**Purpose:** Skin fibrosis amounts to significant morbidity due to the prevalence of trauma and burn injuries. Fibroblasts are chiefly responsible for extracellular matrix (ECM) deposition in the skin and are increasingly recognized to be a heterogeneous population comprised of multiple subpopulations with distinct functions and scarring propensity. While anatomic and embryonic origin are known to determine fibroblast heterogeneity, most prior work in mice has focused on the dorsal dermis. Here, we explore how embryonic lineage and dermal spatial location define fibroblast heterogeneity in the mouse ventral dermis in both homeostasis and in acute and chronic fibroses.

**Methods:** Prrx1 Cre;R26mTmG mice were used to identify two ventral dermal fibroblast lineages; Prrx1-positive fibroblasts (GFP+ PPFs), and Prrx1-negative (RFP+ PNFs). Fibrogenic potential was explored by comparing fibroblast abundance using FACS and histology: 1) throughout 6 developmental timepoints; embryonic day(E)16.5 (non-scarring), E18.5, postnatal days(P) 1, 30, and 60 (scarring) in wounds (acute fibrosis); 3) following irradiation (chronic fibrosis); and 4) after implantation of melanoma cells (chronic fibrosis). Reciprocal transplantation into the (non-scarring) oral dermis and co-localization with collagen type 1 by Imaris 24 hours after transplantation was used to assess whether fibrogenic potential was cell-intrinsic. Single-cell sequencing of PPFs and PNFs from unwounded and scarred ventral skin was performed using 10X Genomics. Manifold-based dimensionality reduction was used to delineate transcriptionally-distinct fibroblast clusters (subpopulations) and non-linear discriminant analyses were applied to determine cluster expression profiles and surface markers and thus identify papillary and dermal subpopulations. Gene-set enrichment analysis was performed to confirm cluster profibrotic potential, and immunofluorescence and FACS were performed to confirm findings at the protein level.

**Results:** PPFs progressively increased as a proportion of the total ventral fibroblasts over development, with the largest difference seen E16.5 to E18.5. In adult ventral skin, there were significantly more PPFs in wounded and irradiated skin, as well as in tumor stroma. PPFs transplanted into the oral mucosa exhibited 23.89% collagen I co-localization, whereas Wnt-1-positive (non-scarring) fibroblasts transplanted into the ventral dermis exhibited low collagen co-localization (1.49%), demonstrating that the fibrogenic potential of PPFs is cell-intrinsic. Single cell analysis revealed unique PPF and PNF subpopulations which responded differently to wounding; the papillary (CD26+) fibroblasts were largely PPFs and expanded with wounding, whereas reticular (Dlk1+) fibroblasts were PNFs and minimally responded to wounding.

**Conclusion:** For the first time, we highlight the functional heterogeneity of ventral dermal fibroblasts. We show how embryonic expression of paired related homeobox 1 (Prrx1) gives rise to two distinct fibroblast lineages comprised of unique fibroblast subpopulations with distinct fibrogenic potential. These data highlight that PPFs are responsible for the majority of connective tissue deposition in ventral skin and targeted modulation of PPF papillary subtypes may reduce the acute fibrosis during wound repair and the chronic fibrosis of tumor stroma formation.

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**Postnatal Engrailed-1 Expression Activates A Pro-Fibrotic Transcriptional Program In Wound Fibroblasts**

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**Purpose:** Skin scars represent a massive biomedical burden on patients, but the cellular mediators of scarring remain poorly understood. We previously showed that embryonic expression of *Engrailed-1* (*En-1*) defines a lineage of fibroblasts (*En-1*-positive fibroblasts; eEPFs) responsible for the deposition of fibrotic scar tissue in dorsal skin. More recently, we demonstrated that a subpopulation of *En-1*-negative fibroblasts (ENFs) activates *En-1* during adult wound healing and contributes to scar formation as *postnatally-derived* EPFs (pEPFs). However, it was not known if such postnatal *En-1* expression is accompanied by the acquisition of a pro-fibrotic phenotype. We thus sought to determine if pEPFs are a viable therapeutic target to minimize fibrosis in postnatal wound healing.

