Fibroblast plasticity during tendon regeneration in zebrafish

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Fibroblasts are specialized cells that function to remodel the extracellular matrix (ECM) during tissue development, homeostasis, and repair. Recent single cell RNA sequencing studies have uncovered previously unappreciated heterogeneity within fibroblast populations. However, the *in vivo* function and plasticity of fibroblasts remain poorly understood. Combining live imaging and scRNA-seq, we identified numerous morphologically and transcriptionally distinct fibroblast populations in the zebrafish trunk arising from the sclerotome compartment of the somite. Further, RNA velocity analysis of our scRNA-seq dataset revealed the presence of a 'progenitor-like' blood vessel-associated 'perivascular fibroblast' population which gave rise to other specialized fibroblasts including tendon fibroblasts, tenocytes. To characterize this differentiation potential of fibroblasts *in vivo*, we developed a laser-induced tendon injury model. Zebrafish can quickly regenerate tenocytes (tendon fibroblasts) and the surrounding ECM post injury. Cell lineage tracing showed newly regenerated tenocytes arise from pre-existing sclerotome-derived fibroblasts. Using live imaging and single cell clonal analysis, we demonstrated that perivascular fibroblasts are actively recruited to the injury site where they proliferate and generate new tenocytes. This contrasts with their behavior during normal development wherein they remain associated with the vasculature, functioning as pericyte progenitors and depositing supportive ECM. Strikingly, other neighboring fibroblasts derived from the same sclerotome lineage, including uninjured tenocytes, show no such regenerative response. Surprisingly, perivascular fibroblast-derived pericytes also do not respond to tenocyte ablation, suggesting perivascular fibroblasts lose their regenerative capacity upon further differentiation. Finally, to elucidate the molecular mechanisms regulating fibroblast response to injury, we used small molecule inhibitors to block Hedgehog signaling. Drug treatment resulted in reduced tendon regeneration by preventing proliferation and trans-differentiation of responding fibroblasts into tenocytes between 1 to 2 days post injury. Altogether, our work demonstrates transcriptional and functional diversity among zebrafish trunk fibroblasts and establishes perivascular fibroblasts as tenocyte precursors and potential therapeutic targets for tendon regeneration.