Use of a Novel IL11 Blocking Agent to Modulate Hypertrophic Dermal Scarring

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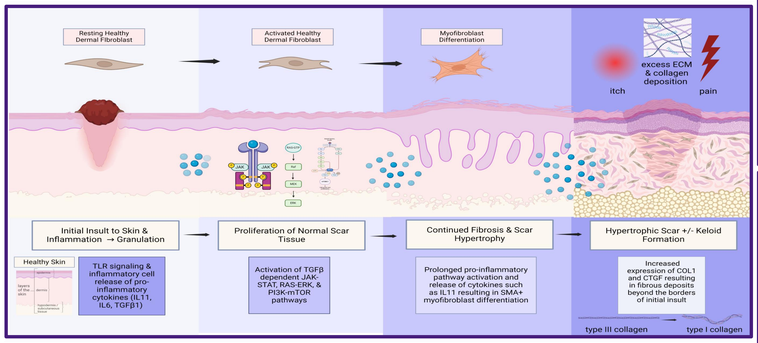
Introduction

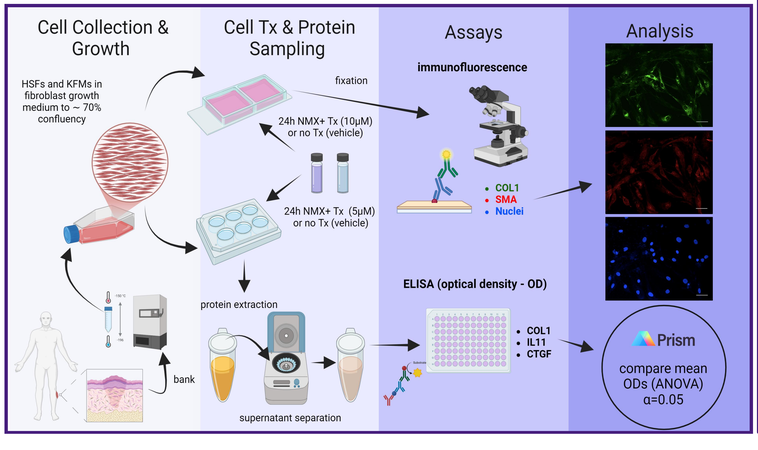
- Keloids are aesthetically distressing and itchy or painful hypertrophic scars that form by aberrant growth and differentiation of myofibroblasts that excessively deposit fibrotic matrix mostly comprising collagen (COL)1 primarily mediated by transforming growth factor beta (TGFβ)1 and connective tissue growth factor (CTGF). Though recurrence is common, the standard of keloid care is monthly intralesional corticosteroid injections (ICSI) until the lesions improve. Still, recurrent use of ICSI may result in lipoatrophy, hypopigmentation, and dermal atrophy. Several inflammatory pathways are involved in the differentiation of fibroblasts to smooth muscle actin (SMA)-positive myofibroblasts leading to fibrosis including JAK-STAT3, RAS-ERK, and mTOR pathways. Though controlled interleukin (IL)11 signaling plays an important role in myofibroblast differentiation of healthy human skin fibroblasts (HSFs) during normal healing, it is highly expressed in fibrotic diseases such as keloids and idiopathic pulmonary fibrosis.
- NMX structural analogs are designed to selectively block the TGFβ1-dependent, autocrine IL11 JAK/STAT3 signaling cascade, and are shown to successfully reduce heart fibrosis and preserve cardiac function.
- Therefore, this project investigates whether novel NMX compounds can disrupt the cascades that differentiate HSFs into keloid-forming myofibroblasts (KFMs) and related dysfunctional COL1 deposition



Hypothesis & Significance

We predict that inhibition of IL11-dependent pro-fibrotic pathways via NMX treatment will effectively suppress myofibroblast differentiation and normalize COL1 production in KFMs. Improving pharmacotherapeutics for early modulation of key inflammatory stimuli of fibrotic processes and transformation of HSF into contractile myofibroblasts that lead to non-compliant keloids could improve current treatment paradigms and more effectively reduce the painful discomfort imparted by hypertrophic scarring.

Methods



Results

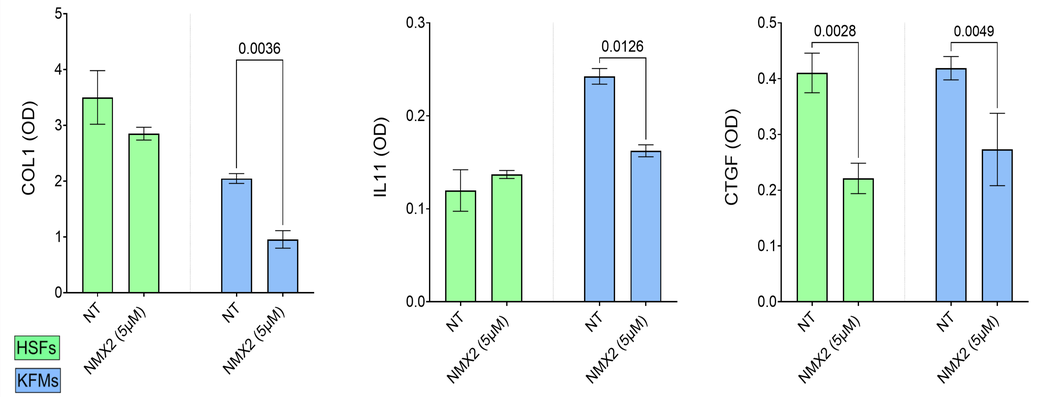


Fig. 1 To compare the impact of NMX2 to the BSA control groups, the amount of CTGF, COL-1, and IL-11 in each cell type was measured. When treated with NMX2 (μM), we observed lower COL1 and CTGF values in KFMs and HSFs but calculated significant decreases only in KFMs for COL1 (53%; p=0.036) and IL11 (33%; p=0.0126). CTGF was significantly lowered in both NMX2-treated HSFs (46%; p=0.0028) and KFMs (35%; p=0.0049).

