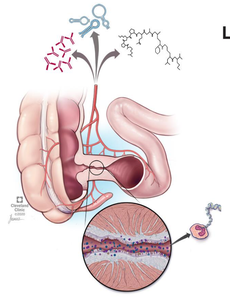
Characterizing the Role of AXL Signaling in Intestinal Fibrosis

Authors: Katherine Hullin[1], Ye Li[1], Samuel D Koch[1], Sean P Colgan[1], Calen A Steiner*[1]
University of Colorado School of Medicine, Department of Gastroenterology and Hepatology
*corresponding author, calen.steiner@cuanschutz.edu

Background

- **Crohn's Disease (CD)** is a chronic inflammatory disease of the GI tract marked by transmural inflammation of the intestinal lining
- It involves a complicated pathogenesis involving autoimmune dysfunction, genetic factors, and environment (microbiome, pathogenic microbial insult)
- A hallmark of Crohn's is **this repetitive cycle of inflammation and resolution**, and the fibrosis burden increasing with each round of inflammation[1]
- A majority of patients will eventually develop complications such as **fibrostenosis** and/or penetrating disease requiring surgery.
- Powerful **anti-inflammatory biologic therapies** have been developed that are effective in **reducing inflammation** BUT **they are not directly anti-fibrotic** and have not had a robust effect on these complications as we had hoped[2]
- So, our lab is trying to uncover alternative targets that can treat and help prevent fibrostenosing lesions.



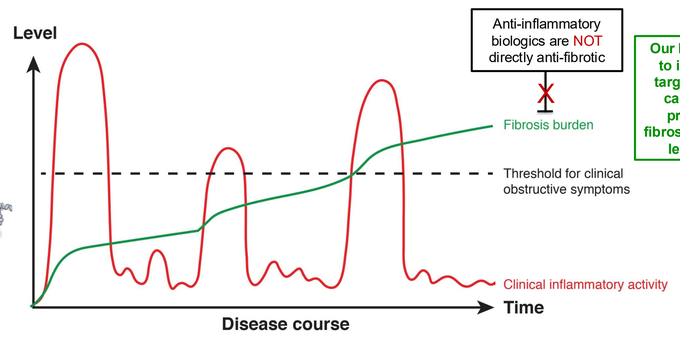


Figure 1: Infographic demonstrating the pattern of injury in Crohn's disease, highlighting the limitations of current anti-inflammatory biologics as not being directly anti-fibrotic and introducing the purpose of our research: to uncover new targets that can act in an anti-fibrotic manner.

Introduction

How is AXL involved in intestinal fibrosis?

- **AXL**, a receptor tyrosine kinase expressed by myofibroblasts, may play a central role in the development of intestinal fibrosis
- When activated by its ligand **GAS6**, AXL commences at phosphorylation cascade to carry out a variety of downstream effects[3]

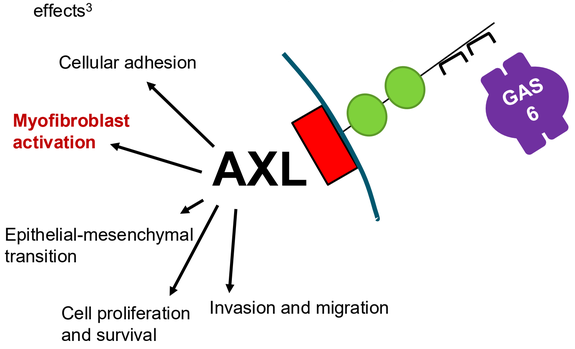


Figure 2: Graphic demonstrating various downstream effects of AXL when phosphorylated

- The activation and downstream effects of AXL signaling are cell-type specific, so the questions we're trying to answer are, in intestinal fibroblasts specifically:
  - What activates AXL?
  - What does AXL do, at the organism and cellular level?
  - How does it do those things?

Methodology

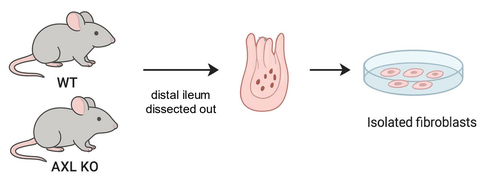


Figure 3: Whole-body AXL knockout (KO) mice were generated, and intestinal fibroblasts were subsequently isolated from the distal ileum of both wild-type (WT) and AXL KO mice. The distal ileum was selected because it represents the most common site of inflammation in CD.

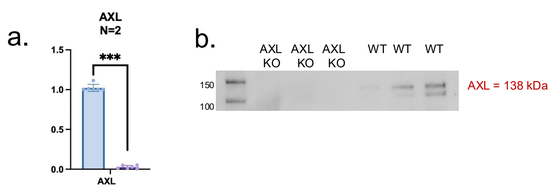


Figure 4: Confirmation of AXL KO; **a)** fibroblasts isolated from distal ileum of AXL KO and WT mice, plated to 100% confluency, cells harvest, RNA isolated, then cDNA and qPCR to quantify AXL expression in WT vs AXL KO mice at the RNA level ($p < 0.001$); **b)** cells from same passage plated in 6-well plate and harvested in RIPA buffer for western blot analysis of AXL expression at the protein level.

