The role of LKB1-AMPK signaling on renal mTOR and cyst progression in PKD

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BACKGROUND The Liver Kinase B1 (LKB1), a well-known tumor suppressor, directly phosphorylates and activates AMP Kinase (AMPK). The LKB1-AMPK pathway is an important regulator of cell metabolism, growth and proliferation. In PKD, the mTOR pathway is thought to contribute to cell proliferation and cyst growth. Downstream components of the mTOR pathway, i.e. ribosomal protein S6, are aberrantly phosphorylated in cyst cells. Fluid accumulation within the cyst cavity is driven by Cl^- secretion via an apical CFTR Cl^- channel. AMPK is an important negative regulator of both mTOR and CFTR. Our hypothesis is that the LKB1-AMPK pathway inhibits mTOR-mediated cell proliferation and CFTR-dependent fluid secretion by cystic cells and slows the progression of PKD.

METHODS We crossed *LKB1*$^{flox/flox}$ and Pkhd1-Cre mice to knock out LKB1 selectively in collecting ducts (CDs). In addition, we isolated cells of *LKB1*$^{flox/flox}$:*ROSA26-Cre*^{ERT2} mouse kidneys to generate an inducible LKB1 knockout cell line. To directly activate LKB1, we used a novel small molecule LKB1 activator called BIT-11. BIT-11 was delivered by daily gavage from 5 to 20 weeks of age in *PKD1*$^{RC/RC}$:*PKD2*$^{+/-}$ mice, an ADPKD model that develops a prominent cystic phenotype by 5 weeks of age that progresses slowly thereafter.

RESULTS *LKB1*$^{flox/flox}$:*ROSA26-Cre*^{ERT2} renal cells were treated with tamoxifen to delete LKB1 expression. The loss of LKB1 significantly decreased P-AMPK, but had no effect on mTOR signaling. CD-specific knockout LKB1 in otherwise normal mice resulted in hydronephrosis; however, renal cyst formation was not observed. On the other hand, direct LKB1 activation with BIT-11 increased P-AMPK and decreased P-S6 in human ADPKD cells. BIT-11 decreased Cl^- secretion across ADPKD cell monolayers and blocked cyst-like tubule dilations in *Pkd1*$^{-/-}$ mouse kidneys in metanephric organ culture. Treatment with BIT-11 caused a significant decrease in kidney weight (percent body weight), blood urea nitrogen and interstitial fibrosis in *PKD1*$^{RC/RC}$:*PKD2*$^{+/-}$ mice.

CONCLUSION The LKB1/AMPK pathway does not appear to regulate basal mTOR levels in the kidney; however, direct activation of the pathway using a novel LKB1 activator decreased mTOR, cell proliferation, Cl^- secretion and cyst growth, suggesting that this may be a potential therapeutic target for the treatment of PKD.