43

Embryonic Expression of Prrx1 Identifies the Fibroblast Responsible for Scarring in the Mouse Ventral Dermis

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PURPOSE: Scarring and fibrosis are tremendous public health concerns, leading to excessive morbidity and mortality, in addition to countless healthcare dollars. Although there are many treatments for cutaneous scarring available, few have proven successful. Despite the lack of molecular therapies, there is a $12 billion annual market for the treatment of scarring in the United States alone. We previously identified a sub-population of fibroblasts—labeled by the embryonic expression of En1—responsible for the bulk of connective tissue deposition in the mouse dorsal dermis during embryonic development, cutaneous wound healing, and melanoma stroma formation. Herein, we identify and characterize the fibroblast sub-population responsible for scarring in the mouse ventral dermis.

METHODS: Fibroblasts with embryonic expression of Prrx1 were lineage traced by crossing Prrx1^{Cre} and ROSA26^{mTmG} mice. Prrx1-positive fibroblasts (PPFs) and Prrx1-negative fibroblasts (PNFs) were characterized using flow cytometry, histology, and ATAC-seq analysis at various stages of embryonic development. This lineage was ablated using a triple transgenic mouse expressing Cre-dependent simian diphtheria toxin receptor (DTR) and diphtheria toxin delivered to full thickness excisional wounds or injected prior to transplantation of melanoma tumor xenografts. Histology with picrosirius red and tensile strength testing of fully healed wounds were performed. A novel method for automated quantification of collagen fiber characteristics was performed.

RESULTS: Lineage tracing of fibroblasts within the ventral dermis revealed a sub-population, labeled by the embryonic expression of Prrx1, acting as the key contributor to connective tissue deposition during scar formation. This lineage increased as a proportion of total fibroblasts within the ventral dermis over the course of gestation, associated with the transition from scarless to scarring repair. Differential patterns of chromosomal accessibility based on ATAC-seq data further demonstrated the heterogeneic nature of fibroblasts within the ventral dermis. Ablation of PPFs resulted in diminished connective tissue deposition when examined after completion of wound healing (*p<0.05) without change in the tensile strength of the scar (p>0.05). PPF ablation prior to transplantation of melanoma tumor xenografts resulted in decreased tumor mass (*p<0.05). Analysis of collagen fiber characteristics demonstrated significant differences after PPF ablation versus control in staining intensity, fiber length/width/persistence, and branch points (*p<0.05), showing collagen matrix patterns similar to normal skin.

CONCLUSIONS: Analogous to findings in the dorsal dermis, fibroblasts of the ventral dermis show functional heterogeneity. Prrx1 identifies the fibroblast sub-population with fibrogenic potential in the ventral dermis. As in the dorsal dermis, selectively ablating this fibroblast sub-population leads to decreased cutaneous scarring. Further research into the role of PPFs holds promise for a novel therapeutic to decrease scarring and fibrosis.

44

Successful Reduction of Radiation Associated Skin Injury Utilizing Topical DFO in a Murine Breast Reconstruction Model

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