Targeted Interleukin-11 Inhibition to Control Keloiding Orthopaedic Wounds

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Introduction: Keloids are a significant yet often underestimated cause of postoperative problems in orthopaedic surgery, directly threatening treatment success and long-term functional recovery. Unlike simple cosmetic issues, keloids on high-tension and mobile areas—common after orthopaedic procedures—can quickly lead to debilitating contractures, limited joint movement, and serious secondary complications such as nerve compression (e.g., ulnar nerve entrapment). Orthopaedic wounds are inherently high-risk due to increased mechanical stress and the frequent presence of internal fixation hardware. The high recurrence rate of keloids with excision alone underscores the urgent need for targeted, non-invasive treatments that address the molecular causes of hyperactive keloid myofibroblasts (KMFs), which promote keloid growth under hypoxia. While canonical transforming growth factor-β (TGFβ) is known to initiate fibrosis, the persistent fibrotic phenotype of KMFs is partly maintained by the downstream interleukin (IL)11/Jak-Stat3 pathway. Additionally, the low O_2 environment stabilizes hypoxia-inducible factor-1α (HIF1α), which activates the transcription of the oncogenic driver Homeobox C6 (Hoxc6), which is upregulated during embryogenesis, particularly in the spinal cord. Otherwise, Hoxc6 is barely expressed in adulthood, mostly confined to fallopian tubes, ovaries, and some neoplasms. The importance of the HIF1α→Hoxc6 axis lies in Hoxc6's role in promoting keloid growth and extracellular matrix (ECM) formation through regulation of genes involved in sustaining Erk/MAPK activity, a common signaling hub downstream of IL11, which is itself downstream of TGFβ. We predict that small-molecule, first-in-class IL11 inhibitors (NM compounds) will disable this Erk-convergent, pro-fibrotic network.

Methods: We compared human KMFs (ATCC) with healthy dermal fibroblasts (DFs; Cell Applications) with confirmed phenotypes. First, we evaluated the effectiveness of NM1157 (10μM) over 48 hours under standard normoxic conditions (21% O_2). Collagen type 1 (COL1) deposition and IL11 secretion were measured using sandwich (Abcam) and competitive (R&D Systems) ELISA kits. The myofibroblast phenotype was assessed by co-immunocytochemistry (ICC) fluorescence co-labeling of COL1 and α-smooth muscle actin (SMA). Next, to understand the underlying disease mechanism in response to environmental stress, a separate experiment modeled the keloid microenvironment using a hypoxia challenge (1% O_2). To obtain quantitative and reproducible data on the HIF1α→Hoxc6 axis, the Jess Simple Western (JSW) platform was used for automated, total-protein-normalized quantification of the mean normalized peak area (MNPA) of the key mechanistic proteins HIF1α and Hoxc6 in lysates of untreated KMFs and DFs under normoxia or hypoxia. Automated immunodetection protocols involved primary antibodies targeting the 109- and 27-kDa bands of HIF1α (Abcam) and Hoxc6 (Novus), respectively.

Results: Baseline KMFs produced 26% more COL1 ($p = 0.0091$) and 30% more IL11 ($p = 0.0002$) than DFs. NM1157 treatment under normoxia significantly reduced COL1 deposition by 59% ($p < 0.0001$) and IL11 secretion by 33% ($p = 0.008$). At the same time, confocal photomicrographs of ICC staining showed a reversal of SMA and COL1 expression in KMFs, aligning with the pattern observed in baseline DFs. JSW analysis showed significantly more HIF1α in both hypoxic KMFs (MNPA: 423.3±25.7) versus normoxia [MNPA: 103.3±37.6; ($p = 0.035$)] and hypoxic DFs (MNPA: 559.5±46.1) versus normoxia [153.3±37.1; ($p = 0.041$)], confirming effective HIF1α stabilization (310% increase in KMFs and 265% increase in DFs). Conversely, significantly higher expression of Hoxc6 was only observed in KMFs under hypoxia (MNPA: 11255.9±36.5), representing a 61.7% increase compared to KMFs under normoxia [MNPA: 6964.4±1385; ($p = 0.012$)]. DFs showed no significant upregulation of Hoxc6 under hypoxia (MNPA: 8253.5±162.0) compared to normoxia [MNPA: 9147.3±519.6; ($p = 0.407$)].

Conclusion: This study validates that KMFs have a unique molecular vulnerability, specifically the pathogenic switch of Hoxc6 downstream of HIF1α stabilization under hypoxia. JSW data clearly show that while HIF1α stabilizes in both hypoxic KMFs and DFs, the significant induction of Hoxc6 occurs only in KMFs, confirming a keloid-specific trait. HIF1α→Hoxc6 promotes fibrotic output by activating Erk/MAPK myofibroblast signaling, which autocrine IL11-Jak/Stat3 co-triggers. The anti-fibrotic effect of NM1157 under normoxia suggests that targeting the core IL11 maintenance loop may be sufficient to disrupt the keloid pro-fibrotic network, thereby overcoming the environmental boost provided by HIF1α→Hoxc6, which is more difficult to modulate. If confirmed, NM1157 could serve as a mechanism-based, non-invasive, and safe pharmacological approach that surpasses general anti-inflammatory or surgical interventions for high-risk keloid and hypertrophic scarring in orthopaedic incisions, ultimately improving long-term function and recovery. Ongoing research will evaluate the effectiveness of NM1157 in reducing the outcome of downstream effectors in hypoxic KMFs and weakening structural ECM deposition in a 3D tri-culture of patient-derived KMFs, microvascular endothelial cells, and alternatively polarized, M2 macrophages.

Significance/Clinical Relevance: Keloids following orthopaedic procedures can cause contractures, nerve compression, and loss of joint mobility, compromising long-term recovery. By identifying a keloid-specific activation of the HIF1α→Hoxc6 axis under hypoxia, this study establishes a mechanistic foundation for developing targeted, non-invasive therapies to prevent or reverse pathologic fibrosis in high-risk orthopaedic wounds.