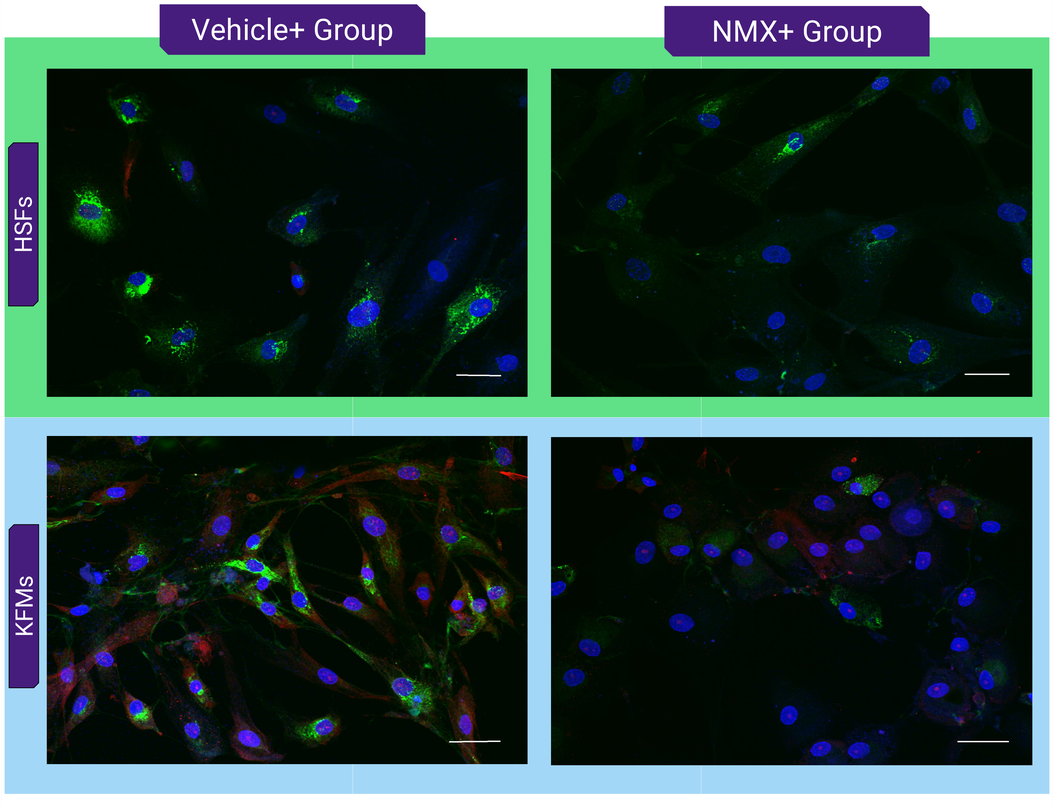


Fig. 2 HSFs and KFMs were immunostained for COL1 (green) and SMA (red) and counterstained with DAPI nuclear dye (blue). Representative confocal photomicrographs were captured at 200x (50-micron bar) to compare vehicle-treated control groups of both cell lines with corresponding groups treated with 10μM of NMX2.

Conclusion

- Uncontrolled signaling of inflammatory and fibrogenic pathways following TGFB1 and IL11 receptor binding such as JAK/STAT3 and RAS-ERK in dermal fibroblasts drives their differentiation into myofibroblast pools that lead to increased fibrous collagen deposition, hypertrophic scarring, and painful keloids.
- At 5μM, NMX2 significantly limits intracellular processing of procollagen and excessive fibrous deposition in KFMs, but not HSFs, as indicated by the decreased production of COL1 and IL11. At this concentration, NMX lowers CTGF in both cell lines, which indicates that NMX must be altering additional fibrogenic cascades in KFMs to cause a significant effect on their aberrant COL1 deposition.
- Immunostaining data for COL1 and SMA suggests that although COL1 is detectable in both KFMs and HSFs, only KFMs express SMA, indicative of their myofibroblast phenotype. To that end, NMX2 effectively abrogates myofibroblast differentiation and minimizes fibrous deposits characteristic of keloid-derived cells. However, the higher NMX concentration of 10μM used for the immunostaining experiment seemed to have a greater effect on COL1 in HSFs than that observed in experiments using 5μM. This is important because it indicates a threshold of how NMX concentration can affect normal skin around the lesion. Notably, the cytotoxicity of NMX compounds has been shown negligible at concentrations as high as 20μM.
- Finding a safer and more effective alternative to ICSI to interfere with inflammatory and fibrotic cascades that promote the differentiation of HSF to contractile myofibroblasts prior to hypertrophic scar or keloid maturation would not only minimize scarring, but also potentially alleviate the pain and discomfort that patients endure to maximize quality of life.

Limitations

- The study is underpowered because only one HSF and one KFM patient cell line were available. Efforts are underway to collect more patient-derived biopsies of hypertrophic scars and keloids. Additionally, further ELISA data of the 10μM NMX2 treated groups should be matched to immuno-labeling to determine if there is a more robust concentration-dependent effect on fibrotic deposition compared to the 5μM NMX2 treated cells.
- In vivo experiments must be designed to observe the safety and effectiveness of local NMX application relative to hypertrophic scar development, invasiveness, and recurrence. However, an animal model of keloid formation does not exist. Therefore, future studies will focus on developing keloid organoids in vitro to gain a better understanding of the impact of NMX on hypertrophic fibroblast growth, fibrous deposition, differentiation, and epithelial-stromal transition dynamics.

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