Ectopic Ossification in the Regenerated Tissue at the Tendon Defect Site

Omachi, T; Sakai, T; Hiraawa, H; Hamada, T; Nakashima, M; Ono, Y; Ishizuka, S; Matsukawa, T; Takamatsu, A; Oda, T; Yamashita, S; Ishiguro, N
+Nagoya University Graduate School of Medicine, Nagoya, JAPAN
tadsakai@med.nagoya-u.ac.jp

INTRODUCTION: Recently, mesenchymal stem cells are known to participate in tendon healing. They are detected in synovium, bone marrow, adipose, and tendon itself and are proved to have multipotency. It is reported that chondrocyte phenotype and ectopic ossifications were acquired in the degenerated tendons. We have reported the chondrogenesis in the regenerated tissue at the tendon defect site in the rat model at the annual meeting of ORS 2011. For longer observation period, we have detected ectopic ossification in it. There have not been precise reports focused on the differential process of the regenerated tissue in tendon healing.

OBJECTIVE: To analyze expressions of the genes related to tendon, cartilage and ossification in the differential process of the regenerated tissue at the tendon defect site.

METHODS: Sprague-Dawley rats (12-15 weeks old) were anesthetized and 2mm square defects were made in the patellar tendons unilaterally. On the other side of the knees, sham operations were performed. Their tendons were harvested at 3days, 1, 2, 3, 6, and 20 weeks after surgery. The paraffin embedded sections of patellar tendons were made. They were stained with HE and Safranin O. Total RNA was extracted from the scar tissue in the defect. Real-time reverse-transcription PCR (RT-PCR) was used with ΔΔCt method to calculate the ratio of expression to sham side. Genes of interest were as follows: scleraxis (SCX), tenomodulin (TNMD) and type-I collagen (Col1) as tendon related; SOX9, aggrecan (AGG) and type-II collagen (Col2) as cartilage related; RUNX2, type-X collagen (Col10) as ossification related genes; GAPDH as internal control. The experimental design was approved by the animal study committee of Nagoya University School of Medicine, Japan.

RESULTS: In the sham side, the patellar tendon was mainly consisted with dense connective tissues. The cells in tendon resided between collagen fibers and the number of the cells were few (Fig. 1a). At 3 days, the defect was filled with regenerated tissue accompanied by many cells and few fibrous components (Fig. 1b). At 1 week, more fibrous components could be found (Fig. 1c). The tissue became more fibrous over time. To the contrary, cartilage and bone mass formation. Both of these studies suggested that initial control of stem cells would be essential for the fate of them to differentiate as tendon cells and for prevention of chondrogenesis and subsequent ossification. We will try to analyze the effect of the BMP 12 sustained releasing collagen sponge to our tendon defect model in the future study.

DISCUSSION: Safranin O stain revealed the existence of proteoglycan at 1 week and continued until 20 weeks (Fig. 2a-c). It is reported that pretreatment of mouse embryo mesenchymal stem cells with the retinoic acid receptor-γ agonist blocked chondrogenesis and bone mass formation. Both of these studies suggested that initial control of stem cells would be essential for the fate of them to differentiate as tendon cells and for prevention of chondrogenesis and subsequent ossification. We will try to analyze the effect of the BMP-12 sustained releasing collagen sponge to our tendon defect model in the future study.

SIGNIFICANCE: The healing pattern of dense connective tissue is not fully studied. This information will be important for the future studies that attempt to acquire better healing of dense connective tissue.

REFERENCES:
5)Lee J et al. 2011 PLoS ONE

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