Effect of Tenascin-C on the repair of full-thickness osteochondral defects of articular cartilage in rabbits

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Group 1 Group 2 Group 3 
4 weeks 
8 weeks 
12 weeks 
H-E       Saf-O H-E       Saf-O H-E       Saf-O

INTRODUCTION:
Tenascin-C (TNC) is a member of the extracellular matrix glycoprotein family that is expressed during embryogenesis. While the expression is repressed in normal adult tissues, it reappears under pathological conditions such as inflammation, infection 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 and rheumatoid arthritis, TNC was highly reappeared in cartilage (2).

Our in vitro studies have demonstrated that TNC promotes the proliferation of cultured chondrocytes, upregulates the mRNA expression of aggrecan in chondrocytes, and increases the amount of glycosaminoglycan in the culture (3). Moreover, we showed that cartilage repair in full-thickness cartilage defects in TNC knockout mice was delayed compared with wild-type mice (4).

We hypothesized that TNC could promote chondrogenesis and cartilage repair in damaged articular cartilage in vivo. To test this hypothesis, we examined the effects of TNC on the repair of osteochondral defects of articular cartilage in rabbits. We used gellan-gellan-sulfate sponge (gellan-GS) of which capacity to bind TNC has been demonstrated, as a new scaffold for slow release of TNC.

MATERIALS AND METHODS:

Implant: Gellan-GS was prepared as previously described (5). Briefly, water-soluble gellan and NaOH were dissolved, followed by the addition of the GS to this solution.

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

Animals: All procedures were performed according to the guidelines approved by our institution. A total of 27 female 12-week-old Japanese white rabbits weighing about 2.5kg were used.

Surgical procedures: The rabbits were anesthetized by intramuscular injection of ketamine at 20 mg/kg body weight and xylazine at 4 mg/kg body weight and underwent surgery on both knees. After a medial parapatellar incision, the patella was dislocated laterally. A full-thickness osteochondral defect (4 mm in diameter and 4mm in depth) penetrating to the subchondral bone was created in the center of the patellar groove using an electric drill. The knees were divided into three groups: the defect was filled tightly with gellan-GS which was sterilized by ethylene oxide gas, impregnated with 5 μg of TNC (Group 1), gellan-GS impregnated with 0.5μg of TNC (Group 2), and gellan-GS alone (Group 3). After operation, the animals were allowed free cage activity without immobilization.

Histopathological examination: The animals were sacrificed by carbon dioxide inhalation at 1 month (n=6 knees in each group), 2 months (n=6 knees in each group), and 3 months (n=6 knees in each group) after operation. Gross observation of the tissue growth was conducted in the operated region. All samples were fixed in 10% formalin at room temperature, decalcified with 10% ethylenediamine tetraacetic acid, dehydrated, embedded in paraffin, and sliced up sagittally at 4 μm. Hematoxylin & eosin (H-E) and Safranin-O staining and collagen type II immunohistochemistry were performed.

Histological grading score: The sections were evaluated blindly by three independent investigators using the modified WAKITANI score (7).

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

RESULTS:

Macroscopic findings of the defects: Neither joint contracture nor infection was found in any rabbits. At 4 weeks, the defects were filled with brown opaque tissue with a concavity toward the center in all groups. At 8 weeks, the defects in Group 2 were covered with a shiny white cartilage-like tissue. In Group 1 and Group 3, only the margins of the defects were covered with cartilage-like tissue with a concavity toward the center. At 12 weeks, the defects in Group 1 and Group 2 were covered with a smooth cartilage-like tissue. In Group 3, the defect was filled with brown rough opaque tissue.

Microscopic findings of the defects: Fig. 1 shows the histological sections stained with H-E and Safranin-O. At 4 weeks, the defects were not filled with repaired tissue in all groups. At 8 weeks, the defects in Group 2 were covered with hyaline-like cartilage. In Group 1, the margin of the defect was covered with hyaline-like cartilage, but the center of the defect was filled with fibrous tissue that was not metachromatically stained. In Group 3, the defects remained un repaired, and the subchondral bone showed no signs of repair. At 12 weeks, the defects in Group 2 were covered with hyaline-like cartilage. In Group 1 and Group 3, the subchondral bone was repaired, but we found no cartilage repair with adjacent host cartilage degeneration.

Comparison of histological grading score: The average scores obtained using the modified WAKITANI score are shown in Fig. 2. At 4 weeks, there was no statistical significance in the average scores in all groups. At 8 weeks, the average histological scores in Group 2 (8.3) were statistically better than in other groups (Group 1: 12.2, Group 3: 13.5) (p <0.05). At 12 weeks, the average histological scores in Group 2 (4.5) were statistically better than in other groups (Group 1: 9.5, Group 3: 9.8) (p <0.01).

DISCUSSION:
This study demonstrated that full-thickness osteochondral defects in rabbits filled with gellan-GS impregnated with 0.5μg of TNC were successfully repaired with hyaline-like cartilage and with subchondral bone formation. Limitations of this study include small sample size and lack of samples from rabbits more than 12weeks after implantation.

In conclusion, this is the first report, to our knowledge, that TNC promote the repair of full-thickness osteochondral defects in vivo. Further studies are needed to determine the optimal dosage and the optimal duration of administration of TNC.

SIGNIFICANCE:
The capacity of TNC to improve the repair of full-thickness osteochondral defects of articular cartilage emphasizes its promise as a practical and important candidate for cartilage repair.

REFERENCES: