CHARACTERIZING THE ROLE OF AXL SIGNALING IN INTESTINAL FIBROSIS

Authors: Katherine Hullin, Ye Li, Samuel D Koch, Sean P Colgan, Calen A Steiner

Purpose of Study: Fibrosis is the final common pathway to intestinal failure in Crohn's disease (CD), yet no medical therapies exist to treat intestinal fibrosis. Activated myofibroblasts are central drivers of fibrosis across organ systems, including the intestine. The receptor tyrosine kinase *Axl* has been implicated in myofibroblast activation and fibrogenic signaling. We previously showed that *Axl* is upregulated in models of intestinal fibrosis and that its inhibition exerts anti-fibrotic effects *in vitro*. Here, we further define the role of Axl signaling in intestinal fibrosis and evaluate its role in wound healing in the intestine.

Methods: Given the importance of activated fibroblasts in intestinal fibrosis, we first examined conditions that activate *Axl* in intestinal fibroblasts, focusing on responses to induced wound. Fibroblasts were isolated from the distal ileum of wildtype mice and injury was modeled *in vitro* using scratch-wound assays. *Axl* activation was assessed by quantifying its phosphorylation via western blot. To evaluate the role of *Axl* in fibrosis, we isolated fibroblasts from the ileum of Axl -null mice. 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. To determine baseline changes in downstream signaling in response to *Axl* KO, expression of profibrotic genes (TGFβ, FN1, MYLK, ACTA2, COL1A1, COL1A2) in the same *Axl* KO fibroblasts as compared to wildtype fibroblasts was measured by qPCR.

Results: *Axl* phosphorylation significantly increases 60 min following mechanical stress ($p = 0.0399$). Compared with wildtype fibroblasts, *Axl* KO fibroblasts show reduced migration, with lower cell density at the wound site over time ($p = <0.0001$). *Axl* KO is also associated with decreased expression of α smooth muscle actin, ACTA2, which is a defining marker for active myofibroblasts ($p = <0.0001$).

Conclusions: In intestinal fibroblasts, *Axl* is activated in response to scratch wounding. *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.