Achilles tendon derived from LacZ of Achilles tendon in rats, treated with BMP-7 for meniscal defect in a rat model. The rats were sacrificed 4 weeks after the surgery. As control groups, transplantation of Achilles tendon was harvested and transplanted into the meniscal defect. The rats were sacrificed 4 weeks after the injection. (Study 2) Transplantation of Achilles tendon treated with BMP-7 in a rat massive meniscus defect model. Native cells in the Achilles tendon contributed to meniscal regeneration in this model.

488 TRANSPLANTATION OF ACHILLES TENDON TREATED WITH BMP-7 PROMOTED MENISCUS REGENERATION IN A RAT MASSIVE MENISCUS DEFECT MODEL

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Purpose: To preserve meniscus function, various meniscal substitutions such as meniscal allograft, collagen meniscus implant, and artificial materials have been tried in animal experiments or clinical studies. Transplantation of tendon is also one of possible treatments. BMP-7 is known to induce cartilage formation. Here we investigated the effect of BMP-7 on ectopic cartilage formation of tendon and outcome of transplantation of Achilles tendon treated with BMP-7 in a rat massive meniscus defect model.

Methods: (Study 1) Ectopic cartilage formation of tendon. After exposure of Achilles tendon in rats, 1 μg of BMP-7 was injected into the tendon located anatomically. The tendon was evaluated histologically at 2, 3, and 4 weeks after the injection. (Study 2) Transplantation of Achilles tendon treated with BMP-7 for meniscal defect in a rat model. Untreated Achilles tendon was harvested and 1 μg BMP-7 was injected. After anterior half of medial meniscus was resected, tendon treated with BMP-7 was transplanted into the meniscal defect. The rats were sacrificed at 4, 8, and 12 weeks after the surgery. As control groups, transplantation of Achilles tendon untreated with BMP-7 or only meniscectomy were performed. (Study 3) Analysis of cell kinetic. Achilles tendon derived from LacZ expressing rats were transplanted into meniscal defect of the wild rats.

Results: (Study 1) Injection of BMP-7 into Achilles tendon induced chondrocyte differentiation of tendon cells at two weeks. The number of chondrocytes evaluated with safranin-o staining, and type II collagen immunostaining, increased at 3 and 4 weeks. (Study 2) Macrophocically, transplantation of Achilles tendon irrespective of treatment of BMP-7 promoted meniscus regeneration. Microscopically, matrix of regenerated meniscus was already greater stained with safranin-o and type II collagen at 4 weeks and the meniscus became close to native meniscus at 12 weeks in the BMP-7 treated tendon transplantation group. Quantification analyses demonstrated that the size of meniscus, histological score for regenerated meniscus, and histological score for articular cartilage were better in the BMP-7 treated tendon transplantation group than in other two groups (p = 0.05; n = 6). (Study 3) When LacZ expressing Achilles tendon was transplanted, LacZ positive cells were detected within the transplanted tendon tissue.

Conclusions: BMP-7 induced ectopic cartilage formation of tendon and transplantation of Achilles tendon treated with BMP-7 promoted meniscus regeneration and prevented cartilage degeneration in a rat massive meniscus defect model. Native cells in the Achilles tendon contributed to meniscal regeneration in this model.

489 CHONDROGENIC, INFLAMMATORY AND FIBROTIC PROCESS IN PATHOLOGY OF IDIOPATHIC FROZEN SHOULDERS

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Purpose: To elucidate the chondrogenic, inflammatory, and fibrotic differentiation process in the pathogenesis of idiopathic frozen shoulders. Methods: The protocols of this study were approved by both institutional review boards of Funabashi Orthopaedic Clinic and Tohoku University. From July 2007 to June 2009, we performed arthroscopic capsular release in 12 patients with idiopathic frozen shoulders, whose condition had failed to improve or had deteriorated after 6-months of conservative treatment. As a control group, 16 patients with rotator cuff tears without limited range of motion were selected. The difference of age distribution between these two groups was not statistically significant. Biopsy materials from the rotator interval capsule, middle glenohumeral ligament (MGHL), and inferior glenohumeral ligament (IGHL) were obtained during arthroscopic surgery. The number of cells was counted and the tissue elasticity of the samples was calculated by scanning acoustic microscopy (SAM). The amount of glycosaminoglycan content was assessed by alcian blue staining. Mast cells were stained with toluidine blue. Gene and protein expressions related to chondrogenesis, inflammation, and fibrosis were analyzed by quantitative polymerase chain reaction (qPCR), in situ hybridization (ISH), and immunohistochemistry (IHC). Furthermore, the total genes of the two groups were compared by DNA microarray analysis. SAM images of IGHL were compared with IHC of collagen type I and alcin blue staining and Pearson’s product-moment correlation coefficient was calculated.

Results: The collagen bundles were dense with less space in idiopathic frozen shoulders, but the bundles were sparse and well-organized in shoulders with rotator cuff tears. Though the number of cells was significantly higher in idiopathic frozen shoulders, there were few cells expressing immunoreactivity of Ki-67 in both groups. The capsular tissue was significantly stiffer in idiopathic frozen shoulders by SAM. Staining intensity of alcin blue was significantly stronger in idiopathic frozen shoulders. Gene expressions related to fibrosis (COL1A1, COL3A1, PDGFB, α-SMA, and Substance P), inflammation (IL-1β), and chondrogenesis (ACAN, COL2A1, COLX1, FO3, and FOSB) were not changed in both groups. Fibroblast-like cells expressed ACAN signals in idiopathic frozen shoulders by ISH. Immunoreactivity of collagen type I and vimentin was stronger in idiopathic frozen shoulders. Immunoreactivity of α-SMA was not detected in fibroblast-like cells, but was detected in blood vessels in both groups. Comparing gray scale images of SAM, high sound speed area or low sound speed area did not correspond with any images in IHC of collagen type I and alcin blue.