Lung Biology Research & Trainee Day
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Category: Predoc
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Title: Novel Ligand BTB Reveals Novel Mechanisms of Myofibroblast Differentiation in the Context of Pulmonary Fibrosis
Abstract: Novel Ligand BTB Reveals Novel Mechanisms of Myofibroblast Differentiation in the Context of Pulmonary Fibrosis Authors: Ashley R. Rackow, R.M. Kottmann Rationale: Respiratory disease represents one of the leading causes of mortality worldwide. Integral to all tissue repair and homeostasis is the proper maintenance of pH. Within the interstitium of the lung, pH can decline for several reasons including infection, tissue repair and fibrosis. Despite this finding, we do not understand how alterations in interstitial pH may contribute to disease pathology or cell biology. We began to study the proton sensing G-protein coupled receptor GPR65, utilizing a specific ligand BTB. We have demonstrated activation of GPR65 with BTB prevents myofibroblast differentiation via inhibition of ROCK/Rho signaling. Inhibition of ROCK/Rho signaling in vivo prevents the development of pulmonary fibrosis and may show efficacy in reversing areas of established fibrosis. For the first time, we demonstrate human lung fibroblasts have a biphasic response to the pro-fibrotic cytokine transforming growth factor beta (TGFβ). Our data demonstrate BTB targets ROCK/Rho in the second phase of induction, indicating it is the latter phase which is critical to the pro-fibrotic phenotype. We hypothesize the biphasic induction of the ROCK/Rho pathway is responsible for driving myofibroblast differentiation, independent from other well known pro-fibrotic mediators. Methods: Primary human lung fibroblasts, isolated from patients with and without pulmonary fibrosis were treated with TGFβ (1 ng) and/or BTB (50 uM). Protein was harvested at the indicated timepoints to assess myofibroblast differentiation and matrix production via alpha smooth muscle actin (αSMA), fibronectin and collagen 1. We also examined if BTB disrupts the pro-fibrotic ROCK/Rho pathway, including ROCK1, mDia and profilin. RhoA activity was assessed by incubating protein lysate with rhotekin-Rho binding domain coated beads to selectively isolate and pulldown active (GTP-bound) RhoA which is then subsequently detected via Western blot. Results: Fibroblasts treated with BTB exhibited decreased protein expression of collagen 1 and alpha smooth muscle actin (αSMA), a marker of myofibroblast differentiation at baseline. BTB blocked TGFβ induced αSMA expression and decreased Col1a1 and fibronectin protein. To examine the potential mechanisms by which BTB blocked myofibroblast differentiation we performed a RhoA activation assay, demonstrating BTB blocks RhoA activity levels. BTB also blocked ROCK1 protein, a key effector of RhoA. Interrogation of other downstream effectors of RhoA revealed BTB also decreases profilin protein, an important mediator of intracellular actin dynamics previously unstudied in relation to myofibroblast differentiation. Conclusions: We have strong data demonstrating BTB blocks myofibroblast differentiation. Previous work underscores the importance of ROCK1 in myofibroblast differentiation, and here we have data suggesting BTB may block myofibroblast differentiation via disruption of ROCK/Rho signaling.