INTRODUCTION:
Tenomodulin is a type II transmembrane protein that is expressed by the cells in the dense connective tissues. Its secreted C-terminal cysteine rich domain acts as a regulator of cell proliferation and differentiation, and has anti-angiogenic effect through inhibition of proliferation of endothelial cells. Scleraxis is a basic helix-loop-helix transcription factor and expressed in the progenitor of tendons. It is an upstream regulator of tenomodulin. Scleraxis and tenomodulin participate in differentiation of tendon in the development and have recently been used as markers of tendon cells. When a tendon is damaged, regenerated scar tissue arises. Usually, the strength of this tissue does not recover to normal. On the other hand, mature tendon is dense connective tissue with poorly formed blood vessels, and is reported that angiogenesis causes deterioration and pain in tendinosis. Tenomodulin has been supposed to maintain the strength of tendon, because of its anti-angiogenic effect. Thus, control of angiogenesis of the regenerated tissues will lead to better tendon healing. Because there have not been precise reports focused on that, the aim of this study is to examine the differentiation state of the cells and the anti-angiogenic effect of tenomodulin in the regenerated tissue.

METHODS:
Tendon defect model: Sprague-Dawley rats (12-15 weeks old) were anesthetized and 2mm square defects were made in the patellar tendons unilaterally. The tendons were harvested at 3days, 1, 2, 3 and 6 weeks after surgery. On the other side of the knees, sham operations were performed and were used as controls. Histology: The paraffin embedded sections of patellar tendons were made. They were stained with HE and Safranin O. The numbers of vessels within 1 mm square of the scar tissues were counted.
qRT-PCR: Total RNA was extracted from the half piece of the scar tissue in the defect. Real-time reverse-transcription PCR was used with ΔΔCT method to calculate the ratio of expression to sham side. Genes of interest were as follows: VEGF as angiogenesis related; scleraxis, tenomodulin and type-I collagen as tendon related; SOX9, aggrecan and type-II collagen as cartilage related genes; GAPDH as internal control.

RESULTS: Histology: In the sham side, the patellar tendons were mainly consisted with dense connective tissues. The cells in tendon were resided between collagen fibers and the number of the cells were few. Vessels were detected around the tendons, but not within them (Fig. 1a). 3 days after the surgery, defects of patellar tendons were filled with regenerated tissues, in which there were many cells and vessels, and few fibrous components (Fig. 1b). At 1 week, more fibrous components could be found obviously (Fig. 1c). The tissues became more fibrous over time, however, its cellularity was decreased conversely (Fig. 1d). Safranin O stain revealed the existence of proteoglycans at week 3days (a), 1(c) and 6(d) weeks after surgery. Vessels were increased within 3 days after surgery. Then they were decreased at 2 weeks and the number of them did not change significantly after that (Fig. 3a) .
RT-PCR: VEGF was upregulated at the third day and returned to the normal level at 3 weeks (Fig. 3b). Tenomodulin was upregulated at 1 week. Scleraxis was up-regulated at 2weeks, later than tenomodulin. Both of them were returned to normal level at 3 weeks (Fig. 4a). SOX9 was up-regulated at 1 week and the expression persisted for 6weeks. Aggrecan was up-regulated at 1 week and the expression decreased gradually (Fig. 4b).

DISCUSSION: Safranin O stain revealed the existence of proteoglycans in the regenerated tissue (Fig. 2). Expression of tendon markers returned to normal at 3 weeks (Fig. 4a). On the other hand, cartilage related markers were kept upregulated until 6 weeks (Fig. 4b). Recently, some stem cells are advocated for tendon healing, which have multipotency to make adipose, bone, tendon, and cartilage tissue. They could partici-pate in the formation of the regenerated tissue, that was rather cartilaginous. The erroneous differentiation of stem cells might occur in this site. Though scleraxis is an upstream regulator of tenomodulin, tenomodulin was upregulated earlier than scleraxis. Considering early increase of the number of vessels and VEGF mRNA expression, the early upregulation of tenomodulin seemed to respond to angiogenesis.

CONCLUSION:
In the regenerated tissues at the tendon defect site, proteoglycans were detected and cartilage related genes were upregulated. It is speculated that to modulate differentiation of stem cells could be important to make the regenerated tissues more tendinous. The earlier upregulation of tenomodulin than its up-stream regulator, scleraxis, seemed to respond to angiogenesis.

REFERENCES:
1) Sharma P. and Maflulli N. 2006. J Musculoskeletal Neuronal Interact