ORIGIN OF MESENCHYMAL CELLS IN TENDON HEALING USING TENDON AND BONE MARROW CHIMERIC GFP RAT MODEL

* Kajikawa, Y; ** Watanabe, N; ** Sakamoto, H; ** Matsuda, K; * Kobayashi, M; * Oshima, Y; * Yoshida, A; ** Kawata, M; * Kubo, T
+*Department of Orthopaedics, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan
**Department of Anatomy and Neurobiology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan

nobuw@koto.kpu-m.ac.jp

INTRODUCTION:
Influx of mesenchymal cells in the injured site of tendon is an important process for tendon healing. It has not yet been determined whether the influx is from circulating blood derived from bone marrow, injured tendon, or adjacent tissues such as subcutaneous tissue, fascia, synovium, tendon sheath, and periosteum. Thus, the cellular origin of mesenchymal cells in the injured tendon can be used to obtain pilot data for tendon repair. In this study, we focused on the tendon cells and circulating cells, and hypothesized that both tendon-derived and bone marrow-derived circulating mesenchymal cells exist in the injury site and contribute to tendon healing.

Green fluorescent protein (GFP) has widely been used in vivo as a cell marker. We were successful to develop 2 types of GFP chimeric rat models; the tendon chimeric rat and the bone marrow chimeric rat. The objective of this study was to follow GFP signal positive tendon cells and circulating cells using these chimeric rat models after tendon injury.

MATERIALS AND METHODS:
Patellar tendons of 12-week-old GFP transgenic rats (Japan SLC) (n=20) were harvested. These tendons were transplanted into the defects of 12-week-old Sprague-Dawley (SD) wild type rats’ (n=20) bilateral patellar tendons to create tendon chimeric rats that GFP signals exist only in the patellar tendons (Fig.1). At 2 weeks after the transplantation, GFP signals were observed to confirm the survival of transplanted tendon cells (tendon chimeric rat). Lateral partial perpendicular injuries were created in the middle of the right patellar tendons of tendon chimeric rats.

Bone marrow transplantation was performed to make bone marrow chimeric rats that GFP signals exist only in the bone marrow and circulation. Seven-week-old wild type rats (n=60) received 100Gy radiation from X-ray generator. 1.0x10^7 of bone marrow cells, which were obtained aseptically from tibias, femurs, and humeri of 7-week-old GFP rat (n=30), were transplanted into radiated wild type rats. The GFP chimeric rate of peripheral blood and bone marrow were measured with FACS calibur 7 days after transplantation (bone marrow chimeric rat). Tendon injuries were created in the right patellar tendons of bone marrow chimeric rats in the same method as the tendon chimeric rats. Left patellar tendons were used as a control.

At 1, 3 and 7 days after the injury of both chimeric rat models, tendons were harvested and 14um of serial coronal frozen sections were made. The sections were stained with hematoxylin and eosin and propidium iodide (PI) and examined with a confocal laser scanning microscopy (LSM 510, Zeiss) to quantify the survival of GFP positive cells.

RESULTS:
In the patellar tendon of the tendon chimeric rats, GFP positive rate (GFP positive / PI positive fibroblast-like cells) was approximately 60-70% (Fig.2-A). This indicated the survival of the transplanted tendon cells in the tendon chimeric rat. At 1 day after the injury in the tendon chimeric rat, hemorrhage was observed and GFP positive cells were not detected (Fig.2-B). At 3 days, the injured sites were repaired by GFP positive fibroblast-like cells. GFP positive rate was 40-50% (Fig.2-C). At 7 days, the number of GFP positive fibroblast-like cells increased to 70-80% in the tissue (Fig.2-D). In the bone marrow chimeric rats, the GFP chimeric rate of peripheral blood and bone marrow was approximately 20% and the patellar tendons had no GFP signal (Fig.3-A). Therefore, this model has GFP signal only in the circulation. At 1 day after the injury, there were a few GFP positive fibroblast-like cells with elongated nuclei in the edges of the injury sites as well as in the injury sites with hemorrhage (Fig.3-B). However, GFP positive fibroblast-like cells were not detected in the tendon sites far from the injury sites. At 3 days, GFP positive fibroblast-like cells increased in the injury site, and the GFP positive rate was 50-60% (Fig.3-C). At 7days, GFP positive rate decreased to 30-50% (Fig.3-D).

DISCUSSION:
In this study, GFP positive fibroblast like cells with elongated nuclei infiltrated in the injury site at 1, 3 and 7 days. Therefore, it was found that tendon-derived mesenchymal cells and circulating mesenchymal cells migrated to the injury site and contributed to the tendon repair. However, the cellular proportion of GFP positive cells after injury was different between these 2 chimeric rat models. In the bone marrow chimeric rat, circulating mesenchymal cells were found in the injury sites at 1 day after injury and increased at 3 days after injury. However, the proportion of the circulating mesenchymal cells decreased at 7 days after injury. In the tendon chimeric rat model, tendon-derived mesenchymal cells first appeared in the injury site at 3 days. The number of GFP-positive cells increased with time and GFP positive rate in the injury sites was increased up to the same rate as uninjured site (60-70%) at 7 days after injury. From these results, it is likely that only circulating mesenchymal cells appear just after injury of tendon, which are replaced by tendon-derived mesenchymal cells with time. In conclusion, the tendon chimeric rat and bone marrow chimeric rat using GFP rats were beneficial to follow tendon cells and circulating cells during tendon healing. Circulating mesenchymal cells primarily contribute to tendon healing for the first 3 days following injury. After this period, tendon-derived mesenchymal cells play a more important role in tendon repair.