Integrated Analysis of TWIST1 in Keloid Pathogenesis: Single-Cell Transcriptomics Reveals Fibroblast Heterogeneity and a Novel MEF2A-TBR1 Regulatory Axis

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BACKGROUND:

Keloid scarring is caused by a fibroproliferative disorder due to abnormal activation of genes, the underlying mechanism of which is still unclear. The basic helix−loop−helix transcription factor Twist−related protein 1 (TWIST1) controls cell proliferation and differentiation in tissue development and disease processes. In this study, we aimed to clarify the essential role of TWIST1 in the pathogenesis of keloids.

METHODS:

1.Single−Cell RNA Sequencing: Analyzed 28,064 cells from keloid and adjacent normal tissues to map cellular heterogeneity (n=4 patients).

2.Functional Assays:In vitro: CCK −8, Transwell, immunofluorescence, and Western blotting in KFBs treated with TWIST1 inhibitor harmine.Molecular Interactions: Co−immunoprecipitation (Co −IP), ubiquitination assays, chromatin immunoprecipitation (ChIP−qPCR), and dual−luciferase reporter assays to validate TWIST1−MEF2A−TBR1 interactions.

3.Pathway Analysis: Gene set enrichment (GSEA) for TGF−β, Eph−ephrin, and tumor−related pathways.

RESULTS:

1.Cellular Heterogeneity:

scRNA−seq identified expanded fibroblast (cluster c9) and VEC subpopulations (clusters c4, c5, c18) in keloids, linked to TGF−β and Eph−ephrin pathway activation.TWIST1 was significantly upregulated in KFBs and VECs ($p<0.01$).

2.TWIST1 Functional Roles:

Fibrosis: TWIST1 promoted collagen synthesis (COL1A1, COL3A1) and myofibroblast activation via TGF −β/Smad3. Harmine (TWIST1 inhibitor) suppressed TWIST1, reducing ECM deposition ($p<0.05$).

Angiogenesis: TWIST1 enhanced Eph −ephrin signaling (EFNB2 −EPHA4) in VECs, driving pathological vascularization.

3.Mechanistic Insights:

TWIST1 stabilized MEF2A by inhibiting MDM2–mediated ubiquitination, prolonging its half –life.MEF2A directly bound the TBR1 promoter, enhancing TGF –β receptor expression (ChIP–qPCR fold enrichment=2.5, $p<0.001$).TWIST1 overexpression rescued TBR1 expression in KFBs, while MEF2A knockdown reversed this effect ($p<0.01$).

CONCLUSION:

Our research highlights a significant function of TWIST1 in the development of keloid and its related fibroblasts, partially facilitated by elevated MEF2A –dependent TBR1 expression. Blocking the expression of TWIST1 in KFBs could potentially pave a novel therapeutic avenue for keloid treatment.

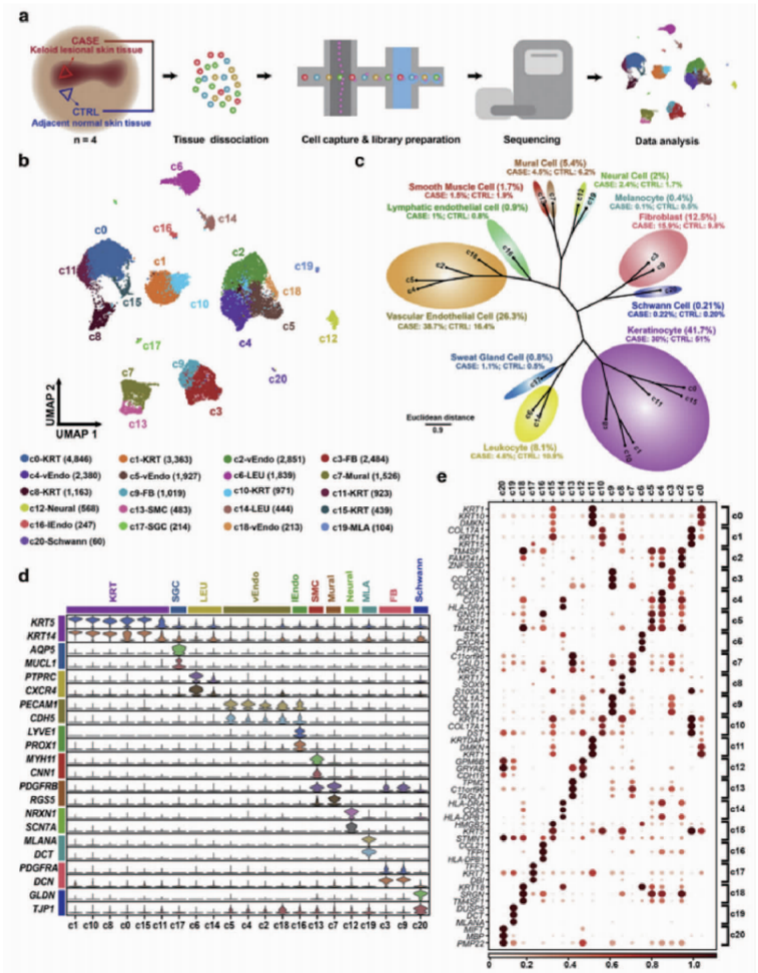

Fig.1Presents a comprehensive analysis of the cellular diversity and heterogeneity within keloid skin tissue through scRNA

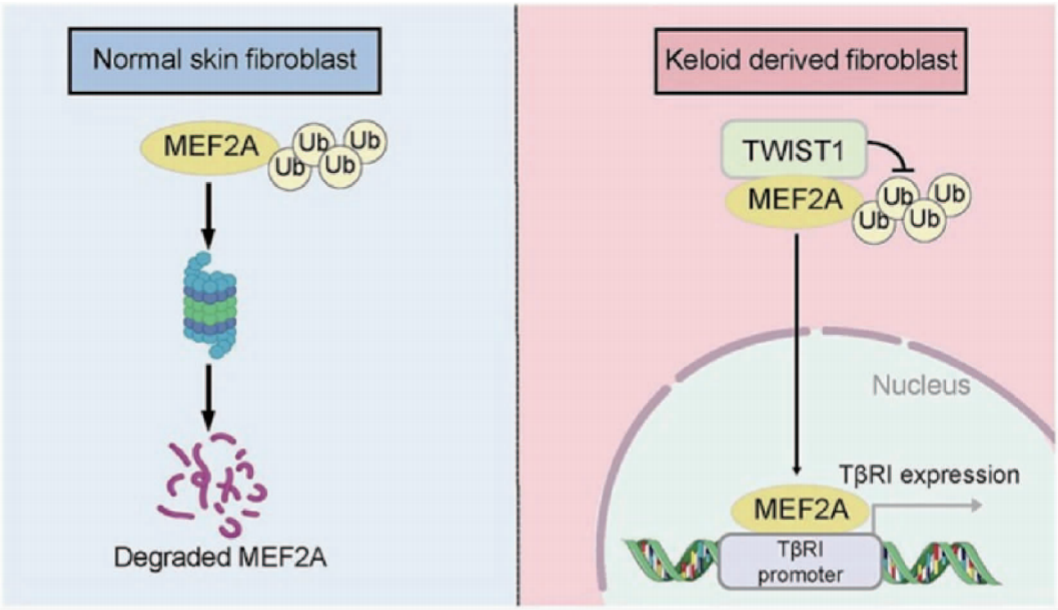

Fig.2 Schematic representation of TWIST1 promoting expression of TGF–β receptor 1 by regulating the stability of MEF2A