**Methods:** *En-1* CSG;Ai6 (*En-1*-positive cells GFP+, *En-1*-negative cells no reporter) mice were systemically induced with tamoxifen prior to dorsal excisional wounding.
This novel transgenic mouse model reliably distinguishes ENFs (GFP-CD26), embryonically-derived EPFs (GFP-CD26+), and postnatally-derived EPFs (GFP-). The scars and surrounding unwounded tissue were harvested upon complete wound healing (day 14), enzymatically-digested, and then sorted by fluorescence-activated cell sorting. Five sorted fibroblast populations (pEPFs from wounded skin; eEPFs from unwounded and wounded skin; and ENFs from unwounded and wounded skin) were then analyzed by bulk RNA-sequencing (experimental schematic in n = 2 biological replicates, 6 pooled mice each).

**Results:** Hierarchical clustering and principal components analysis of differentially expressed genes after wounding revealed that pEPFs clustered more closely with eEPFs than with ENFs. Both postnatally- and embryonically-derived EPFs showed increased expression of fibrosis-related genes in response to wounding, including Dpp4 (CD26). In contrast, ENFs showed increased expression of mechanotransduction signaling-related genes (Notch ligands Jag1, Dll1), suggesting that they are responsive to wound mechanical cues. Supporting these findings, gene set enrichment analysis of ranked whole genomes revealed that scar ENFs enriched for terms related to ECM adhesion and Notch signaling, while postnatal EPFs enriched for terms related to ECM production. Finally, we compared transcriptional activity of genes known to differentiate ENFs and eEPFs. Once again, pEPFs diverged from ENFs, exhibiting a gene expression profile more closely resembling that of eEPFs.

**Conclusion:** Postnatal En-1 activation in ENFs during wound healing is accompanied by the acquisition of a profibrotic transcriptional profile similar to that of embryonically-derived EPFs. These RNA-seq data also support our recent finding that ENFs activate En-1 through a canonical mechanotransduction mechanism involving YAP and Notch, before transitioning to an ECM-producing phenotype (pEPF). Thus, inhibition of mechanotransduction signaling may mitigate scarring by blocking this phenotypic switch. In future studies, we will compare the chromatin profiles of ENFs, eEPFs, and pEPFs to determine whether postnatal En-1 activation recapitulates the epigenomic shift that occurs during embryogenesis.

**Dermal Wounding Reveals Focal Adhesion Kinase Dependent Tissue-Resident Fibroblast Progenitors**

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**Purpose:** Wound healing is a complex process that involves the extensive coordination of different cell types, most significant of which is the fibroblast. Fibroblasts play a key role in wound healing by assisting in wound closure and tissue remodeling. Significant research efforts have gone into identifying and characterizing specific subsets of fibroblasts involved in wound healing. However, unanswered questions in wound healing remain including where these cells originate from and what their progenitor phenotypes are. Our lab has previously shown that scar fibrosis is dependent on focal adhesion kinase (FAK) signaling and mechanotransduction pathways. The aim of our research was to characterize fibroblast progenitor-type phenotypes in the setting of dermal injury and to understand the role of FAK-signaling in fibroblast proliferation during wound healing.

**Methods:** We used the Rainbow mouse model (Rosa26VT2/GK3), which has a four-color reporter construct at the Rosa-26 locus. Following the induction of Cre-recombinase, cells express one of four fluorescent proteins, and all daughter cells are labeled with the same color. We created wounds in the dorsal dermis of Rainbow mice using a stented wound-healing model which mimics human wound healing kinetics. We used local application of tamoxifen liposomes to induce Cre recombination in tissue-resident cells at the time of injury. Confocal imaging was conducted on whole mount and sectioned wound specimens after tissue clearing. Using an unbiased FACS strategy, we isolated wound-healing fibroblasts based on their rainbow color and their position (outer edge vs. center of wound) for RNA-seq at various timepoints post-operatively (POD 2, 7 and 14). Topical FAK-inhibitor versus vehicle control was applied to Rainbow mouse dorsal dermal wounds and imaged with confocal microscopy.