single injection (group II) at 8 weeks. **Conclusion.** TNC administered exogenously remained in the cartilage of knee joints for 1 week, and could decelerate articular cartilage degeneration in murine models of OA. We also showed that sequential administration of TNC was more effective than a single injection. TNC could be an important molecule for prevention of articular cartilage damage.

Keywords

tenascin-C, osteoarthritis, cartilage degeneration, animal model

Introduction

It is well known that cartilage degeneration results in development of osteoarthritis (OA). Because articular cartilage has limited potential for regeneration, the development of effective treatments for OA is an important goal. Various agents have been developed to prevent cartilage degeneration and slow the progression of OA in animal models, including hyaluronic acid,^{1–5} bone morphogenetic protein-7,^{6–8} celecoxib,^{9,10} rebamipide,¹¹ and statins,¹² but there is no widely accepted disease-modifying OA drug.¹³

Tenascin-C (TNC) is an extracellular matrix glycoprotein. Although its expression is repressed in normal adult tissues, it reappears under pathological conditions such as wound healing, regeneration, inflammation, and tumorigenesis.^{14–17} TNC expression is also associated with the development of articular cartilage, but markedly decreases during maturation of chondrocytes and almost disappears in adult articular cartilage.^{18,19} In diseased joints, including those with OA and rheumatoid arthritis (RA), high levels of TNC have been shown to reappear in both articular cartilage and the

synovium.^{19,20} TNC concentrations of synovial fluid from patients with cartilage lesions of the knee joints are also elevated.²¹ Using an enzyme linked immunosorbent assay, we previously demonstrated that the TNC level of joint fluids increases in parallel with the radiographic progression of OA.²² Our *in vitro* studies have demonstrated that 10 µg/mL TNC promotes chondrocyte proliferation and increases proteoglycan content in culture.²³ We have also shown that cartilage repair in full-thickness cartilage defects in TNC knockout mice was delayed compared with wild-type mice, and the deficiency of TNC delays articular cartilage repair *in vivo*

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model.²⁴ Moreover, using a rabbit model, we demonstrated that treatment of full-thickness osteochondral defects by filling the defect with a gellan sulfate sponge filled with 10 $\mu\text{g}/\text{mL}$ exogenous TNC resulted in cartilage repair, in which hyaline-like cartilage was formed over newly formed subchondral bone tissue.²⁵ These results strongly suggest that TNC could promote cartilage repair in articular cartilage defects and could potentially prevent cartilage degeneration.

We speculated that knee injections of exogenous TNC would suppress the loss of cartilage matrix and consequently prevent progression of OA. We hypothesized that TNC could prevent cartilage degeneration in murine models of OA *in vivo*. Furthermore, we investigated the expression and distribution of exogenous TNC injected in the knee joints.

Methods

Purification of TNC

TNC was purified from culture supernatant of the U-251MG glioma cell line by ammonium sulfate precipitation, Sephacryl S-500 gel filtration, Mono Q ionexchange chromatography, and using a hydroxyapatite column as previously described.^{26,27} Purification was monitored by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of all column fractions. In SDS-PAGE, the anti-TNC antibody (4F10TT; IBL, Takasaki, Japan) reacted with TNC with molecular weights of 230 kDa, showing full length TNC (Fig. 1).

Animals

One hundred one male BALB/c strain mice (174 knees) weighing about 22 g were used and maintained according to guidelines approved by the animal experiment and care committee of our institution. This study protocol was approved by the Institutional Ethics Review Board of Mie University (approval number 24-30).

Surgical Procedures

A surgical procedure to create an experimental OA model was performed on 8-week-old mice. The mice were anesthetized with an intraperitoneal injection of sodium pentobarbital (0.05 mg/g body weight). Both knee joints were exposed following a medial capsular incision and gentle lateral displacement of the extensor muscle, without transection of the patellar ligament. Then, the anterior cruciate ligament and medial collateral ligament were transected using a surgical microscope and microsurgical technique. After replacement of the extensor muscle, the articular capsule and skin were closed independently.²⁴

Figure 1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis of the chromatography fraction in the purification of tenascin-C (TNC). The 230 kDa full length TNC band was found (red arrow).

