Discovery of a Tendon/Ligament Stem Cell Causing Lumbar Spinal Stenosis

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INTRODUCTION:
Currently the identity of the stem cell giving rise to mature tenocytes in tendons and ligaments remains unknown. While several lineage markers of tenocytes have been identified, the identity of the tendon/ligament stem cell remains elusive. Lumbar spinal stenosis (LSS) is a common condition of narrowing of the spinal canal due in part to ligament hypertrophy resulting in nerve compression and lower extremity pain in 11% of older adults in the US. While this pathogenesis suggests that the LSS is caused by derangements in the self-renewal or differentiation capacity of a TLSC, identification of the TLSC is a prerequisite to understanding LSS pathogenesis.

METHODS:
Here we screen cell surface markers and fractionate tendon/ligamentum cells using a 7 color FACS panel and develop transplantation-based serial self-renewal and differentiation hierarchy assays to assess TLSC stemness, transplantation-based tendon organoid formation assays to determine the tenocyte production capacity of each of these cells. We also established a mouse model of injury induced LSS. Hypertrophic and normal ligamentum flavum from patients was collected for analysis of TLSC identity and alterations in self-renewal and differentiation. Transcriptome analysis was performed to profile the TLSC gene-expression pattern. Immunofluorescence staining was used to identify TLSC and mature tenocyte localization in native tendon tissue.

RESULTS:
We identify a postnatal tendon/ligament stem cell (TLSC) defined by the markers (LIN-Thy-Sca1-CD73+CD140a-). TLSCs display formal evidence of stemness in the transplantation-based self-renewal and sit at the apex of their differentiation hierarchy in serial transplantation assays. While undergoing self-renewal, they are capable of generating mature tenocytes during in vivo tendon organoid formation assays. The identity of the TLSC and its associated stemness properties were similar in all tendon/ligament tissues examined, suggesting that this represents a universal TLSC. Human counterpart of TLSCs were identified in ligamentum flavum specimens and display a conserved differentiation hierarchy and stemness features. In a mouse model of injury induced LSS, this TLSC is markedly expanded in the ligamentum flavum, and isolation and transplantation of the TLSC from the LSS mouse model to secondary hosts demonstrates that LSS enhances the tenocyte production capacity of this TLSC. This TLSC is expanded in the ligamentum flavum of LSS patients. Further, xenografting of equivalent numbers of human ligamentum flavum TLSCs from LSS and control patients demonstrates that the tenocyte production capacity of LSS patient derived TLSCs is enhanced on a per cell basis, demonstrating that the key feature of LSS pathology is intrinsic to the TLSC identified here.

DISCUSSION:
Here we have identified the stem cell (TLSC) in postnatal tendon/ligament tissue via *in vivo* stemness assays. Alterations of the self-renewal and differentiation of this TLSC provides an etiology for hypertrophic ligamentum flavum in LSS. Previously reported tendon stem or progenitor lineage markers such as Ctsk, Tppp3 or Scx can partially label tendon and TLSCs, though are not fully specific for this population.

SIGNIFICANCE:
Altogether, this work establishes new assays to formally assess stemness in tendon/ligament cells and uses these assays to identify a TLSC shared among tendon/ligament tissues in humans and mouse. Derangements in this cell are central to LSS pathology which provides an insight to the cellular basis of LSS patients and therefore makes TLSCs a potential therapeutic target for LSS, a disorder which currently lacks any disease modifying medical therapy.

REFERENCES:
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IMAGES:

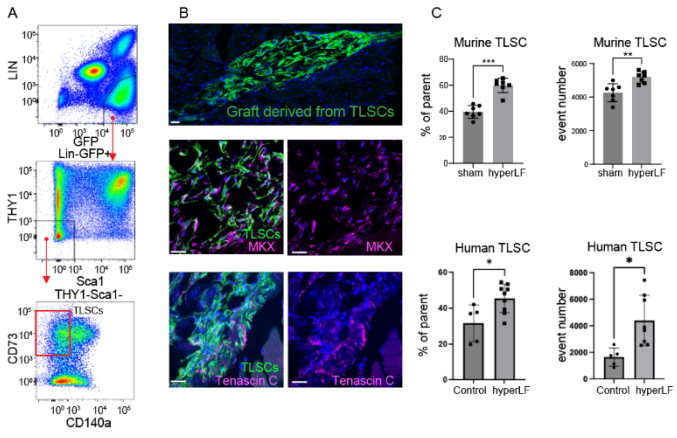

Figure 1| Identification of TLSCs and their derangements in LSS. **A**, Flow cytometry of cells from ligamentum flavum digests from P28 mice to identify TLSCs. LIN indicates CD31, CD45 and Ter119. Plots are representative of three independent experiments. **B**, Representative immunofluorescence staining of MKX and Tenascin C in TLSC derived tendon organoids 8 weeks after intramuscular transplantation. Scale bars, 50μm. **C**, Frequency and absolute numbers of TLSCs in ligamentum flavum between normal and hypertrophic ligamental flavum groups in both human surgical specimens (lower row) and in mice that underwent a surgical model of ligamentum flavum hypertrophy. Data are mean ± s.d.; two-tailed unpaired t-test.