INTRODUCTION:

The pathogenesis of tendinopathy remains unclear. Ectopic expression of chondro-osteogenic BMP-2, -4 and -7 was observed in clinical samples and animal model of tendinopathy. We reported that BMP-2 promoted non-tenogenic differentiation of tendon-derived stem cells (TDSCs) in vitro. We hypothesized that TDSCs isolated from the collagenase-induced (CI) tendinopathy animal model proliferated slower and showed higher chondro-osteogenic differentiation potential compared to TDSCs isolated from healthy animals. Based on these observations, we hypothesized that the sensitization of the chondro-osteogenic BMP/Smad signaling pathway in TDSCs might account for the higher chondro-osteogenic differentiation potential of TDSCs isolated from the CI model. This study therefore aimed to compare the activation state of chondro-osteogenic BMP/Smad pathway at basal level and upon BMP-2 stimulation in TDSCs isolated from CI model and healthy animals.

METHODS:

The study was approved by the animal research ethics committee of the authors’ institution. TDSCs were isolated from patellar tendon of 4-6-week-old male Sprague-Dawley rat with intratendonous injection of collagenase for 2 weeks (CI group) and from healthy patellar tendon (HT group). The isolated cells were confirmed to be stem cells by their colony-forming ability, multi-lineage differentiation potential and they showed similar expression of CD90, CD44 and CD45. TDSCs (HT) and TDSCs (CI) at passage 4 were used in this study. TDSCs from both sources were cultured in basal complete medium (LG-DMEM containing 10% FBS, 100U/ml penicillin, 100μg/ml streptomycin) were subjected to mRNA and protein analysis of expression of BMPs and BMP receptors using qRT-PCR and Western blotting (WB), respectively. To investigate the response of both cell types to BMP-2 stimulation, TDSCs from both sources were treated with rhBMP-2 (100ng/ml) for 0, 15, 30 and 60 minutes and the expression of phosphorylated Smad 1/5/8 (pSmad 1/5/8) was examined by WB while the nuclear translocation of pSmad 1/5/8 was examined by immunocytochemical staining (ICC) and image analysis. The comparison of targeted mRNA and protein expression between the TDSCs (CI) group and TDSCs (HT) group was done using Mann-Whitney U test or ANOVA for repeated measures.

RESULTS:

TDSCs (CI) expressed significantly higher mRNA levels of Bmp2 (p=0.006), Bmp4 (p=0.010), Bmpr1a (p=0.004) and Bmpr1b (p=0.016) but not Bmpr2 (p=0.873) compared to TDSCs (HT) (Figure 1). TDSCs (CI) also expressed significantly higher protein levels of BMP-4 (p=0.021), BMP-7 (p=0.021), BMPR-IB (p=0.050), BMPR-IB (p=0.034) and BMPR-II (p=0.034) compared to TDSCs (HT) (Figure 2). There was higher expression of total Smad 1/5/8, but not pSmad 1/5/8, in TDSCs (CI) compared to TDSCs (HT) (Figures 3). The addition of BMP-2 in both TDSCs (HT) and TDSCs (CI) increased the expression of pSmad 1/5/8 (Figure 3). The expression of total Smad 1/5/8 also increased upon BMP-2 stimulation in both groups (Figure 3). There was higher expression pSmad 1/5/8 and total Smad in TDSCs (CI) compared to TDSCs (HT) and similar results were obtained in 4 independent experiments (Figure 3). Similar to the results in Western blotting, the expression of pSmad 1/5/8 was weak in both groups at basal level, as shown in immunocytochemistry (Figure 4). The addition of BMP-2 increased the expression of nuclear pSmad 1/5/8 in both TDSCs (CI) and TDSCs (HT) (Figure 4).

DISCUSSION:

Our results showed that TDSCs (CI) expressed higher levels of chondro-osteogenic BMPs (BMP-2, BMP-4, BMP-7) and BMP receptors (BMPR-IA, BMPR-IB, BMPR-II) compared to TDSCs (HT). TDSCs (CI) were also more sensitive to BMP-2 stimulation as indicated by the higher expression of phosphorylated Smad 1/5/8.

SIGNIFICANCE:

The sensitization of the BMP/Smad pathway might account for the higher chondro-osteogenic differentiation potential and altered fate of TDSCs in the CI animal model of tendinopathy. This might be a possible mechanism contributing to the pathogenesis of tendinopathy.

REFERENCES:


ACKNOWLEDGEMENT:

This research project was made possible by equipment/resources donated by The Hong Kong Jockey Club Charities Trust.