Expression of Wnt Pathway Mediators in Metaplastic Tissue in Animal Model and Clinical Samples of Tendinopathy – Potential Effects of Wnts on the Erroneous Differentiation of Tendon-Derived Stem Cells (TDSCs) in the Pathogenesis

INTRODUCTION:
Tissue metaplasia is observed in both animal model and clinical samples of tendinopathy. BMP2-induced non-tenogenic differentiation of tendon-derived stem cells (TDSCs) and has been suggested to contribute to the pathogenesis. The Wnt signaling pathway plays a vital role in pathological calcification and was shown to cross-talk with the BMP signaling pathway. This study aimed to examine the spatial-temporal expression of Wnt pathway mediators in an ossified failed tendon healing animal model and clinical samples of tendinopathy. The effect of Wnt3A on the osteogenic differentiation of TDSCs was also examined.

METHODS:
The study was approved by the animal research ethics committee and clinical research ethics committee of the authors’ institution. Ossified failed tendon healing was induced by the injection of collagenase into the patellar tendon of rats. Saline injection was performed in another set of rat and served as controls. At week 2, 4, 8, 12 and 16, the tendons were harvested for immunohistochemical staining of Wnt3A, Lrp5, Tcf7 and β-catenin. Patellar tendon samples were obtained from 15 patients with tendinopathy (14 unossified and 1 ossified) and 15 controls undergoing ACL reconstruction with healthy patellar tendon. The Wnt signaling pathway and its effect on TDSCs was examined.

RESULTS:
There were increased expression of Wnt3A (Figures 1, 3), Lrp5 (results not shown), Tcf7 (results not shown) and β-catenin (Figure 2, 4) in the healing tendon cells, chondrocyte-like cells and ossified deposits in the animal model and clinical samples of tendinopathy. Wnt3A increased ALP activity (results not shown), calcium nodule formation (Figure 5) and expression of osteogenic markers (Figure 6) in TDSCs.

DISCUSSION:
Activation of the Wnt signaling pathway and its effect on TDSCs might contribute to tissue metaplasia. The erroneous differentiation of TDSCs might reduce the pool of stem/progenitor cells for tendon repair after injury, resulting in failed healing in tendinopathy.

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Figure 1 Photomicrographs showing immunohistochemical staining of Wnt3A in the (A) saline control at week 16, and the collagenase-induced injured tendon at (B) week 2, (C) week 4, (D) week 8, (E) week 12 and (F) week 16. Magnification: 400x, arrowhead: chondrocyte-like cells; CR: ossified region; ▲: blood vessels, n=5/time point.

Figure 2 Photomicrographs showing immunohistochemical staining of β-catenin in the patellar tendon in the (A) saline control at week 16, and the collagenase-induced injured tendon at (B) week 2, (C) week 4, (D) week 8, (E) week 12 and (F) week 16. Magnification: 400x, arrowhead: chondrocyte-like cells; CR: ossified region; ▲: blood vessels, n=5/time point.

Figure 3 Photomicrographs showing immunohistochemical staining of Wnt3A in the (A) healthy human patellar tendon, (B) un-ossifying and (C) ossifying clinical tendinopathy samples, arrowhead: chondrocyte-like cells; △: blood vessels, CR: ossified region; magnification: 400X, Scale bar=50μm.

Figure 4 Photomicrographs showing immunohistochemical staining of β-catenin in the (A) healthy human patellar tendon, (B) un-ossifying and (C) ossifying clinical tendinopathy samples, arrowhead: chondrocyte-like cells; △: blood vessels, CR: ossified region, magnification: 400X, Scale bar=50μm.

Figure 5 Effect of Wnt3A on the mineralization of TDSCs. (A-D) Alizarin red S staining. A, C: Wnt3A treated; B, D: control. C, D Magnification: 100x. (E) Quantization of bound Alizarin red S. * p<0.05, n=5/group.

Figure 6 Boxplots showing the mRNA expression of Bglap (A), Spp1 (B) and Alpi (C) in TDSCs upon treatment with Wnt3A in vitro (n=5/group). Relative expression is equal to the expression of the osteogenic markers in relative to the β-actin expression. *p<0.05, Blue: Control; Green: Wnt3A.