76 Interleukin-4 Promotes Human Prostate Fibroblast Extracellular Matrix Protein Deposition

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Abstract

Background. Pathological deposition of extracellular matrix (ECM) by peri-urethral prostate fibroblasts and myofibroblasts contributes to lower urinary tract fibrosis and consequent urinary voiding dysfunction. Inflammatory mediators, such as chemokines and interleukins, can promote ECM accumulation. IL-4 and IL-13 are abundant in the prostate microenvironment and their cognate receptors are expressed in association with high levels of collagen deposition in periurethral prostate tissue. Moreover, IL-4 andIL-13 signal through a shared axis, suggesting that may coordinately play key roles in the promotion of fibrotic pathology. Based on this data, we hypothesized that the IL-4/IL-13 axis may promote expression of ECM proteins through JAK/STAT activation, thereby contributing to lower urinary tract dysfunction (LUTD).

Methods. N1 or SFT1 immortalized human prostate fibroblasts were serum-starved for 24 hr then grown in serum-free media with or without 2 hr pre-treatment with antibodies against IL-4Ra or IL-13Ra1, then supplemented with vehicle (PBS or citric acid), IL-4 (40ng/ml), IL-13 (40ng/ml), or TGFB (4ng/ml, as positive control). Cells were then lysed for protein or RNA purification, and subjected to immunoblotting, sircol assays, or qRT-PCR analysis.

Results. Sircol assays demonstrated significantly higher levels of soluble collagens I-V produced by N1 and SFT-1 cells treated with IL-4 ($p<.001$) or IL-13 ($p<.0001$) compared to vehicle-treated cells. qRT-PCR and immunoblot assays showed that IL-4 and IL-13 robustly up-regulated COL1 ($p<.001$) and moderately up-regulated COL3 transcripts and protein ($p<.05$) compared to vehicle-treated cells. Pre-treatment with antibodies against IL-4Ra or IL-13Ra1 ablated the observed IL-4 or IL-13-mediated collagen transcript and protein expression to levels similar to those of vehicle-treated cells ($p<.05$). IL-4 and IL-13 also promoted robust phosphorylation of STAT6, which can induce the expression of the IL-4, IL-13, IL-4Ra and collagen genes.

Conclusions. IL-4 and IL-13 signal through the JAK/STAT pathway to phosphorylate STAT6, suggesting that the observed IL-4/IL-13 induced expression of high levels of extracellular matrix transcripts and proteins by human prostate fibroblasts likely occurs through JAK/STAT signaling. Several JAK/STAT inhibitors are FDA approved and are in current clinical use to treat human disease. Therefore, further studies that positively link ECM deposition with IL-4/IL-13 mediated JAK/STAT signaling may provide new therapeutic approaches to treat LUTD.

Methods

(A) N1 were produced through the immortalization of human prostate stromal fibroblasts and SFT1 cells are NAB2/STAT6 spontaneously immortalized cells cultured from a solitary fibrous tumors.
(B) IL-4/IL-13 axis inhibition was achieved using antibodies directed again the IL4Ra or IL13Ra1 receptors.
(C) N1 or SFT1 cells were serum-starved for 24 hours then grown in serum free media with or without a 2 hour pre-treatment with antibodies against IL-4Rα1 or IL-13Rα1.
(D) Cells are then supplemented with either:
  (A) Vehicle (PBS or Citric Acid)
  (B) IL-4 (40ng/mL)
  (C) IL-13(40ng/mL)
  (D) TGFb (4ng/mL, positive control)
(E) Cells are then lysed for protein or RNA purification, and assessed via qRT-PCR, immunoblotting, and Sircol assay (used to detect soluble collagen levels).
(F) N1 cells were also tested for long-term (72 hour) STAT6 activation and COL1/3 production by serum-starving for 24 hours followed by 20ng/mL IL-4/IL-13 treatment for 24 hrs. The media was removed, cells washed, and grown in serum-free media for 24, 48, or 72 hours
(G) Protein from these long-term assays was examined using immunoblotting for COL1, COL3, STAT6, and pSTAT6, and GAPDH (loading control)

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Background

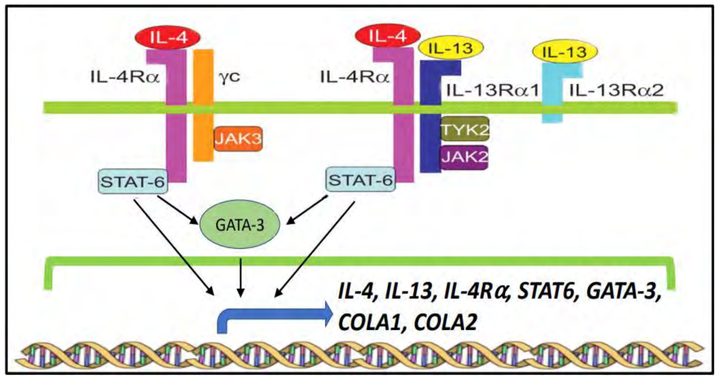


Figure 1: Fibrosis is consequent to chronic or unresolved inflammation associated with aging, metabolic syndrome, or urinary tract infection. Inflammatory cells secrete a medley of pro-fibrotic proteins into the prostatic microenvironment including interleukins which have been implicated in inflammation-mediated fibrosis. Among these, IL-4 and IL-13 are of particular interest because they share a common signaling axis which we hypothesize promotes expression of ECM proteins through JAK/STAT activation.

