Intraarticular injected synovial stem cells differentiate into meniscal cells directly and promote meniscal regeneration without mobilization to distant organs in rat massive meniscal defect

INTRODUCTION:

Repair for massive meniscal defect remains a challenge owing to a lack of cell kinetics for the meniscus precursors in the knee joint. The synovium plays pivotal roles during the natural course of meniscal healing and contains mesenchymal stem cells (MSCs) with high chondrogenic potential (1). We investigated whether intraarticular injected synovium-MSCs enhanced meniscal regeneration in rat massive meniscal defect. To track the injected cells, we created transgenic expressing dual luciferase and LacZ.

METHODS:

Establishment of luc/LacZ® synovium-MSCs. Transgenic rats expressing dual luciferase and LacZ (Luc/LacZ) were developed by cross-breeding ROSA/luciferase Tg Lewis rats with ROSA/LacZ Lewis rats. MSCs were isolated from synovium of Luc/LacZ® Tg rat.

Meniscectomy and MSCs injection. Wild type male Lewis rats at 12 weeks of age were used (n=27). Anterior half of medial meniscus was excised in both knees (Fig.2A). Immediately after the skin incision was closed, 5x10⁶ Luc/LacZ® MSCs in 50 µL PBS were injected into the right knee joint. For control, the same volume of PBS was injected into the left. These groups were compared macroscopically and histologically at 2, 4, 8 and 12 weeks.

In vivo bioluminescent imaging. Luc/LacZ® MSCs were injected into meniscectomized knee or intact knee. To detect photons from Luc+ cells, D-luciferin was injected into the penile vein of anaesthetized rats.

Quantitative real-time PCR. The mRNA levels of LacZ obtained from expanded synovium-MSCs and various organs (brain, lung, liver, spleen, kidney, and knee synovium) were determined by SYBR® green-based real-time quantitative RT-PCR.

RESULTS:

MSCs derived from the synovium of Luc/LacZ Tg rats expressed luciferase and LacZ (Fig.1). Two to 8 weeks after five million Luc/LacZ® synovium-MSCs were injected into massive meniscectomised knee of wild type rat, macroscopically, the menisci regenerated much better than it did in the control group (Fig.2). After 12 weeks, the regenerated menisci were LacZ positive, produced type II collagen, and showed meniscal features by transmission electron microscopy (Fig.3). In in vivo luminescence analysis, photons increased in the meniscus-resected knee over three days, then decreased without detection in all other organs (Fig.4A, B). LacZ gene derived from MSCs could not be detected in other organs except in synovium by real-time PCR (Fig.4C).

DISCUSSION:

A number of reports have previously described the injection of bone marrow-MSCs into the joint for meniscus injury (2); however, the kinetics and role of injected MSCs remain unknown. To refine the analysis, we created transgenic rats expressing dual Luc/LacZ genes.

To avoid spontaneous healing, we resected meniscus massively. Though meniscal size also increased in the control group, the synovium-MSC injected groups showed better results from the standpoint of type II collagen expression and electron microscopic features.

For clinical application, interspecies differences have to be considered. We used a rat model, and rat meniscus had a greater spontaneous healing potential. To demonstrate the effectiveness of intraarticular injection of synovium-MSCs for meniscus regeneration, further experimental studies in larger animals are needed.

CONCLUSION:

Synovium-MSCs injected into the massive meniscectomized knee adhered to the lesion, differentiated into meniscal cells directly, and promoted meniscal regeneration without mobilization to distant organs.

REFERENCES:

(1) Sakaguchi, Sekiya, Muneta et al. Arthritis Rheum. 2005
(2) Murphy, Hunziker, Barry et al. Arthritis Rheum 2003