Analysis of TNC in Knee Joints

TNC was labeled using HiLyte Fluor 555 Labeling Kit (Dojindo Labo, Kumamoto, Japan). NH_2 -Reactive HiLyte Fluor 555 has a succinimidyl ester group, and can easily make a covalent bond with an amino group of the target

protein or other macromolecules without any activation process. Filtration tube is used for sample protein in removing small molecules such as Tris buffer and amine compounds that interfere with the assay or labeling reaction. Fluorescently labeled TNC (10 μ L) was administered into one knee in murine models of OA (18 mice). Three mice were used per time point. Cartilage was obtained from knee joints at 1 and 4 days, or 1, 2, and 4 weeks post-operatively. The frozen sections were mounted on silane-coated glass slides and air-dried. Hoechst 33342 (Sigma, St. Louis, MO) was applied for 5 minutes for nuclear staining.

Intra-articular Administration of TNC

After the articular capsule was closed, 10 μ L of phosphate-buffered saline (PBS) solution containing 100 μ g/mL (group I) or 10 μ g/mL (group II) of TNC was injected into intra-articular spaces of one knee joint. Contralateral knee was injected 0 μ g/mL (group III, vehicle control) of TNC. All mice were allowed to walk freely without any splintage after operation. They were kept in the laboratory animal house at 24°C to 25°C, provided with standard mouse chow and water ad libitum and maintained under a 12-hour light-dark cycle. Mice were sacrificed at 2 weeks, 4 weeks, 6 weeks, and 8 weeks after surgery (Fig. 2a).

Additionally, we injected 10 μ g/mL of TNC 3 weeks after surgery (group IV, 2 injections), and weekly for 3 weeks (group V, weekly injections). Mice were sacrificed at 8 weeks after surgery (Fig. 2b).

Histopathologic Examination

All samples were fixed in 10% formalin at room temperature for 2 days, decalcified with 10% ethylenediamine tetraacetic acid (EDTA), dehydrated, embedded in paraffin, and sliced coronally at 4 μ m. Sections were stained with hematoxylin and eosin and safranin-O. Specimens from all groups were simultaneously stained and destained. We also performed an immunohistochemical analysis. The immunostaining procedure of type II collagen and TNC have been described previously.^{24,25} The production, characterization, and immunostaining technique of the monoclonal anti-type II collagen (Kyowa Pharma Chemical Co, Ltd, Tokyo, Japan) and the monoclonal anti-TNC antibody (4F10TT) have been described previously.²⁸ Briefly, sections were incubated in methanol containing 0.3% H₂O₂ for 30 minutes to block intrinsic peroxidase activity and then treated with 0.04% proteinase K (Sigma-Aldrich, St. Louis, MO)/0.1 M PBS solution for 10 minutes at 37°C to retrieve the antigens, followed by an overnight incubation with the primary antibody (1 mg/mL) at 4°C. The immunostaining procedure of type II collagen was performed using a standard technique (Histofine mouse stain kit; Nichirei Co.,

Figure 2. (a) Outline for the analysis (groups I-III).

Concentrations of tenascin-C (TNC) were 100 μ g/mL (group I), 10 μ g/mL (group II), and 0 μ g/mL (group III, vehicle control). Mice were sacrificed at 2, 4, 6, and 8 weeks after surgery. (b) Outline for the analysis (groups IV and V). We injected 10 μ g/mL of TNC twice (group IV) and weekly for 3 weeks (group V). Mice were sacrificed at 8 weeks after surgery.

Tokyo, Japan) to block intrinsic mouse immunoglobulin activity. The sections were incubated in methanol containing 0.3% H₂O₂ for 30 minutes to block intrinsic peroxidase activity and then treated with 0.04% proteinase K (Sigma-Aldrich)/0.1 M PBS solution for 10 minutes at 37°C. After washing, sections were treated with Histofine blocking reagent A and B for 60 minutes at 37°C, followed by an overnight incubation with the primary antibody (1 mg/mL) at 37°C. The sections were incubated with Histofine simple stain mouse MAX-PO (Nichirei Co.) for 60 minutes at 37°C. Finally, color was developed with diaminobenzidine/H₂O₂ solution.