Results

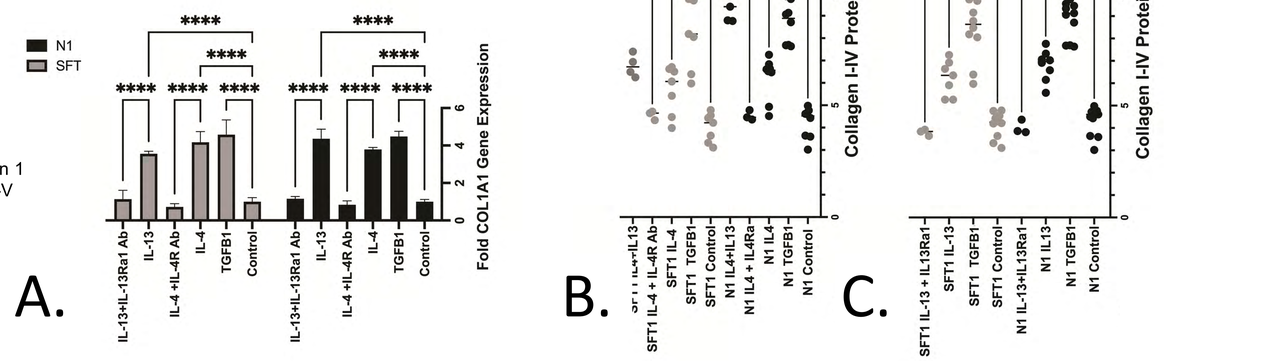
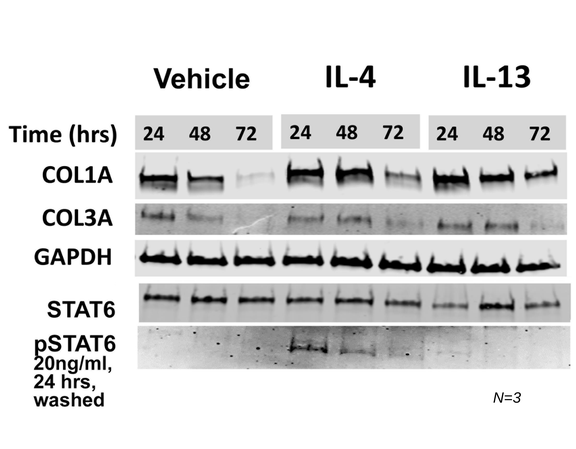


Figure 2. qRT-PCR (A) and Sircol (B,C) assays demonstrate that fibroblasts treated with IL-4 and IL-13 show increased collagen 1 gene transcription (A) and collagen types I-V protein expression (B, C).



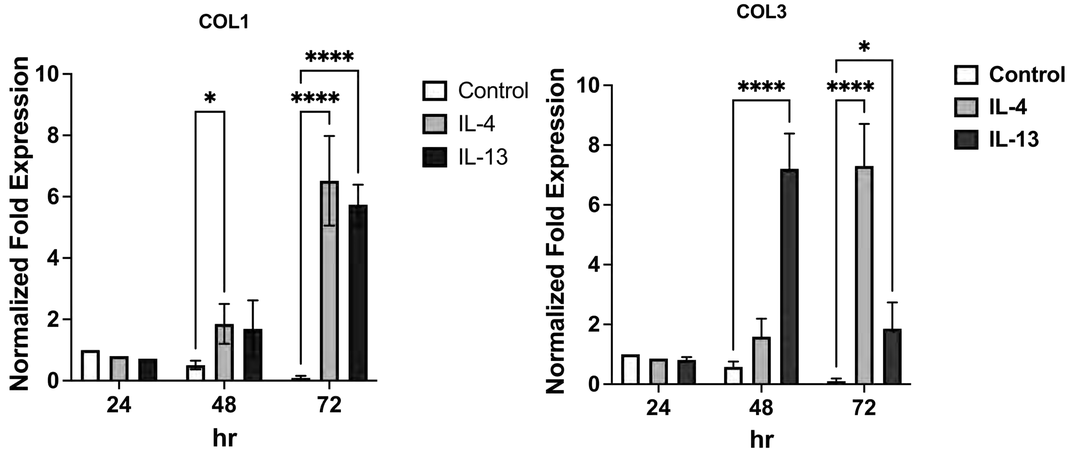


Figure 3: Immunoblot (left) shows significant long-term COL1 and COL3 production and extended STAT6 activation (pSTAT6) following initial treatment with IL-4/IL-13. Cells were treated with vehicle, IL-4 or IL-13 for 24 hr, washed, and assessed for COL1, COL3, pSTAT6, STAT6 or GAPDH (loading control) for 24, 48 or 72 hr.

Conclusions and Future Directions

Previous work has shown that IL-4 and IL-13 signal through the JAK/STAT pathway to phosphorylate STAT6, suggesting that the observed IL-4/IL-13 induced expression of high levels of extracellular matrix transcripts and proteins by human prostate fibroblasts likely occurs through JAK/STAT signaling. Based on the work shown here we can confirm that IL-4 and IL-13 result in activation of STAT6 resulting in increased expression of ECM proteins including COL1 and COL3 by both N1 and SFT1 prostate fibroblasts cells. The expression of these proteins can be attenuated with IL-4Rα1 and IL-13Rα1 antibodies which prevent binding of IL-4 and IL-13 respectively. Additionally, we've shown that IL-4 and IL-13 treatment result in both long-term activation of STAT6 and continued expression of COL1 and COL3.

Several JAK/STAT inhibitors, e.g., tofacitinib (Xeljanz) are FDA approved and are in current clinical use to treat human disease. Future studies will include the use of these inhibitors *in vitro* to assess their effect in preventing the long-term ECM deposition currently seen in IL-4/IL-13 stimulated fibroblasts and *in vivo* using mouse models. If successful, these studies may provide a new therapeutic approach for preventing or regressing lower urinary fibrosis and consequent urinary voiding dysfunction.