Tenascin-C Prevents Articular Cartilage Degeneration in Murine Models of Osteoarthritis

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Introduction: Tenascin-C (TNC) is an extracellular matrix glycoprotein. While the expression is repressed in normal adult tissues, it reappears under pathological conditions such as wound healing, regeneration, inflammation and tumorigenesis (1). In articular cartilage, TNC expression is also associated with the development, but markedly decreases during maturation of chondrocyte, and is finally almost disappeared in adult articular cartilage. In diseased joints including those with osteoarthritis (OA), TNC was highly reappeared in cartilage (2). Our in vitro studies have demonstrated that 10μg/ml TNC promotes chondrocyte proliferation and increases proteoglycan content in culture (3). Moreover, we showed that the deficiency of TNC progresses during cartilage degeneration in the spontaneous OA in aged joints and surgical OA model (4). We hypothesized that TNC could prevent cartilage degeneration in murine models of OA in vivo.

Methods: Purification of TNC: TNC was purified from culture supernatant of human glioma cells U-251 MG as previously described (5).

Animals: Eight-week-old male BALB/c strain mice weighing about 22g were used and maintained according to guidelines approved by the animal experiment and care committee of our institution.

Surgical procedures: All mice were anesthetized with an intramuscular 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.

Intra-articular injection of TNC: The knees were divided into two groups. The concentrations of TNC were 10μg/ml (Group I) and 0μg/ml (Group II, vehicle control). After articular capsule were closed, TNC in 10μl PBS were injected into the knee joint. The control group had injection of only PBS. All mice were allowed to walk freely without any splintage after operation.

Histopathological examination: Mice were sacrificed at 2 weeks, 4 weeks, 6 weeks, 8 weeks after operation (Table.1). All samples were fixed in 10% formalin at room temperature, decalcified with 10% ethylenediamine tetraacetic acid, dehydrated, embedded in paraffin, and sliced up coronally at 4 μm. Safranin-O (Saf-O) staining, collagen type II immunohistochemistry, TNC immunohistochemistry were performed. Histological grading score: Specimens were evaluated blindly by three independent investigators using modified Mankin score (6).

Statistical analysis: Statistical significance was determined using the Mann-Whitney U-test. A p-value <0.05 was considered significant.

Results: Microscopic findings: To evaluate the chondroprotective effect of TNC, the isolated knee joints from the two groups were analyzed microscopically. Histological examinations were made using Saf-O staining. The articular cartilage had a smooth surface and evenly stained with Saf-O in both 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 both two groups at 8 weeks (Fig.1a).

Type II collagen expression was maintained in both groups at 2 weeks. At 4 and 6 weeks, strong immunoreactivity was found in Group I. However it was decreased in Group II at 4 and 6 weeks and both two groups at 8 weeks.

In TNC immunohistochemistry, TNC was highly expressed in cartilage in Group II at 4 and 6 weeks and both two groups at 8 weeks. The enhancement of TNC staining was observed at the damaged surface (Fig.1b).

Comparison of histological grading score: The joint lesions were rated on a scale of 0-14 using modified Mankin scoring system, giving combined score for cartilage structure, cellular abnormalities, and matrix staining. At 2 weeks, there was no statistical significance in the average scores and we found no development of OA in both groups. However progressive cartilage damage was seen in Group II at 4 and 6 weeks after surgery. Average histological scores were significantly better in Group I than in Group II (4 weeks:Group I 1.4, Group II 3.4 (p<0.001), 6 weeks:Group I 1.9, Group II 5.0 (p<0.001)). At 8 weeks, no significant differences in average scores between two groups were observed, but progressive cartilage damage was seen in both two groups (Group I 5.5, Group II 5.1 (P=0.34)) (Fig.2).
**Discussion:** This study demonstrated that 10μg/ml of TNC could prevent articular cartilage degeneration for 6 weeks. These findings indicate that intra-articular injection of TNC prevents matrix breakdown and the development of OA. Limitations of this study include small sample size and lack of samples from mice more than 8 weeks after operation. In conclusion, this is the first report, to our knowledge, that TNC prevents articular cartilage degeneration in murine models of OA, and our hypothesis was verified. Further studies are needed to determine the optimal dosage and duration of administration of TNC.

**Significance:** Intra-articular injection of TNC prevented articular cartilage degeneration for 6 weeks in murine models of OA and TNC could be an important candidate for prevention articular cartilage degeneration.

**Acknowledgments:**

**References:**
(1) Chiquet-Ehrismann R et al. Cell.1986
(4) Okamura N et al. Osteoarthritis Cartilage.2010
(5) Aukhil I et al. Matrix.1990

<table>
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<tr>
<th>Table 1 Numbers of sacrificed mice at each time points</th>
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<tr>
<td>Group I: TNC 10μg/ml (n)</td>
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<td>--------------------------</td>
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<tr>
<td>2w 5</td>
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<td>4w 10</td>
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Figure 1. Histological analysis of surgically-induced OA in the knee joints of mice after administering of TNC or the vehicle control. 
(a) Safranin-O staining. (b) TNC immunostaining. 
(Original magnification × 200)

Figure 2. Histological scoring of cartilage destruction according to modified Mankin score. Error bars indicate standard deviation. *P < 0.001