Evaluating IL-11 and TGFβ1 Distribution Relative to Synovial Fibrosis Status

Molly Chaffee [1], Kayla Vo [2], Ryan J. Schroeder [2], Luis Marrero [3].

Tulane University [1], Louisiana State University Health Sciences Center School of Medicine [2], Department of Orthopaedics [3]

LSU Health NEW ORLEANS School of Medicine Department of Orthopaedics

Introduction

Synovial fibrosis (SFb), a painful contracture limiting joint motion and quality of life, is a hallmark of arthrofibrosis (AF), a common complication after joint repair. SFb is categorized by low (<41%), moderate (42-54%), and high (>54%) collagen deposition levels and is a significant challenge in osteoarthritis (OA) patients. As shown in Figure 1, transforming growth factor beta 1 (TGFβ1) drives SFb and regulates essential cell processes. Interleukin (IL) 11, synthesized downstream of the TGFβ1-mediated JAK/STAT3 cascade, promotes fibrosis if dysregulated. Novomedix's (NMX) novel inhibitors selectively target IL11 without disrupting TGFβ1-mediated functions. These inhibitors effectively reduce IL11-driven collagen deposition in OA-derived fibrotic synoviocytes.

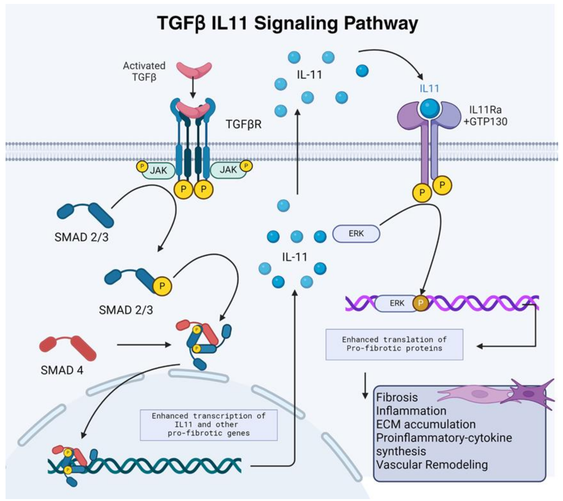


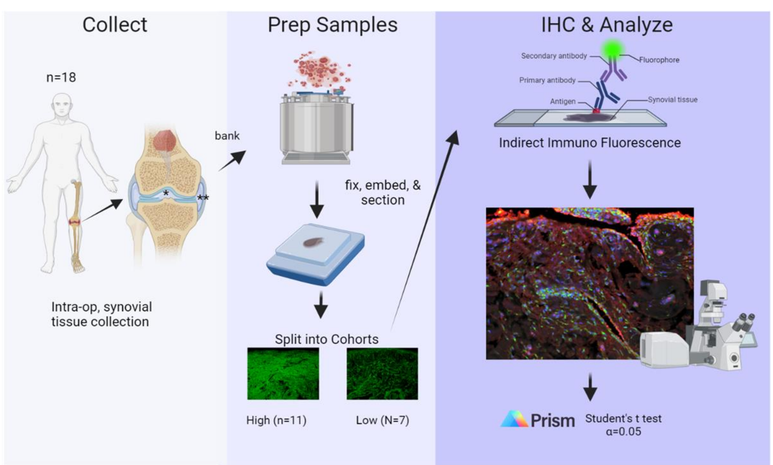
Figure 1: The TGFb1 mediated IL11 Signaling Pathway

Objective and Significance

To assess the potential of NMX for in vivo SFb treatment, this study analyzes IL11 co-expression with TGFβ1 in banked knee OA samples, hypothesizing a correlation between IL11 expression and SFb severity.

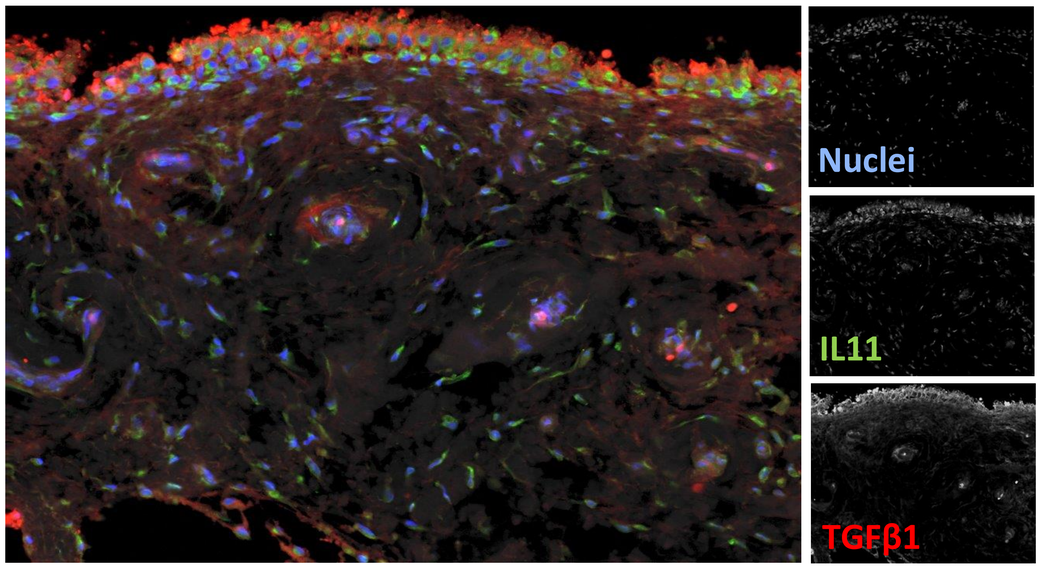
Methods

SFb cohorts were based on pre-defined histological scores. Co-detection of TGFβ1 and IL11 by indirect immunofluorescence used anti-TGFβ1 (mouse monoclonal) and anti-IL11 (rabbit polyclonal) antibodies. Sections were then stained with anti-mouse Alexa 594 and anti-rabbit Alexa 647 secondary antibodies for TGFβ1 and IL11, respectively, along with DAPI nuclear counterstain. Samples were mounted and imaged using a confocal microscope (Olympus) at 200x magnification. Co-expression of TGFβ1 and IL11 was quantified through background-corrected signal analysis using Slidebook™ software.



