Mechanical Over-loading Induced Non-tenocyte Differentiation Of Tscs Is Not Reversible By Rest

Jianying Zhang, James H-C. Wang.
University of Pittsburgh School of Medicine, Pittsburgh, PA, USA.

Disclosures: J. Zhang: None. J.H. Wang: None.

Introduction: Tendinopathy affects millions of Americans in both athletic and occupational settings and costs billions of healthcare dollars every year. Despite its prevalence, the cellular and molecular mechanism of this tendon disorder is poorly understood. Our previous in vitro and in vivo studies showed that mechanical over-loading may cause tendinopathy by upregulating non-tenocyte related genes in affected tendons, which induces aberrant differentiation of tendon stem cells (TSCs) into non-tenocytes [1] [2]. However, it is not known whether this loading-induced, non-tenocyte differentiation effect is transient or long lasting. If long lasting, it may lead to the development of non-tendinous tissues, a hallmark of degenerative tendinopathy. Therefore, we undertook this study to test the hypothesis that upregulation of non-tenocyte related gene expression by mechanical-over loading is not reversible by rest.

Methods: In vitro cell stretching experiments - Human TSCs (hTSCs) were stretched to 4% and 8% for 12 hrs using a custom designed cell stretching apparatus described in our previous study [1]. These two stretching magnitudes are considered as moderate and excessive mechanical loading on cells, respectively. Stretched cells were allowed to rest for 15 days in cell cultures. Then, cells were harvested and analyzed by qRT-PCR to determine the expression of tenocyte and non-tenocyte related genes. In vivo cell implantation experiments - hTSCs were cyclically stretched to 4% and 8% for 12 hrs, harvested, resuspended in PBS and then injected into the patellar or Achilles tendons of nude rats. Five days after implantation, cells were harvested and their gene expression profiles were analyzed by qRT-PCR using human specific primers.

Statistical data analysis - One-way ANOVA was used followed by Fisher’s PLSD test for multiple comparisons. When P-values were less than 0.05, the two groups compared were considered to be significantly different.

Results: In the in vitro study, 4% stretching followed by 15 days of rest elevated the expression of only collagen I and collagen III (tenocyte related genes), while collagen II, Sox-9, PPARγ and Runx2 (non-tenocyte related genes) registered no change in expression levels when compared to the control cells that were not stretched (Fig. 1A). However, hTSCs stretched to 8% showed upregulated expression of both tenocyte and non-tenocyte related genes, even after 15 days of rest (Fig. 1B). These upregulated gene expression profiles were similar to those at the end of stretching published in our previous study [1].

When hTSCs stretched to 4% were implanted into rat patellar and Achilles tendons in vivo only tenocyte-related genes (collagen I and tenascin C) were upregulated (Fig. 2A, 3A). However implantation of hTSCs that had been subjected to 8% stretching resulted in the upregulation of both tenocyte and non-tenocyte related genes after implantation (Fig. 2B, 3B). These results are consistent with the above findings of our in vitro study.
**Discussion:** Both in vitro and in vivo studies we performed on hTSCs showed that mechanical over-loading (8%) can specifically induce non-tenocyte differentiation of TSCs. More importantly, once TSCs begin differentiation, the process becomes irreversible, even after removal of the mechanical load. These results indicate that mechanical over-loading may lead to the development of tendinopathy by inducing the non-reversible upregulation of non-tenocyte gene expression in TSCs. This may lead to the development of degenerative tendinopathy, manifested by the formation of lipids, cartilage-like tissues, and calcification, either alone or in combination.

**Significance:** Mechanical over-loading appears to be a risk factor for the development of tendinopathy. Therefore, the findings of this study suggest that tendinopathy treatments can be improved by optimizing training regimens for professional athletes and laymen, to prevent the occurrence of such loading-induced tendinopathy and devising methods to curb the non-tenocyte differentiation of TSCs.

**Fig. 1** Gene expression profiles induced by mechanical over-loading is not reversed after rest *in vitro*. Expression of tenocyte and non-tenocyte related genes after 4% (A) and 8% (B) stretching, followed by 15 days of rest. Blue bars are controls without loading. (*P < 0.05, with respect to controls). The gene expression patterns are similar to those at the end of mechanical loading [1].
Fig. 2 Gene expression profiles induced by mechanical over-loading are not reversed after implantation in vivo. Tenocyte and non-tenocyte gene expression in rat patellar tendons in vivo. Blue bars are controls without loading. hTSCs stretched to 4% (A) showed specific up-regulation of collagen I (Coll I) and Tenascin C (Tena C), but not the non-tenocyte related genes. However, 8% stretching (B) up-regulated both tenocyte and non-tenocyte genes to varying degrees. Note that human-specific primers were used in qRT-PCRs to specifically amplify human genes in rats. (*P<0.05, with respect to the respective controls).
Fig. 3 Gene expression profiles induced by mechanical over-loading are not reversed after implantation into Achilles tendons *in vivo*. Tenocyte and non-tenocyte gene expression in rat Achilles tendons. Blue bars are controls without loading. Up-regulation of collagen I (Coll I) and Tenascin C (Tena C) is observed in hTSCs stretched to 4% (A). However, 8% stretching (B) up-regulated both tenocyte and non-tenocyte genes. Human-specific primers were used to specifically amplify human genes in rats. (*)P<0.05, with respect to the respective controls.