Transcriptional Profiles of Keloid Tissue and Matched Normal Skin Tissue Reveal Heterogeneity in Fibroblast Subtype Enrichment, Gene Expression, and Immune Cell Responses

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Running Title
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BACKGROUND
Keloid disease (KD) is a fibroproliferative disorder of the skin involving abnormal wound healing that leads to excessive scar tissue growth which causes pain, itching, and decreased quality of life. While previous RNA sequencing (RNA-Seq) studies have characterized differential gene expression between keloid and normal skin tissue, no prior studies have evaluated how fibroblast subtype enrichment variability may impact the observed gene expression in the tissue or immune cell responses.

OBJECTİVES
1. Characterize how fibroblast subtype composition in a largely African American (AA) cohort differs across keloid and matched adjacent normal skin tissue (MANST).
2. Characterize how fibroblast subtype composition affects differential gene expression across keloid and MANST.
3. Group keloids into subclasses defined by fibroblast subtype enrichment patterns.

METHODS
We performed RNA-Seq on keloid and MANST from 14 patients (10 AA and 4 European American) with keloids in the head and neck (H&N) region. Enrichment of the five established fibroblast subtypes and immune cells was measured with single sample gene set enrichment analysis (ssGSEA). Keloid subclasses were identified through hierarchical clustering of keloid tissue based on fibroblast subtype enrichment. Differentially expressed genes (DEGs) across tissue type and keloid subclass were determined by linear mixed-effects modeling after false-discovery-rate correction. Pathway analysis on the resulting DEGs was performed with Qiagen Ingenuity Pathway Analysis (IPA).

RESULTS
We found three fibroblast subtypes enriched (mesenchymal, secretory reticular, and pro-inflammatory B) and one reduced (pro-inflammatory A) in keloid tissue relative to MANST. After model adjustment for fibroblast subtype enrichment scores and correction for false discovery rate (FDR <0.05), there were 136 differentially expressed

genes (DEGs). IPA of these DEGs implicated pathways involving fibroblast activation, proliferation, and epithelial to mesenchymal transition, consistent with prior findings in non-AA cohorts. Clustering of keloid tissue revealed five different keloid subclasses (High, Intermediate 1, Intermediate 2, Intermediate 3, Low) characterized by varied enrichment score patterns across all five fibroblast subtypes. Additionally, these subclasses were present in RNA-seq data from a previously published study of H&N keloids. DEGs between the subclasses could be traced back to activation of TGFB1, a key gene in keloid pathology. Finally, activated mast and natural killer (NK) cell ssGSEA enrichment scores were strongly associated with keloid subclasses enriched for mesenchymal and secretory-reticular fibroblasts.

CONCLUSİONS

These findings reveal significant fibroblast subtype enrichment heterogeneity in keloids related to differential immune responses. Further studies are needed to determine whether these keloid subclasses may underly differences in KD progression or varied responses to therapy.