Administrations Of Tenascin-c Delay 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 cartilage degeneration (4) and intra-articular injection of 10μg/ml TNC prevented articular cartilage degeneration for 6weeks in murine models of OA (5).

In this study, we investigated expression and distribution of exogenous injected TNC in the knee joints, and we hypothesized that additional doses injections of TNC could be more effective than one shot injection in murine models of OA.

Methods: Purification of TNC: TNC was purified from culture supernatant of human glioma cells U-251 MG as previously described (6).

Animals: A total of 31 male 8-week-old 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.

Pharmacokinetic analysis of TNC in the knee joints: TNC was labeled with HiLyte FluorTM 555 Labeling Kit (Dojindo, Kumamoto, Japan). Labeled TNC was injected into the knee joint in murine models of OA. Cartilage and synovial tissues were obtained from knee joints at 1,4 day and 1,2 and 4 weeks after intra-articular injection of labeled TNC. For fluorescence microscopy of labeled TNC in OA samples, 6μm frozen serial sections were mounted on saline-coated glass slides, air-dried, then Hoechst33342 (SigmaSt.Louis, MO) was applied for 5 minutes for nuclear staining.
Intra-articular injection of TNC: The knees were divided into three groups. The concentrations of TNC were 10μg/ml and 0μg/ml (vehicle control). Group I (single injection): We injected 10μg/ml of TNC into the knee joints after articular capsule were closed. Group II (twice injections): We injected 10μg/ml of TNC twice after articular capsule were closed and 3 weeks later after operation. Group III (control): The control group had injections only PBS. All mice were allowed to walk freely without any splintage after operation. (Fig.1)

Histopathological examination: Mice were sacrificed at 8 weeks after operation. (Group I: n=12, Group II: n=9, Group III: n=10) 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 4μm. Safranin-O staining, collagen type II immunohistochemistry, TNC immunohistochemistry were performed.

Histological grading score: Specimens were evaluated blindly by three independent investigators using Mankin scoring system.

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

Results: Expression and Distribution of Injected TNC in the knee joints: To detect the distribution of injected TNC, cartilage and synovial tissues that had received an intra-articular injection of the labeled TNC were evaluated by fluorescence microscopy 1, 4 days, and 1, 2, and 4 weeks after injection. In frozen sections, red fluorescence emission was observed in the cartilage and synovial cells during 2 weeks after injection. TNC was maintained for at least 2 weeks after injection. By contrast, red fluorescence emission was not observed in the cartilage and synovial cells after injection of labeled bovine serum albumin (control). (Fig.2)

Microscopic findings: To evaluate the chondroprotective effect of TNC, the isolated knee joints from the three groups were analyzed microscopically. Histological examinations were made using Safranin-O staining.

At 8 weeks, proteoglycan loss and alterations in surface structure were observed in group I and group III. The twice administrations of TNC markedly protected the articular cartilage from proteoglycan depletion. (Fig.3a)

Type II collagen expression was maintained in group II. However, it was decreased in group I and group III. (Fig.3b)

In TNC immunohistochemistry, TNC was highly expressed in cartilage in group I and group III. The enhancement of TNC staining was observed at the damaged surface.

Comparison of histological grading score: The joint lesions were graded on a scale of 0-15 using Mankin scoring system, giving combined score for cartilage structure, cellular abnormalities, and matrix staining. At 8 weeks, progressive cartilage damage was seen in both groups and no significant differences were observed in average scores in group I and group III (group I: 5.4, group III: 5.6). On the other hand, average histological scores were significantly better in group II than in group I (group II: 3.9). The twice injections were more effective than single injection at 8 weeks. (Fig.3c)

Discussion: This study demonstrated that TNC was maintained in the cartilage and synovial cells for at least 2 weeks after injection.
After single injection of TNC into the knee joint, TNC could prevent cartilage degeneration for only 6 weeks. The twice injections could prevent cartilage degeneration for 8 weeks, so twice injections were more effective than single injection at 8 weeks.

Limitations of this study include small sample size and lack of samples from mice more than 8 weeks after operation.

In conclusion 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 in murine models of OA and TNC could be an important candidate for prevention articular cartilage degeneration.

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**Fig. 1:** Intra-articular injection of TNC

**Fig. 2:** Expression and distribution of injected TNC in the knee joints. (Original magnification × 1000)
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