Synovial Mesenchymal Stem Cells Promote Healing Of A Rat Massive Meniscus Defect Augmented By Achilles Tendon

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Introduction: For the treatment of meniscal tear, meniscectomy is commonly performed because suture repair is only applicable to meniscal tears which have longitudinal orientation or vascular zone. To preserve the functions of meniscus after meniscectomy, various type of meniscal substitute such as meniscal allografts, collagen meniscus implant, artificial materials, and autologous tendon grafts have been tried in animal experiments and clinical studies. We previously reported that transplantation of Achilles tendon treated with BMP-7 promotes meniscus regeneration in a rat model of massive meniscus defect (1). However, there remains the problem about the healing of transplanted tendon and native meniscus or peripheral capsule. Mesenchymal stem cells (MSCs) derived from synovium had a high proliferation (2) and chondrogenic potential (3). Transplantation of synovial MSCs into a full-thickness cartilage defect promoted cartilage regeneration due to direct differentiation into chondrocytes (4) and trophic factors for residual chondrocytes (5). In this study, we investigated whether synovial MSCs promotes meniscus regeneration augmented by tendon transplantation in a rat model of massive meniscus defect.

Methods: This study was approved by the Animal Experimentation Committee of our institution. Preparation of MSCs: Synovium was harvested from rat knee joints, digested with collagenase, and the nucleated cells were cultured for 7 days. After 2-3 passages, the adherent colony-forming cells were collected and used for injections. For the administration, 1 million synovial MSCs were suspended in 30μl PBS.

Animal and surgery: Wild type male Lewis rats were used. Achilles tendon was harvested and placed in the solution suspended with synovial MSCs for 10 minutes before transplantation. After anterior half of medial meniscus was resected, the pretreated tendon was transplanted into the meniscus defect and sutured with joint capsule. Residual MSCs suspended in the solution was also injected into the knee joint after closing the patellar tendon. The rats were allowed to walk freely in their cages, and sacrificed at 2, 4 and 8 weeks after the surgery. As controls, same number of rats had transplantation of tendon without MSCs (Tendon group), or only meniscectomy (Control). Macroscopic and microscopic findings of regenerated meniscus were analyzed. Cartilage degeneration was also analyzed for the tibial plateau.

In vivo bioluminescent imaging: To chase the MSCs, in vivo imaging system (IVIS) was used to detect photons from synovial MSCs derived from luciferase transgenic rats (Luc⁺ synovial MSCs). Detection of GFP and LacZ expressed synovial MSCs: To clarify cell distribution of injected cells, synovial MSCs derived from GFP transgenic rats (GFP⁺ synovial MSCs) and LacZ transgenic rats (LacZ⁺ synovial MSCs) were used. X-Gal staining was performed to detect LacZ⁺ synovial MSCs.

Results: Synovial MSCs promotes meniscus regeneration by transplantation of Achilles tendon. Macroscopically, in the untreated group, the meniscus defect was only filled with synovial tissue 2 and 4 weeks after meniscectomy (Fig. 1A, left panel). Irrespective of treatment with MSCs, the meniscus defect was covered with transplanted tendon at 2 weeks. The integration was observed between the transplanted tendon and the native meniscus with filled synovial tissue in the tendon + MSC group (Fig. 1A, left panel, white arrow), though no integration was observed in the tendon group (Fig. 1A, left panel, black arrow). The meniscus covering ratio was smaller in the untreated group than in the other 2 groups after transplantation (Figure 1B, right panel). Histologically, coarse synovial tissue was observed at the end of the native meniscus in the untreated group throughout the study (Fig. 2A). In the tendon group, transplanted tendon and native meniscus were clearly separated at 2 weeks, and the border of both tissues had been observed clearly even at 4 and 8 weeks. In the tendon + MSC group, the space between transplanted tendon and native meniscus was filled with the abundant aggregated cells at 2 weeks (Fig. 2A, arrows), and the integration was completely obtained at 4 and 8 weeks (Fig. 2A, arrowheads). Distribution of type II collagen expression in the regenerated meniscus revealed that those in the tendon + MSC group at 8 weeks were most similar as normal meniscus (Fig. 2B).

Transplanted tendon treated with synovial MSCs prevented cartilage degeneration. Macroscopically, cartilage erosion was observed even at 2 weeks in the untreated group, and it progressed severely at 4 and 8 weeks (Fig. 1C, arrows). In the tendon
group, cartilage lesion was not detected at 2 and 4 weeks, but observed at 8 weeks (Fig. 1C, arrows). In the tendon + MSC group, cartilage erosion was not detected even at 8 weeks. Hayashi’s score, evaluation for macroscopic cartilage lesion, revealed that cartilage erosion was significantly prevented in the tendon + MSC group than in other two groups at 8 weeks (Fig. 1D).

MSCs could be detected around the knee joint 10 weeks after the surgery. IVIS demonstrated that MSC-derived photons were detected around the knee joint after the surgery (Fig. 3A), and they increased at one week. Then, they decreased but remained up to 10 weeks. MSC-derived photon could not be detected in any other organs.

MSCs were confirmed at the integration site of the transplanted tendon and native meniscus, or around transplanted tendon. Macroscopically and histologically, GFP+ synovial MSCs were detected at the border of the transplanted tendon and native meniscus 2 weeks after the surgery (Fig. 3B). Macroscopically, dark blue areas for LacZ were detected not only at the integration site of the transplanted tendon and the native meniscus, but also around the transplanted tendon 2 and 4 weeks after the surgery (Fig. 3C). LacZ positive cells were observed at the integration site and around the transplanted tendon histologically. Figure legend Fig 1 Macroscopic analysis (A) Macroscopic features (B) Meniscus covering ratio = Area A/ Area B (C) Tibial plateau stained with India ink (D) Macroscopic evaluations of cartilage lesion based on Hayashi’s score Fig 2 Histologic analyses (A) Regenerated meniscus stained with Safranin-O (B) Regenerated meniscus immunostained with type II collagen. Boxed areas in the left panels are shown at a higher-magnification view in the right panels. N; native meniscus, R; regenerated meniscus Fig 3 Cell tracking (A) IVIS analysis (B) Detection of GFP+ synovial MSCs (C) Detection of LacZ synovial MSCs

Discussion: Transplantation of autologous tendon increased the size of regenerated meniscus, which indicated that transplanted tendon served as a scaffold for meniscus defect. Synovial MSCs survived around the tendon for a long period, which enhanced the healing process of the transplanted tendon and promoted differentiation of tendon cells into meniscal cells. Both transplantation of tendon and administration of synovial MSCs promoted meniscus regeneration and prevented cartilage degeneration.

Significance: Transplantation of autologous tendon with synovial MSCs could be one of the promising procedures to regenerate meniscus and to prevent cartilage degeneration in the meniscus deficient knee.

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