ATAC-seq Reveals Heterogeneity of Fibroblasts During Transition from Scarless Fetal to Scar-Forming Adult Wound Repair

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INTRODUCTION: Cutaneous wounds in early gestation heal without a scar in a process resembling regeneration.[1] Although myriad studies have been performed to understand this phenomenon, the exact mechanism for fetal scarless repair is unknown. We previously characterized a fibroblast lineage in the dorsal skin of adult mice defined by embryonic expression of *Engrailed-1* (*En1*) thought to be responsible for scar formation.[2] Here, we investigate the role of this lineage during fetal wound healing.

MATERIALS AND METHODS: *En1*-derived fibroblasts were traced by crossing $En1^{Cre}$ and $ROSA26^{mTmG}$ mice. A murine model of fetal scarless wound healing allowed for investigation of *En1*-derived fibroblast behavior before and after the scarless to scarring transition. *En1*-derived fibroblasts were characterized using flow cytometry. ATAC-seq (Assay for Transposase-Accessible Chromatin with high throughput sequencing) was also performed in isolated pre- and post-gestational fibroblasts at a series of time points.

RESULTS: Dorsal wounds created at embryonic day (E)16.5 healed scarlessly with minimal connective tissue deposition. However, wounds created at E18.5 healed with substantial scar deposited primarily by *En1*-lineage-derived fibroblasts. The abundance of *En1*-lineage-derived fibroblasts and the expression of CD26, a previously identified marker of the *En1* lineage, steadily increased from E12.5 through postnatal day 1. Differential transcriptional activity shown by ATAC-seq further demonstrates the heterogeneic nature of fibroblasts within the dorsal dermis.

CONCLUSION: The *En1* lineage of fibroblasts plays a critical role in the transition from scarless wound healing during fetal development. These results hold promise for the development of therapeutic approaches to fibrotic disease and adult wound healing.

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