Single-cell RNA Sequencing Revealed Key Factors of EMT to Promote Fibroblasts Activation and Immune Infiltration in Keloids

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BACKGROUND:

Keloid is a fibrotic disorder of soft tissues secondary to abnormal wound healing. Previous studies have predominantly focused on fibroblasts, yet research on epidermal keratinocytes (KCs) remains limited. Existing evidence indicates that KCs in keloid differ from normal KCs in morphology and transcriptional profiles, with keloid KCs exhibiting EMT-like characteristics. However, the molecular drivers of EMT in keloid KCs and how EMT-primed KCs contribute to keloid pathogenesis and progression remain poorly understood.

METHODS:

We collected lesional tissues from 10 keloid patients and 5 normal skin samples for singlecell RNA sequencing and downstream bioinformatics analysis. In vitro molecular experiments (quantitative polymerase chain reaction [qPCR],western blotting [WB], enzyme-linked immunosorbent assay [ELISA]) and cellular assays (scratch assay, CCK8 proliferation assay, colony formation assay, immunocytochemistry) were performed. We established a bleomycin-induced skin fibrosis mice model and a keloid xenograft nude mice model. After administering the FOSL1 inhibitor SR11302 for two weeks, fibrosis-related indicators were assessed to evaluate the therapeutic efficacy of the drug.

RESULTS:

Single-cell sequencing revealed distinct keratinocyte compositions between keloid and normal skin. The proportions of basal-mig and spinous-mig keratinocyte subpopulations were significantly increased in keloid. Pathway enrichment analysis demonstrated that genes in these subpopulations were enriched in wound healing, epithelial-mesenchymal transition (EMT), and related pathways. Notably, basal-mig exhibited a pronounced EMT tendency. SCENIC analysis suggested that the transcription factor FOSL1 plays a critical role in the differentiation of basal-mig and spinous-mig subpopulations. In cell models, FOSL1 overexpression significantly enhanced the proliferation and migration of normal keratinocytes. ChIP-seq further revealed that FOSL1 binds to MED1 to form a super-enhancer complex, regulating downstream gene transcription. In keratinocytes, FOSL1 overexpression markedly increased the transcription and secretion of Matrix Metalloproteinase 3 (MMP3). Co-culture of FOSL1-overexpressing keratinocytes with normal and keloid fibroblasts enhanced fibroblast proliferation, migration, and inflammatory factors secretion, whereas these effects were significantly attenuated by MMP3 antibody treatment. In skin fibrosis mice models, FOSL1 inhibitor SR11302 can significantly alleviate fibrotic pathology.

CONCLUSION:

Keratinocytes overexpressing FOSL1 can upregulate the secretion of MMP3 and subsequently facilitate the activation of fibroblasts and immune cells infiltration, thereby promoting the initiation and progression of keloid.