Axin2+ Tendon Cells Proliferate, Differentiate, and Self-Renew In Vivo

Mor Grinstein*[1,3], Benjamin freedman*[2], Jenna Galloway[1,4]

1. Center for Regenerative Medicine Massachusetts General Hospital and Harvard Stem Cell Institute.[2.] Department of Orthopaedic Surgery, Beth Israel Deaconess Medical Center[3.] Tel Aviv Medical Center, CORAL, Israel. [4.] Department of Orthopaedic Surgery, Massachusetts General Hospital, Boston. *Equal contribution

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INTRODUCTION: Tendons connect and transfer force between the muscles and bones of the body, making them highly prone to injury. These injuries have slow and imperfect healing with limited treatment options, resulting in scarring and reduced mobility. Although adult tendon stem/progenitor cells have been identified through their characterization in cell culture and transplantation assay, our understanding of the function of unique native tendon cell populations and the pathways regulating their activities during normal homeostatic conditions and healing is largely unknown. The identification of unique cell populations that participate in tendon injuries' healing processes will help us to develop novel regenerative biology approaches to tendon injury and age-related tissue degeneration. Recently, we discovered a unique latent, injury-responsive, *Axin2*$^{+}$ tendon cell population regulated by their own Wnt secretion. These *Axin2*$^{+}$ progenitor cells proliferated and migrated to the wounded area, after tendon injury, adopting a rounded cell morphology, differentiating into tendon cells, and were able to transition into elongated tendon cells in the injured tendon area. To test the *Axin2*$^{+}$ tendon cell progenitor activity to proliferate, differentiate, and self-renew, we developed a unique cell transplantation assay that was transplanted into an injured tendon with the isolated *Axin2*$^{+}$ tendon progenitor cells. In this study, we sought to test the potential of the unique identified progenitor cell population, the *Axin2*$^{+}$ tendon cells, to proliferate differentiate, and self-renew after tendon injury, to test their progenitor characteristics in tendon injuries. The unique transplantation platform will enable us to deepen our study to better understand the function of these cells in tendon healing at different ages and in human tendons.

METHODS: To collect Axin2+ cells we tam-treated *Axin2CreErt2;TdTom* 3-month-old mice, We isolated 100-500 *Axin2*^{TdTom} cells from Achilles tendons from 4-month-old mice by FACS. We excluded dead cells and CD31+/CD45+/TER119+ cells. *Axin2*^{TdTom} cells from each mouse were plated and expanded separately for 60 days. Prior to transplantation, we performed flow cytometry on *Axin2*^{TdTom} cells to confirm they were TdTomato+ and quantify cell number. After obtaining approximately 4 million *Axin2*^{TdTom} cells, we combined *Axin2*^{TdTom} cells with alginate-based hydrogels and transplanted them into injured tendons of host immunodeficient mice Foxn1$^{Nu/Nu}$. Under general anesthesia with isoflurane, both hind limbs were shaved, prepped with 1:1 betadine/alcohol solution, and draped. Buprenorphine and bupivacaine (around the intended surgical site) were administered 30-60 minutes prior to the procedure. Microscissors were used to separate the plantaris longus from the Achilles tendon prior to performing a full-thickness partial width (50%) injury to the central midsubstance of the Achilles tendon with an 0.3 mm biopsy punch. A 1% oxidized RGD modified (DS20) alginate gel (VLVG, NovaMatrix) was synthesized [86,87] . Ultrapure alginate samples were first dissolved in Dulbecco's Modified Eagle Medium at 2.857% (wt/vol). Gels were then punched into 3mm diameter disks and rinsed in DMEM prior to implantation on the same day. The skin was closed using 4-0 vicryl and the animal returned to a regular cage activity. After 10 and 20 days, The Achilles' tendons were harvested for evaluation. All the procedures were done according to IACUC 2013N0000062 MGH.

RESULTS: After 10 and 20 days, we examined the site of transplantati
(TdTom) (Fig 1A-D). After quantifying the number of TdTom^{+} and tota
per area at 10 and 20 days post-injury and transplantation with approximat
cells differentiated and/or retained *Axin2*$^{+}$ identity, we performed smFIS
Axin2 and co-immunostained for TdTom. We found that 90%± 15 of the
Axin2^{TdTom} cells demonstrate the ability to differentiate into *Tnmd*-expres
that this latent tendon-resident cell population also have self-renewal pot

DISCUSSION: The novel developed transplantation assay using an alg
healing in tendons of immunodeficient host mice as Following transpla
cells and *Axin2*^{TdTom} cells that expressed *Axin2*+, providing strong eviden

SIGNIFICANCE/CLINICAL RELEVANCE: The finding of a new
promise for future therapeutics with these cells in the treatment of injure