INTRODUCTION:

The removal of central one-third of patella tendon is commonly performed to harvest the autograft for anterior cruciate ligament (ACL) reconstruction, and is also used as a model to study tendon healing. The repairing process after acute injury is well documented, comprising of inflammatory, proliferative, matrix synthesis and remodeling phases. However, the tendon heals with poor tissue quality and mechanical strength.

Ectopic ossification after midpoint tenotomy of rat or mice Achilles tendon has been reported. Clinically, calcification and tendinopathic-like changes were observed in some patients after acute injury such as Achilles tendon rupture, and in patellar tendon donor site after ACL reconstruction. However, in previous studies using the central one-third patellar tendon injury model, there were no reports on the presence of chondrocytes or calcification in the tendon mid-substance.

In this study, we aimed to examine if chondrocyte phenotype and ossification was present inside wound after removal of the central one-third of the patellar tendon with follow up of 3 months.

METHODS:

Animal surgery

This study was approved by the Animal Research Ethics Committee of the authors’ institution. SD male adult rats (6-8 weeks, n=18) were operated with central one-third of the patellar tendon (1 x 4mm) removed. The contralateral knee with skin injury served as sham control.

Histological assessment

At week 2, 4 and 12 post-injury, the patellar tendons were harvested (n=6 for each time point). 5-µm thick paraffin sections were used for histology. The presence of ectopic ossification was examined with von Kossa stain and immunohistochemical staining of collagen type X while chondrogenesis was examined by cell morphology, immunohistochemical staining of collagen type II and sox 9.

Measurement of calcified area

Image analysis using Image Pro Plus was performed on von Kossa stained sections to measure % calcified area in whole tendon. Statistical analysis was done using SPSS. The data in injury groups were compared with control at week 12 using Kruskal-Wallis test followed by post-hoc comparison using Mann-Whitney U test. p<0.050 was regarded as statistically significant.

RESULTS:

Chondrocyte-like cells were observed at week 4. Calcific areas as confirmed by von Kossa staining and surrounded by chondrocyte-like cells could be observed in three out of six samples (50%) at week 12. The median percentage area with positive von Kossa signal for 6 samples was 0.001% (range: 0-1.46%). There was marginally statistically insignificant difference in the percentage area with von Kossa stain in the week 12 injury group compared to that in the control group (overall: p=0.020; post-hoc comparison: p=0.089). Strong sox 9 and weak collagen type II were observed in tendon fibroblasts at week 4 and were reduced or absent at week 12. Instead, sox 9, collagen type II and type X were expressed in chondrocyte-like cells and calcific areas at week 12. (Figure 1)

DISCUSSION:

This study reported chondrometaplasia and ossification in the central one-third patellar tendon injury. Chondrometaplasia was observed in some samples from week 4 after injury while ectopic ossification was observed in 50% of samples at week 12. We further confirmed that these calcific deposits in the mid-substances were formed by endochondral ossification as shown by the expression of collagen type X, which is consistent with previous report in Achilles tendon repair.

The expression of sox 9 and collagen type II in tendon fibroblasts preceded their expression in chondrocyte-like cells and calcific area after injury, suggesting that erroneous healing tendon cell differentiation to chondrocytes or osteoblasts under aberrant mechanical and biological environment could be the mechanism of ectopic chondrogenesis and ossification. The erroneous deposition of the extracellular matrix as a result of presence of chondrocyte-like cells and calcification might negatively impact the material property of patellar tendon after acute injury, and partially account for the poor tissue quality. Further studies could follow the ossification process longitudinally for longer time. Functional outcome such as gait pattern could be investigated.

In conclusion, ectopic chondrogenesis and ossification were observed in some samples after acute patellar tendon injury. The erroneous deposition of extracellular matrix due to the acquisition of different cell phenotypes might partially account for the poor tissue quality after acute injury. As these changes were also observed in clinical and animal models of chronic tendinopathy, similar biological pathway might be activated in both conditions.

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REFERENCES:


Figure 1. Histological images showing (A-D) von Kossa staining and (E-H) immunohistochemical staining of collagen type II in rat central one-third patellar tendon injury model in (A, E) contralateral control at week 12; (B, F) week 2 post-injury; (C, G); week 4 post-injury; (D, H) week 12 post-injury. Magnification: 200X.

Von Kossa staining was observed in the calcified areas in 3 out of 6 samples at week 12 after injury. Collagen type II was expressed at week 4 in matrices surrounding chondrocyte-like cells and tendon fibroblasts inside wound, and at week 12 post-injury in calcified area and around uncalcified chondrocyte-like cells. There was no von Kossa staining or immunopositive signal of collagen type II in the week-12 control. Arrows = chondrocyte-like cells, arrowhead = tendon fibroblasts, and “CR” = calcified region.