Results

AXL KO is associated with impaired migration of fibroblasts in response to scratching

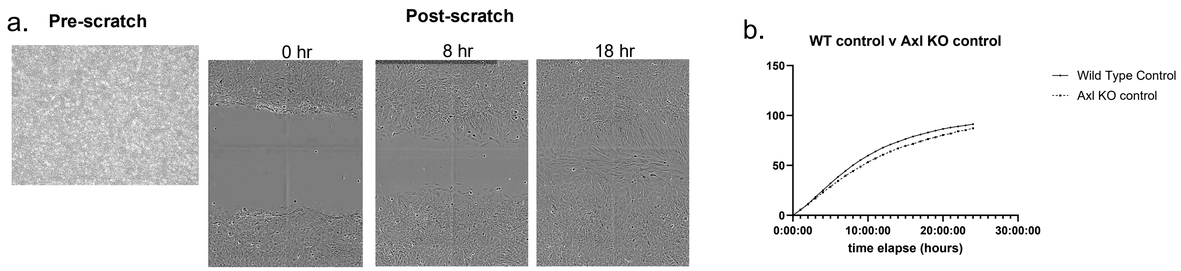


Figure 5: Fibroblasts from the distal ileum of wildtype and *Axl* KO mice were used in scratch-wound assays, and relative wound density was quantified to examine the ability of these fibroblasts to close an induced wound *in vitro* over 24 hr. Cells were plated in 96 well plates, at100% confluency each well was scratched and images were taken every hour for 24hrs until the cells filled in the scratched area. **A)** Representative images of WT fibroblasts at 0, 8, and 18 hours post-scratch. **B)** Graph showing the readout, measured as density comparing the wound region to the outside, controlling internally for the rate of proliferation of different cell types and relative confluency between wells. AXL KO fibroblasts show decreased density over time in comparison to WT cells post-scratch ($p = <0.0001$).

AXL is phosphorylated in response to mechanical stress

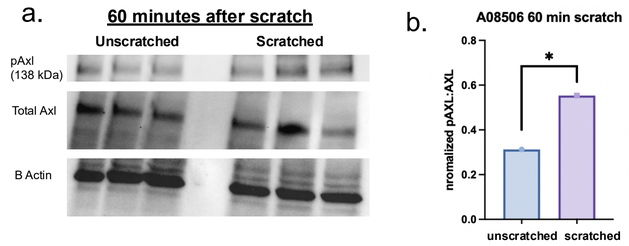


Figure 6: Intestinal fibroblasts isolated from the distal ileum of WT mice, plated to 100% confluency in a 6-well plate, 3 wells scratch and 3 wells left unscratched, and harvested 60min later. **A)** Western blot assessed for phosphorylated AXL (pAXL), total AXL, and beta actin in unscratched vs scratched cells. **B)** Ratio of phosphorylated AXL to total AXL normalized to beta actin and quantified using ImageJ, comparing the amount of pAXL in scratched wells to pAXL in unscratched wells ($p = 0.0399$).

AXL is phosphorylated in response to treatment with its ligand, Gas6, for 1hr

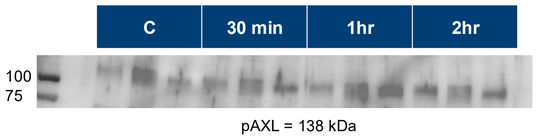


Figure 7: Intestinal fibroblasts isolated from distal ileum of three different WT mice, plated in 12-well plates to 100% confluency, then treated with 100ng/mL of Gas6 for 30 minutes, 1 hour, and 2 hours, and harvested in RIPA buffer for western blot analysis. Western blot stained for phosphorylated AXL across all 3 timepoints with untreated wells as the control.

Conclusion

- In intestinal fibroblasts, *Axl* is activated in response to scratch wounding and treatment with its ligand, Gas6
- *Axl* KO also impairs fibroblast migration and is correlated with decreased activation of fibroblasts
- Together these findings indicate that *Axl* signaling plays a key role in the activation and wound healing function of intestinal fibroblasts, making it a promising target for antifibrotic therapies in the treatment of intestinal fibrosis

Acknowledgements

1. Steiner, C. A., Stenosis Therapy and Anti-Fibrotic Research (STAR) Consortium. (2022). Biomarkers for the prediction and diagnosis of fibrostenosing Crohn's disease: A systematic review. Clinical Gastroenterology and Hepatology, 20(4), 817–846.e10. https://doi.org/10.1016/j.cgh.2021.05.054
2. Rieder, F., et al. (2017). Mechanisms, management, and treatment of fibrosis in patients with inflammatory bowel diseases. Gastroenterology, 152(2), 340–350.e6. https://doi.org/10.1053/j.gastro.2016.09.047
3. Rothlin, C. V., et al. (2014). Tyro3, Axl, and Mertk receptor signaling in inflammatory bowel disease and colitis-associated cancer. Inflammatory Bowel Diseases, 20(8), 1472–1480. https://doi.org/10.1097/MIB.0000000000000050

Contact info: katherine.hullin@cuanschutz.edu