#20 Interleukin-4 Represses Extrinsic Pathway-Mediated Apoptosis in Human Prostate Fibroblasts

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Abstract

Introduction and Objective: Myofibroblasts, major cellular agents of fibrosis, are resistant to apoptosis, and instead persist, accumulate, and promote pathological deposition of the extracellular matrix (ECM). IL-4, which is abundant in the aging prostate microenvironment, represses Fas-ligand (FasL)-mediated extrinsic apoptotic pathways in Th2 macrophages. IL-4 and IL-13 signal transduction occurs through a shared axis, suggesting that both interleukins may play key roles in myofibroblast resistance to apoptosis and continued persistence. Based on these studies, we hypothesized that the IL-4/IL-13 axis may repress myofibroblast apoptosis in fibrotic tissues, thereby contributing to lower urinary tract dysfunction (LUTD).

Methods: Primary human prostate fibroblasts were serum-starved for 24 hr then grown in serum-free media with or without 2 hr pre-treatment with pro-apoptotic TRIO cocktail (TNFalpha, Trail, and Fas ligand @50ng/ml each) followed by growth for an additional 2hr or 48hr with or without added IL-4 (40ng/ml) or IL-13 (40ng/ml). Cells were then photographed and/or lysed and subjected to immunoblotting for pro- and cleaved (activated) caspase 3 or caspase 8, GAPDH or tubulin (loading controls), or FasL receptor.

Results: Primary prostate cells expressed high levels of FasL receptor. When treated with vehicle or IL-4, cells exhibited no caspase cleavage and low levels of cell death. When pre-treated with TRIO followed by supplementation with vehicle, cells exhibited high levels of cell death and caspase cleavage/activation (p<.0001) compared to non-TRIO treated. Cells pre-treated with TRIO followed by supplementation with IL-4 demonstrated significantly less caspase 3 (p<.001) and caspase 8 (p<.01) cleavage/activation and reduced levels of cell death.

Conclusions: Low concentrations of IL-4 protected primary prostate fibroblasts from undergoing complete programmed cell death in response to activation of extrinsic apoptotic pathways and caspase 8 activation. Prostate fibroblasts abundantly expressed FasL receptor, and further investigation should reveal whether other extrinsic pathway death receptors are similarly expressed. IL-4 also repressed activation of caspase 3, which can be activated by caspase 8 and help induce intrinsic pathway-mediated (mitochondrial) apoptosis. Future studies will elucidate potentially targetable signaling mechanisms coupled to IL-4/IL-13-mediated repression of apoptotic pathways contributing to myofibroblast persistence, pathological ECM deposition, and fibrosis contributing to LUTD.

Background: Apoptotic Pathways

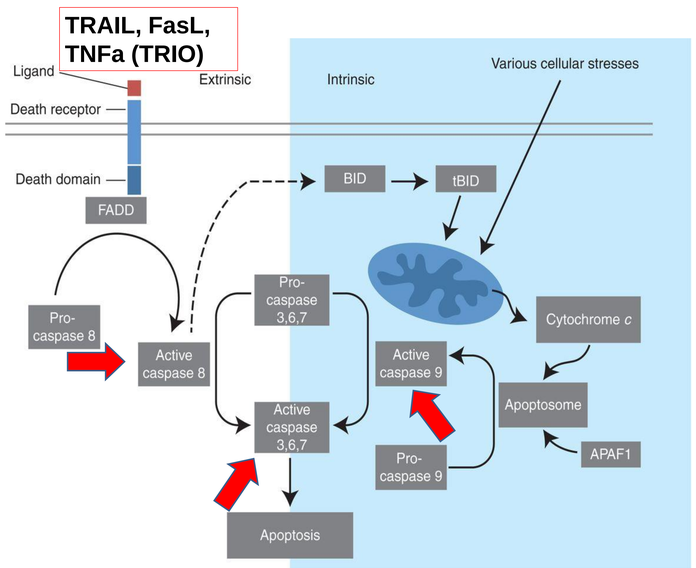

Figure 1. Based on data from immune cells, Il-4 may be protective against apoptosis mediated by the extrinsic pathway (caspase 8 and 3) and perhaps caspase 9. This project seeks to test whether IL-4 (and/or IL-13) may similarly repress extrinsic pathway apoptosis in prostate fibroblasts, perhaps contributing to myofibroblast apoptotic resistance and thereby promoting fibrosis.

