

Pathway-Level Expression Differences in Tenocytes Associate with Phenotypic Variation Between Canonical and Regenerative Healing

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INTRODUCTION: Tendon injuries are prevalent, and in adult mammals they often heal through the formation of scar tissue with reduced mechanical integrity.¹ However, adult Murphy Roths Large (MRL/MpJ, (MRL)) mice demonstrate enhanced regenerative capacity, seen as improved structural and mechanical tendon repair compared to canonically healing C57BL/6 (B6) mice after both acute and overuse injuries.¹ The pro-regenerative, MRL-like phenotype was stimulated in B6 cells seeded on substrates coated with the temporarily bioactive MRL decellularized provisional extracellular matrix (ECM) obtained 7 days post hole-punch injury and treated with proteins found to be particularly enriched in the secretome of healthy MRL tenocytes.¹ This MRL-like phenotype is characterized by increased mechanosensitivity, proliferation, migration, intercellular communication, and ECM deposition.¹ Minimal work has been done to characterize pathways enriched in the B6 tendon environment compared to that of the MRL mice, determine their roles in driving scar-mediated healing, and assess their potential to further augment the efficacy of an MRL-informed protein therapeutic. Thus, this study aims to identify these B6-enriched pathways, with the hypothesis that they will be associated with the lower proliferation, cell migration, ECM deposition, and intercellular communication observed previously in B6 tenocytes.

METHODS: Tenocytes were isolated from naive patellar tendons of male B6 and MRL mice, passaged three times, seeded in triplicate at 90% confluency on Nunc™EasYFlask™ culture flasks, and incubated for 24 hours with phenol red-free, serum-free media supplemented with 2X GlutaMAX™. Male mice were used since MRL males mice demonstrate a more heightened regenerative capacity compared to MRL females.¹ The conditioned media was then collected, concentrated via ultrafiltration centrifugation with a 3 kDa cutoff, normalized across samples to the total protein concentration found using a bicinchoninic acid assay, and outsourced to RayBiotech for analysis of cytokine concentrations.¹ Differentially expressed cytokines were considered for further analysis if they had ≥ 2 -fold change or were only detected in one genotype. The results were uploaded to Ingenuity Pathway Analysis (IPA; Qiagen) ($p \leq 0.05$ and $|Z\text{-score}| \geq 1.5$) to determine significant pathways.

RESULTS: IPA analysis revealed four upregulated pathways and seven downregulated pathways in the B6 mouse tendon environment compared to MRL. Upregulated pathways consisted of the coordinated lysosomal expression and regulation (CLEAR), phosphatase and tensin homolog (PTEN), peroxisome proliferator-activated receptor (PPAR), and gap junction signaling. Downregulated pathways included interleukin-15 (IL-15) production and myelination, sheddase, epithelial membrane protein (EMP), nuclear factor kappa B (NF-κB), and Sertoli cell-Sertoli cell junction signaling.

DISCUSSION: PTEN, the primary negative regulator of the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) pathway, reduces cell proliferation, migration, and stemness when upregulated (Fig. 1).² Elevated PTEN signaling is also associated with reduced extracellular signal-related kinase 1/2 and p38 mitogen-activated protein kinase activity, both of which were enriched in the MRL secretome in our prior analysis.^{1,3} Additionally, PTEN upregulation correlates with decreased myelination, CREB, and NF-κB, alongside increased autophagy via CLEAR signaling (Fig 1).⁴⁻⁷ These pathways further interconnect, with NF-κB downregulation promoting CLEAR and PPAR signaling while suppressing IL-15, whose ectodomain release is regulated by the sheddase pathway (Fig. 1).^{6,8,9} Through their effects on the PI3K/Akt pathway and pro-inflammatory cytokines, downregulation of NF-κB, IL-15, EMP signaling, and the sheddase pathway converge to limit cell proliferation, migration, and ECM deposition (Fig 1).⁶⁻¹¹ Reduced intercellular communication through tight junctions, adherens junctions, gap junctions, and desmosomes—well described in Sertoli cells—parallels the diminished junctional signaling observed in B6 tenocytes.^{1,12}

Taken together, these findings suggest that PTEN functions as a central node connecting multiple pathways that collectively suppress regenerative processes in B6 tenocytes. The pathway-level changes identified in the IPA analysis are consistent with the reduced proliferation, migration, ECM deposition, and intercellular communication we previously observed in B6 compared to MRL tenocytes. One exception is gap junction signaling, which IPA predicted to be upregulated in B6 tenocytes, whereas increased connexin-43 expression was observed in MRL tenocytes.¹ Due to the complexities of the IPA denoted pathway interactions and cellular processes, further research into the specific differences in gap junction signaling in B6 and MRL tenocytes is needed.

These findings highlight PTEN as a central suppressor of regenerative processes in B6 tenocytes. Inhibition of PTEN, which has been shown to enhance tissue repair through increasing cell proliferation and migration in the eye, lung, muscle, and nervous system, could similarly promote tendon regeneration.¹³ Moreover, PTEN inhibition could increase CREB expression, which has been shown to reduce scar formation in the tendon environment, and increase ECM deposition through its connections to pathways regulating pro-inflammatory cytokines.¹⁴ By modulating these interconnected pathways, PTEN inhibition represents a promising strategy to shift B6 tenocytes toward a more MRL-like regenerative phenotype and improve tendon healing outcomes.

SIGNIFICANCE/CLINICAL RELEVANCE: We identified pathways differentially regulated in B6 tenocytes that correlate with the expected phenotype of canonically healing tendons. Inhibition of PTEN, central to the pathway connections, could promote a more pro-regenerative phenotype in canonical healers.

REFERENCES: ¹Marvin+ 2024 *bioRxiv*, ²Goto+ 2025 *Nat Commun*, ³Kotlowski+ 2016 *J Diabetes Res*, ⁴Gaesser+ 2017 *Exp Neurol*, ⁵Liu+ 2014 *PLOS ONE*, ⁶Guo+ 2024 *Sig Transduct Target Ther*, ⁷Zhang+ 2020 *Cell Death Discov*, ⁸Kang+ 2018 *Cell Commun Signal*, ⁹Saftig+ 2011 *Eur J Cell Biol*, ¹⁰Pan+ 2020 *Int J Mol Med*, ¹¹Amin+ 2019 *Cancers (Basel)*, ¹²Lie+ 2014 *Int J Biochem Cell Biol*, ¹³Borges+ 2020 *Regen Med*, ¹⁴Wu+ 2023 *BMC Musculoskeletal Disord*, ¹⁵Bian+ 2019 *J Cell Mol Med*, ¹⁶Wang+ 2019 *J Cell Mol Med*, ¹⁷Guo+ 2024 *Sig Transduct Target Ther*

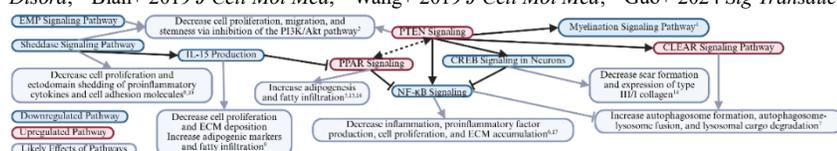


Figure 1: Interconnections of pathways found to be differentially regulated in B6 tenocytes compared to MRL tenocytes. Created in BioRender.