Results

Low Fibrosis



High Fibrosis

Figure 2: Representative 200x confocal photomicrographs of TGFβ1, IL11 and DAPI Nuclear counterstain in the synovium of kOA patients grouped by low and high fibrosis scores.

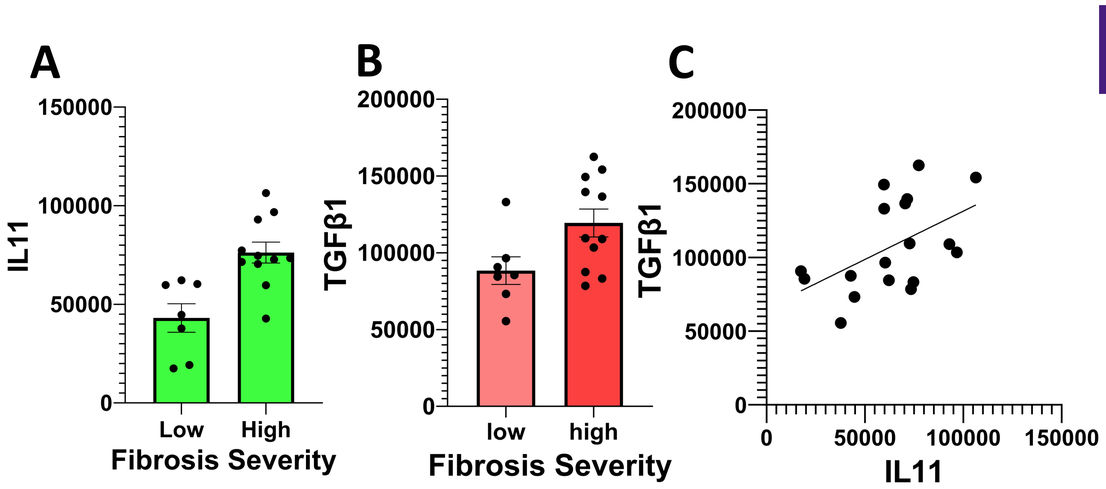


Figure 3: Student T test calculated that (A) the mean expression of TGF1 observed in the synovium of patients classified with high SFb severity was 35% higher (p = 0.0360) compared to the signal measured from the low severity SFb group and (B) IL11 expression in the high SFb severity were registered at a 77% increase (p = 0.0016) from patients with less severe SFb. Pearson's correlation revealed (C) a moderate but significant correlation between TGFβ1 and IL11 (R = 0.51; p = 0.0314).

Discussion and Limitations

- Increased expression of IL11 relates to TGFβ1 in agreement with SFb severity. While this study does not prove causality, it suggests a relationship between IL11 and SFb, highlighting the diseased synovium as an effective target for NMX administration.
- The study is limited by sample size and doesn't account for confounding variables such as synovitis grade and presence of additional pro-fibrotic factors such as connective tissue growth factor.
- Further studies will investigate the effectiveness of NMX on aberrant collagen deposition, contraction, and myofibroblast differentiation rate of patient-derived synovial fibroblasts.

Conclusion

- IL11 levels in patient synovial tissue correlate to TGFβ1 levels and severity of SFb. While this study does not prove causality, it provides further evidence that IL 11 and SFb are interrelated.
- Further studies will investigate the effectiveness of the NMX compound in patient synovial tissue.
- indicates the potential supplementation of NMX to assist manipulation under anesthesia and arthroscopic lysis of adhesions in the management of debilitating arthrofibrosis.

Acknowledgements

We would like to thank members of the Marrero Laboratory and the Morphology and Imaging Core for technical assistance and thoughtful discussions. Collection, preservation, and storage of samples in the LSU Integrated Musculoskeletal Biobank (LIMB) and used in study was supported by an award from the LSU Health Research Enhancement Program. A special thanks to Dr. Marrero for your constant guidance and support that has made this project possible

References

1. Blaney Davidson EN, van der Kraan PM, van den Berg WB. TGF-beta and osteoarthritis. Osteoarthritis Cartilage. 2007;15(6):597-604. doi:10.1016/j.joca.2007.02.005

1. Schafer S, Viswanathan S, Widjaja AA, et al. IL-11 is a crucial determinant of cardiovascular fibrosis. Nature. 2017;552(7683):110-115. doi:10.1038/nature24676

1. Ng et al. Interleukin-11 signaling underlies fibrosis, parenchymal dysfunction, and chronic inflammation of the airway, Experimental & Molecular Medicine (2020) 52:1871–1878; 15

1. Geng et al. PD-L1 on invasive fibroblasts drives fibrosis in a humanized model of idiopathic pulmonary fibrosis, JCI Insight 2019

1. Hodgeson S, O'Brien S, Simkin J, Plakotaris E, McCarthy C, Dasa V, Marrero L. Differences in synovial fibrosis relative to range of motion in knee osteoarthritis patients. J Orthop Res. 2022 Mar;40(3):584-594. doi: 10.1002/jor.25061. Epub 2021 May 11. PMID: 33913554; PMCID: PMC8553814.