Results

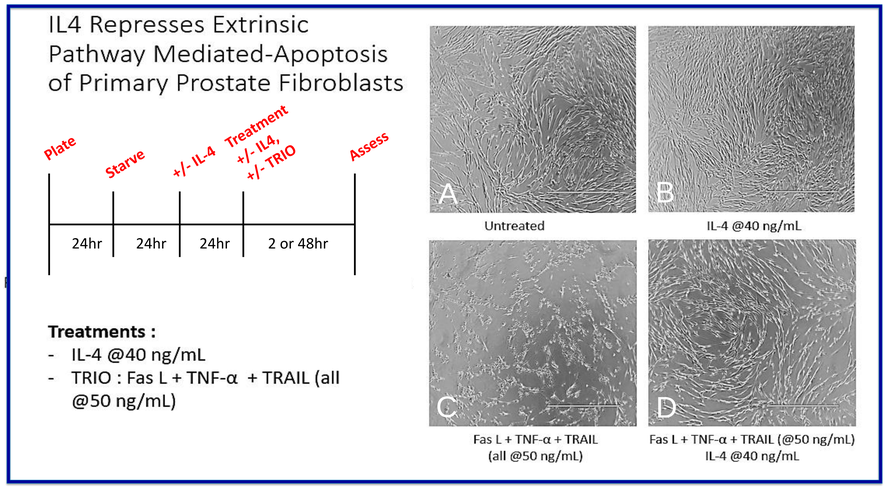

Figure 2. Primary prostate fibroblasts form near-confluent monolayers when grown in vehicle (A) or 40ng/ml IL-4 (B) for 48 hr. Cells first pre-treated for 2 hrs with pro-apoptotic TRIO cocktail then grown for 48 hr exhibit high levels of cell death (C). However, Cells first pre-treated for 2 hrs with pro-apoptotic TRIO cocktail then supplemented withIL-4 for 48 hr show greatly reduced cell death (d) compared to cells not supplemented with IL-4 (C). compared to cells just treated with TRIO. The treatment timeline shows the treatment process in days with the final day being when the images were captured.

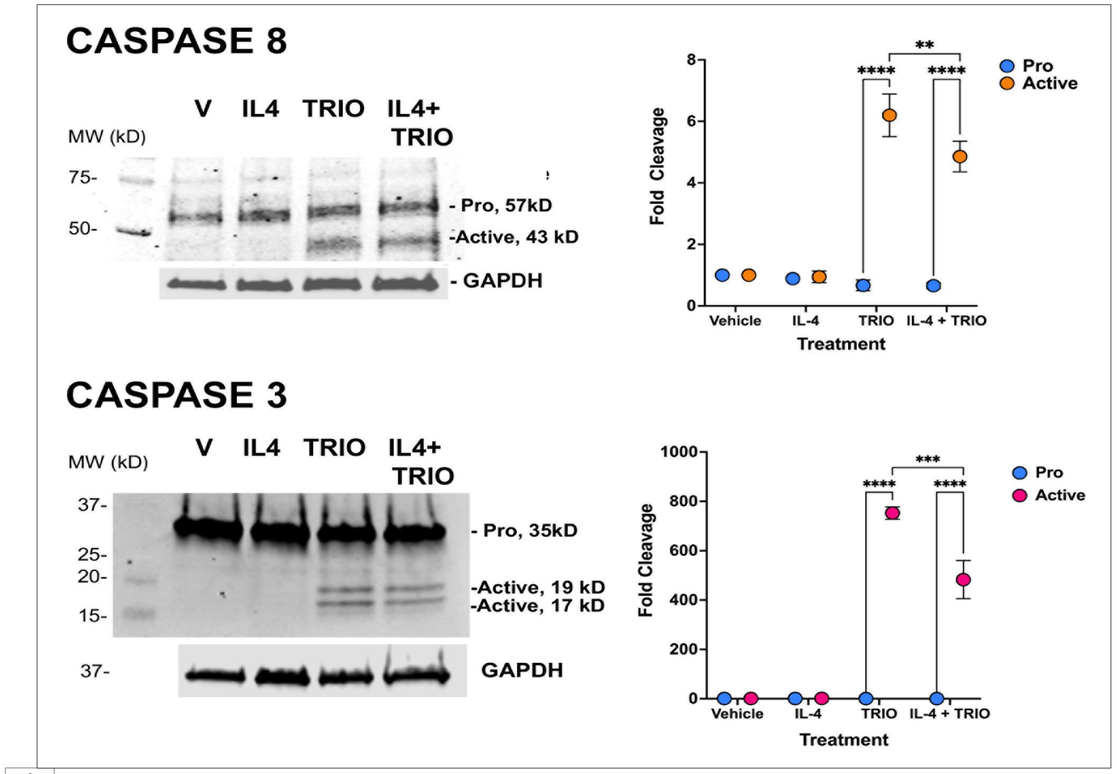

Figure 3. Cells were treated as in Figure 2 except that subsequent growth in IL-4 was 2 hr rather than 48 hr. Purified protein was immunoblotted against antibodies specific for Caspase 8 or Caspase 3. Cleaved/Active Caspase 8 is evident as a 43 kD band, and for Caspase 3 as 17 kd and 19kd bands. Graphs depict fold cleavage for each caspase. Treatment with IL-4 significantly reduced activation of both Caspase 8 and Caspase 3. *, p<.05; **, p<.01; ***, p<.001; ****, p<.0001.

Conclusions and Future Directions

Conclusions and Future Directions

Primary human prostate fibroblasts treated with IL-4 are protected from undergoing complete programed cell death in response to activation of extrinsic apoptotic pathways and caspase 8. IL-4 repressed activation of caspase 3, which can be activated by caspase 8.

Future directions will include targeting potential targets in the signaling mechanism behind IL-4 apoptotic repression in human prostate fibroblast, creating a study that analyzes the possibility of an IL-4 dose response, and analyzing the expression of extrinsic pathway death receptors.

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NIH National Institute of Diabetes and Digestive and Kidney Diseases