The Role of Progranulin in Tendon Inflammation and Tendon Healing

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Disclosures:

LB Qualifying Statement: Data just became available over past couple weeks and therefore was not available for original ORS deadline - significance of this data however, warrants presentation at the 2014 meeting and for this reason we chose to submit as a late-breaking abstract

Introduction: Progranulin (PGRN) is a ~60kDa protein that is secreted as a ~90kDa molecule after post-translational modifications have occurred. Previous studies have shown that in a PGRN knock-out (KO) mouse are more susceptible to collagen-induced arthritis and PGRN, ameliorates the phenotype. Preliminary studies suggest its mechanism of action to be that of an antagonist in the inflammatory cascade, potentially through inhibiting the interaction of TNF-α ligand with its receptor. We hypothesize that deficiency of PGRN will lead to the development of a degenerative phenotype, similar to its effect on cartilage in the arthritic mouse model. We therefore expect that PGRN deficient mice would show increased tendinopathy and fibrosis in tendon tissue compared to WT. The tendon healing will be significantly delayed in PGRN deficient mice with increase in localized inflammation. Further, degenerated human tissue will have a correspondingly altered expression of PGRN compared to healthier tissues.

Methods: In Vitro: Primary tenocytes cultures were isolated from wild-type (WT) and PGRN knock-out (pgrn−/−) mouse tendons by treating the extracted tendon tissue with 0.2% collagenase and trypsin for 1hr at 37°C, and cultured in DMEM with 20% FBS. A MTT proliferation assay (BD Biosciences) was performed in triplicate on days 1, 3, and 7 post plating. One-Way ANOVA was performed to calculated significance difference. In Vivo: 3-.-. Three mouth old mouse patellar tendons were tenotomized. Wounded tendons were harvested at 2 (n=3) and 4 (n=3) weeks from Wt and KO mice. Samples of wounded and non-wounded tissue from each of the mice were embedded in paraffin, mounted, and stained for H&E. All animal studies were done under approved IACUC protocol. Human Tissue: Biceps tendons were collected from arthroplasty and tenodesis operations (IRB study#S13-01258) and were stained for PGRN expression. Slides were examined under 10X magnification.

Results: Tenocyte primary cell showed a statistically significant (*p<0.05) difference in rates of proliferation between WT and KO cells. H&E staining showed a gross difference in tendon tissue quality, with that WT tendon mice showing highly compact and organized fibrils, while KO mouse tendon showed fibrosis and disorganized fibrils. Moreover, in mouse wounding experiments, H&E staining of harvested wounded tendons 2 and 4 weeks after wounding showed an attenuated proliferative response and attenuated scar formation in the KO mouse compared to WT (figures not shown in abstract). Similarly, human biceps tendons, showed differences PGRN expression, areas with less fibrosis and healthier tendon tissue stained more strongly for endogenous PGRN expression compared to more degenerated tissue.

Discussion: These results provide support for PGRN having a role in the pathogenesis of tendon degeneration and repair. PGRN deficient had more degenerated tendon tissues, had less cellular proliferation surrounding their wounded tendons, formed thinner scars, and tenocytes isolated from these respective mice showed significant differences in rates of proliferation. Moreover, human biceps tendons had histology consistent with a less degenerate phenotype were found to stain more strongly for PGRN on immunohistochemistry studies. Future studies extending from these preliminary findings will include rescue experiments whereby PGRN will be introduced back to the KO mouse and its effect on the tissues and wounds, investigated. Tissue will be harvested at different time points to understand the role of PGRN in inflammation, proliferation and tendon remodeling. Detailed histological and biochemical analysis will be conducted to understand the PGRN dependent morphological and molecular mechanism in tendon physiology and wound healing.

in conclusion, PGRN appears to have a significant role in tendon physiology and healing and may be a potential molecular target for clinical applications in the future. Future applications may include alternatives to operative care for traumatic tendon injuries in the body, for instance, in the treatment of rotator cuff tears.

Significance: Preliminary results from this study may play a future role as a therapeutic molecular target for non-operative treatment regimens in tendon healing, including applications in rotator cuff tears, Achilles tendon ruptures, and tendinopathy pain.

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**Figure 1** – PGRN deficient tenocytes have significantly reduced rate of proliferation.
Figure 2 – PGRN deficient tendons (left panel) show more fibrosis and gross disorganization compared to Wt (right panel).

Figure 3 – Human tendon show higher endogenous PGRN expression in healthier tissues (left panel) while more degenerated samples (right panel) showed little to no PGRN staining on immunohistochemistry studies.