Histologic Grading Score

Specimens were evaluated by 3 independent investigators using the Mankin score providing a combined score for cartilage structure, cellular abnormalities, and matrix staining.²⁹ In addition, a modified Chambers system was used³⁰. A score of 0 represents normal cartilage, 0.5 = loss of proteoglycan with an intact surface, 1 = superficial fibrillation without loss of cartilage, 2 = vertical clefts and loss of surface lamina (any % or joint surface area), 3 = vertical clefts/erosion to the calcified layer lesion for 1% to 25% of the quadrant width, 4 = lesion reaches the calcified cartilage for 25% to 50% of the quadrant width, 5 = lesion reaches the calcified cartilage for 50% to 75% of the quadrant width, 6 = lesion reaches the calcified cartilage for >75% of the quadrant width. Histologic grading scores were generated from separately from the medial tibial plateau, the medial femoral condyle, the lateral tibial plateau, and lateral femoral condyle. An overall mean scores were reported.

Figure 3. Distribution of the injected tenascin-C (TNC; red). Nucleus was labeled with blue.

Statistical Analysis

Histologic grading scores were evaluated by 3 independent observers in a blinded manner. Interclass correlation coefficients (ICCs) for the interobserver variability among 3 observers were 0.965 for the Mankin score and 0.944 for the modified Chambers system. The scores evaluated by single observer were demonstrated. The sample sizes were 37 in group I, 36 in group II, 73 in group III, 5 in group IV, and 5 in group V. Additional 18 mice were injected fluorescently labeled TNC. The analysis unit was a group dependent of TNC concentration in each period. Statistical significance was determined using the Kruskal-Wallis test, followed by Steele-Dwass test. A *P* value <0.05 was considered significant.

Results

Animal Welfare

All mice returned to normal activity and weightbearing shortly after waking up from anesthesia. Neither joint contracture nor infection was found in any mice. All knees were evaluated.

Expression and Distribution of Injected TNC in Knee Joints

To detect the distribution of injected TNC, cartilage that had received an intra-articular injection of labeled TNC was

evaluated by fluorescence microscopy at 1 and 4 days and 1, 2, and 4 weeks after injection. In frozen sections, red fluorescent emissions were observed in the cartilage matrix and synovium during 1 week after injection. Although remaining of fluorescent staining in articular cartilage was scant 1 week after injection, TNC administrated exogenously remained for at least 1 week after injection. In contrast, red fluorescent emissions were not observed in the cartilage in the control group (Fig. 3).

Microscopic Findings

To evaluate the protective effect of TNC on cartilage, the isolated knee joints were analyzed microscopically. The articular cartilage had a smooth surface and was evenly stained with safranin-O in all groups at 2 weeks. At 4 and 6 weeks, TNC administration markedly protected the articular cartilage from proteoglycan depletion. However, proteoglycan loss and alterations in surface structure were observed among groups I, II, and III at 8 weeks (Fig. 4a).

Type II collagen expression was maintained in all groups at 2 weeks. At 4 and 6 weeks, strong immunoreactivity was found in groups I and II. However, it was decreased in group III at 4 and 6 weeks and all groups at 8 weeks. In TNC immunohistochemistry, TNC was highly expressed in cartilage in group III at 4 and 6 weeks and all groups at 8 weeks. The enhancement of TNC staining was observed at the damaged surface (Fig. 4b).

Figure 4. Histologic analysis of surgically induced osteoarthritis (OA) in knee joints of mice after administration of tenascin-C (TNC) or vehicle control. (a) Safranin-O staining, (b) TNC immunostaining, with scale bar 100 µm.

TNC was also injected twice (group IV) and weekly (group V) into knee joints after surgery. At 8 weeks, group

IV and V showed comparable protection against proteoglycan depletion from articular cartilage (**Fig. 5**).

Figure 5. Histologic analysis (safranin-O staining) of surgically induced osteoarthritis (OA) in knee joints of mice after repeated administration of tenascin-C (TNC; groups IV and V) and single administration of TNC (group II) (scale bar 100 µm).

