Calmodulin-dependent kinase IV (CaMK4) overexpression promotes mTOR-mediated proliferation of cyst epithelial cells in polycystic kidney disease

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Introduction: Autosomal dominant polycystic kidney disease (ADPKD) is characterized by progressive enlargement of fluid-filled cysts causing nephron loss and a decline in renal function. Mammalian target of rapamycin (mTOR) is overactive in cyst-lining cells and contributes to abnormal cell proliferation and cyst enlargement; however, the mechanism for mTOR stimulation remains unclear. Calcium/calmodulin-dependent kinase IV (CaMK4), a multifunctional kinase, can stabilize and activate mTOR and has been shown to contribute to human disease; however, the role of CaMK4 in PKD had not been examined.

Methods: CaMK4 levels were compared in ADPKD and normal human kidneys and in PKD and normal mouse kidneys using immunoblot analysis and immunostaining. Effects of CaMK4 knockdown using shRNA and pharmacological inhibition with KN-93 on mTOR signaling were determined by changes in the phosphorylation of S6 kinase (S6K) and S6.

Results**:** We discovered that CaMK4 was overexpressed in kidneys of ADPKD patients and PKD mice. Inhibition of CaMK4 with KN-93 reduced mTOR, P-S6K, and P-S6 levels, and inhibited proliferation and *in vitro* cyst growth of ADPKD cells. The inhibitory effect of KN-93 was independent of the AMP kinase (AMPK) pathway, and the combination of KN-93 and metformin, an AMPK activator, caused additive effects on mTOR inhibition and *in vitro* cyst growth of human ADPKD cells. CaMK4 knockdown using shRNA reduced mTOR abundance and phosphorylation of S6K (P-S6K) and decreased the phosphorylation of glycogen synthase kinase β (P-GSK3β) at an inhibitory residue. Treatment with a GSK3β inhibitor increased mTOR and reversed the effect of KN-93 on mTOR abundance.

Conclusion: Our data suggest that CaMK4 overexpression increases the mTOR abundance mediated by the inhibition of GSK3β, leading to increased mTOR signaling and aberrant proliferation of cystic cells in PKD.

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