Histologic Grading Scores

Joint lesions were graded on scales of 0 to 14 using the Mankin score, and 0 to 6 using the modified Chambers system. At 2 weeks, there was no significant differences in both scores between groups, and no group showed development of OA (**Fig. 6**). However, progressive cartilage damage was seen in group III at 4 and 6 weeks after surgery. Mean histologic scores were significantly better in groups I and II than in group III (Figure 6). At 8 weeks, progressive cartilage damage was seen in all groups, but no significant differences in both scores among groups were observed (**Fig. 6**).

When we injected TNC twice (group IV) or weekly (group V), the mean Mankin scores were significantly better in groups IV and V compared with group II, but there were no significant differences in mean scores between groups IV and V. TNC injections in groups IV and V were equally effective (**Fig. 7a**). However, the modified Chambers system showed no difference between the groups (**Fig. 7b**).

Discussion

We demonstrated that a single injection of TNC into the knee joint could prevent articular cartilage degeneration for 6 weeks in murine OA models. Furthermore, we investigated the distribution of exogenously injected TNC in knee joints. These findings indicate that TNC affects the cartilage surface, and protected the articular cartilage from proteoglycan depletion. Lightner *et al.*³¹ reported that TNC binds to chondroitin sulfate (CS) proteoglycans. In samples of severe OA, CS staining was found only in the deep zone of articular cartilage. TNC was detected in matrices surrounding the chondrocytes of deep zones overlying CS staining. Our previous study²³ also showed the co-localization of CS with TNC staining in cartilage matrix around chondrocytes in part of the survival zones of damaged cartilage. As OA progresses, CS is reduced, and TNC is upregulated at the same sites. Our previous *in vitro* studies indicated that 10 µg/mL TNC promotes chondrocyte proliferation and

Figure 6. Histologic scoring of cartilage destruction according to (a) Mankin score (2 weeks, $P = 0.514$; 4 weeks, $P < 0.001$; 6 weeks, $P < 0.001$; 8 weeks, $P = 0.669$) and (b) modified Chambers system (2 weeks, $P = 0.285$; 4 weeks, $P < 0.001$; 6 weeks, $P < 0.001$; 8 weeks, $P = 0.278$). Error bars indicate 95% confidence intervals. * $P < 0.001$, ** $P = 0.039$, *** $P = 0.002$.

increases proteoglycan content in chondrocytes derived from OA patients.²³ Patel *et al.*³² reported that addition of 10 µg/mL TNC upregulates ADAMTS4 mRNA in cultured primary chondrocytes. However, in contrast to ADAMTS4, ADAMTS5 does not show any significant changes with TNC treatment. Majumdar *et al.*³³ and Glasson *et al.*³⁴ demonstrated that ADAMTS5 is the primary aggrecanase responsible for aggrecan degradation in a murine model of

Figure 7. Histologic scoring of cartilage destruction at 8 weeks according to (a) Mankin score ($P = 0.006$) and (b) modified Chambers system ($P = 0.264$). Error bars indicate 95% confidence intervals. * $P = 0.020$, ** $P = 0.043$.

OA. These findings suggest that 10 $\mu\text{g/mL}$ TNC mediates anabolic pathways in OA models.

TNC has also been reported to function as a proinflammatory extracellular matrix glycoprotein, and was recently identified as an endogenous damage-associated molecular pattern that activates TLR4 in inflammatory diseases.^{35,36} TNC expression is specifically and rapidly induced in response to tissue injury. Whereas TNC expression is transient during acute inflammation, persistently high levels of TNC expression occur in the inflamed synovium of joints from patients with RA.^{36,37} In erosive arthritis induced by immunization and intra-articular injection of methylated bovine serum albumin, intra-articular TNC injection promotes joint inflammation in wild type mice, and TNC-null mice show rapid resolution of acute joint inflammation.³⁶ However, these researchers injected only the fibrinogen-like (FBG) domain intra-articularly.³⁶ The FBG domain of TNC was demonstrated to induce joint inflammation *in vivo*.³⁶ However, our previous study showed that full-length TNC did not cause the severe inflammation that was seen in experiments with TNC fragments.²⁵ The proinflammatory

function of TNC-dependent innate immunologic reactions might contribute to cartilage regeneration. A number of growth factors, including transforming growth factor- β (TGF- β), insulin-like growth factor-I, bone morphogenetic protein (BMP)-2, BMP-7, and basic fibroblast growth factor (bFGF) have been used for cartilage repair.^{38,39} Our recent study has shown that TNC is a useful molecule for cartilage repair.²⁵ Previous studies demonstrated that TNC could induce stimulation of chondrogenesis.^{39,40} However, TNC lacking the FBG domain or TNC comprised solely of FBG domains failed to stimulate chondrogenesis. The FBG domain of TNC is necessary but not sufficient for induction of chondrogenesis.⁴⁰ Imanaka-Yoshida *et al.*⁴¹ reported that understanding the clinical significance of TNC may not be straightforward, as it may contribute to both favorable and undesirable effects on pathological events in a context-dependent manner. It is clear that TNC is a key molecule controlling cellular activity during tissue remodeling.

The present study has some limitations. First, we studied the small sample size and lack of samples from mice more than 8 weeks after surgery. Second, we could not demonstrate directly that injected TNC remained intact over time. Third, we could not demonstrate injected TNC remained intact after labeling.

Conclusion

This is the first study, to our knowledge, to report that TNC prevents articular cartilage degeneration in murine models of OA, and our hypothesis that TNC could prevent cartilage degeneration was verified only for 6 weeks. In addition, sequential administration of TNC was more effective than a single injection. TNC could be an important molecule for prevention of articular cartilage damage. Further studies are needed to determine the optimal dosage and frequency of administration of TNC. In addition, we need to analyze known culprits of articular cartilage degeneration, including proteases, inflammatory cytokines, and determine whether they are delayed or expressed at lower levels in TNC-injected knees. We should also analyze known master regulators of articular cartilage function and see whether TNC injection maintained their expression over time after surgery.

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Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: TY receives a royalty from IBL.

Ethical Approval

Ethical approval for this study was obtained from the Institutional Ethics Review Board of Mie University (approval number 24-30).

Animal Welfare

The present study followed international, national, and/or institutional guidelines for humane animal treatment and complied with relevant legislation.

References

- Shimizu C, Yoshioka M, Coutts RD, Harwood FL, Kubo T, Hirasawa Y, *et al.* Long-term effects of hyaluronan on experimental osteoarthritis in the rabbit knee. *Osteoarthritis Cartilage*. 1998;6:1-9.
- Kobayashi K, Amiel M, Harwood FL, Healey RM, Sonoda M, Moriya H, *et al.* The long-term effects of hyaluronan during development of osteoarthritis following partial meniscectomy in a rabbit model. *Osteoarthritis Cartilage*. 2000;8:359-65.
- Plaas A, Li J, Riesco J, Das R, Sandy JD, Harrison A. Intraarticular injection of hyaluronan prevents cartilage erosion, periarticular fibrosis and mechanical allodynia and normalizes stance time in murine knee osteoarthritis. *Arthritis Res Ther*. 2011;13:R46.
- Amiel D, Toyoguchi T, Kobayashi K, Bowden K, Amiel ME, Healey RM. Long-term effect of sodium hyaluronate (Hyalgan) on osteoarthritis progression in a rabbit model. *Osteoarthritis Cartilage*. 2003;11:636-43.
- Hulmes DJ, Marsden ME, Strachan RK, Harvey RE, McInnes N, Gardner DL. Intra-articular hyaluronate in experimental rabbit osteoarthritis can prevent changes in cartilage proteoglycan content. *Osteoarthritis Cartilage*. 2004;12:232-8.
- Hayashi M, Muneta T, Ju YJ, Mochizuki T, Sekiya I. Weekly intra-articular injections of bone morphogenetic protein7 inhibits osteoarthritis progression. *Arthritis Res Ther*. 2008;10:R118.
- Sekiya I, Tang T, Hayashi M, Morito T, Ju YJ, Mochizuki T, *et al.* Periodic knee injections of BMP7 delay cartilage degeneration induced by excessive running in rats. *J Orthop Res*. 2009;27:1088-92.
- Hayashi M, Muneta T, Takahashi T, Ju YJ, Tsuji K, Sekiya I. Intra-articular injections of bone morphogenetic protein7 retard progression of existing cartilage degeneration. *J Orthop Res*. 2010;28:1502-6.
- Jiang D, Zou J, Huang L, Shi Q, Zhu X, Wang G, *et al.* Efficacy of intra-articular injection of celecoxib in a rabbit model of osteoarthritis. *Int J Mol Sci*. 2010;11:4106-13.
- Moon SJ, Park JS, Jeong JH, Yang EJ, Park MK, Kim EK, *et al.* Augmented chondroprotective effect of coadministration of celecoxib and rebamipide in the monosodium iodoacetate rat model of osteoarthritis. *Arch Pharm Res*. 2013;36:116-24.
- Moon SJ, Woo YJ, Jeong JH, Park MK, Oh HJ, Park JS, *et al.* Rebamipide attenuates pain severity and cartilage degeneration in a rat model of osteoarthritis by downregulating oxidative damage and catabolic activity in chondrocytes. *Osteoarthritis Cartilage*. 2012;20:1426-38.
- Yudoh K, Karasawa R. Statin prevents chondrocyte aging and degeneration of articular cartilage in osteoarthritis. *Aging*. 2010;2:990-8.
- Davies PS, Graham SM, MacFarlane RJ, Leonidou A, Mantalaris A, Tsiridis E. Disease-modifying osteoarthritis drugs: in vitro and in vivo data on the development of DMOADs under investigation. *Expert Opin Investig Drugs*. 2013;22:423-41.
- Chiquet-Ehrismann R, Mackie EJ, Pearson CA, Sakakura T. Tenascin: an extracellular matrix protein involved in tissue interactions during fetal development and oncogenesis. *Cell*. 1986;47:131-9.
- Erickson HP. Tenascin-C, tenascin-R and tenascin-X: a family of talented proteins in search of functions. *Curr Opin Cell Biol*. 1993;5:869-76.
- Chiquet-Ehrismann R. Tenascins, a growing family of extracellular matrix proteins. *Experientia*. 1995;51:853-62.
- Belanger I, Beaulieu JF. Tenascin in the developing and adult human intestine. *Histol Histopathol*. 2000;15:577-85.
- Mackie EJ, Murphy LI. The role of tenascin-C and related glycoproteins in early chondrogenesis. *Microsc Res Tech*. 1988;43:102-10.
- Pfänder D, Heinz N, Rothe P, Carl H, Swoboda B. Tenascin and aggrecan expression by articular chondrocytes is influenced by interleukin 1 β . *Ann Rheum Dis*. 2004;63:240-4.
- Salter DM. Tenascin is increased in cartilage and synovium from arthritic knees. *Br J Rheumatol*. 1993;32:780-6.
- Schmidt-Rohlfing B, Gavenis K, Kippels M, Schneider U. New potential markers for cartilage degradation of the knee joint. *Scand J Rheumatol*. 2002;31:151-7.
- Hasegawa M, Hirata H, Sudo A, Kato K, Kawase D, Kinoshita N, *et al.* Tenascin-C concentration in synovial fluid correlates with radiographic progression of knee osteoarthritis. *J Rheumatol*. 2004;31:2021-6.
- Nakoshi Y, Hasegawa M, Akeda K, Iino T, Sudo A, Yoshida T, *et al.* Distribution and role of tenascin-C in human osteoarthritic cartilage. *J Orthop Sci*. 2010;15:666-73.
- Okamura N, Hasegawa M, Nakoshi Y, Iino T, Sudo A, Imanaka-Yoshida K, *et al.* Deficiency of tenascin-C delays articular cartilage repair in mice. *Osteoarthritis Cartilage*. 2010;18:839-48.
- Ikemura S, Hasegawa M, Iino T, Miyamoto K, Yoshida T, Imanaka-Yoshida K, *et al.* Effect of tenascin-C on the repair of full-thickness osteochondral defects of articular cartilage in rabbits. *J Orthop Res*. 2015;33:563-71.
- Aukhil I, Slemp CC, Lightner VA, Nishimura K, Briscoe G, Erickson HP. Purification of hexabrachion (tenascin) from cell culture conditioned medium, and separation from a cell adhesion factor. *Matrix*. 1990;10:98-111.
- Yoshida T, Yoshimura E, Numata H, Sakakura Y, Sakakura T. Involvement of tenascin-C in proliferation and migration of laryngeal carcinoma cells. *Virchows Arch*. 1999;435:496-500.
- Hasegawa K, Yoshida T, Matsumoto K, Katsuta K, Waga S, Sakakura T. Differential expression of tenascin-C and tenascin-X in human astrocytomas. *Acta Neuropathol*. 1997;93:431-7.
- Mankin HJ, Dorfman H, Lippiello L, Zarins A. Biochemical and metabolic abnormalities in articular cartilage from osteoarthritic human hips. II. Correlation of morphology with biochemical and metabolic data. *J Bone Joint Surg Am*. 1971;53:523-37.

30. Glasson SS, Chambers MG, Van Den Berg WB, Little CB. The OARSI Histopathology Initiative—recommendations for histological assessments of osteoarthritis in the mouse. *Osteoarthritis Cartilage*. 2010;18(Suppl 3):S17-23.
31. Lightner VA, Erickson HP. Binding of hexabrachion (tenascin) to the extracellular matrix and substratum and its effect on cell adhesion. *J Cell Sci*. 1990;95:263-77.
32. Patel L, Sun W, Glasson SS, Morris EA, Flannery CR, Chockalingam PS. Tenascin-C induces inflammatory mediators and matrix degradation in osteoarthritic cartilage. *BMC Musculoskelet Disord*. 2011;12:164-77.
33. Majumdar MK, Askew R, Schelling S, Stedman N, Blanchet T, Hopkins B, *et al*. Double-knockout of ADAMTS-4 and ADAMTS-5 in mice results in physiologically normal animals and prevents the progression of osteoarthritis. *Arthritis Rheum*. 2007;56:3670-4.
34. Glasson SS, Askew R, Sheppard B, Carito B, Blanchet T, Ma HL, *et al*. Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. 2005;31;434:644-8.
35. Sofat N, Robertson SD, Hermansson M, Jones J, Mitchell P, Wait R. Tenascin-C fragments are endogenous inducers of cartilage matrix degradation. *Rheumatol Int*. 2012;32:2809-17.
36. Midwood K, Sacre S, Piccinini AM, Inglis J, Trebaul A, Chan E, *et al*. Tenascin-C is an endogenous activator of Toll-like receptor 4 that is essential for maintaining inflammation in arthritic joint disease. *Nat Med*. 2009;15:774-80.
37. Hasegawa M, Nakoshi Y, Muraki M, Sudo A, Kinoshita N, Yoshida T, *et al*. Expression of large tenascin-C splice variants in synovial fluid of patients with rheumatoid arthritis. *J Orthop Res*. 2007;25:563-8.
38. Kock L, van Donkelaar CC, Ito K. Tissue engineering of functional articular cartilage: the current status. *Cell Tissue Res*. 2012;347:613-27.
39. Fukuda A, Kato K, Hasegawa M, Hirata H, Sudo A, Okazaki K, *et al*. Enhanced repair of large osteochondral defects using a combination of artificial cartilage and basic fibroblast growth factor. *Biomaterials*. 2005;26:4301-8.
40. Murphy LI, Fischer D, Chiquet-Ehrismann R, Mackie EJ. Tenascin-C induced stimulation of chondrogenesis is dependent on the presence of the C-terminal fibrinogen-like globular domain. *FEBS Lett*. 2000;480:189-92.
41. Imanaka-Yoshida K, Yoshida T, Miyagawa-Tomita S. Tenascin-C in development and disease of blood vessels. *Anat Rec*. 2014;297:1747-57.