Phosphorylation insensitive 4E-BP1 reduces hyperproliferative phenotype in vitro

Sara J Holditch[1], Carolyn N Brown[1], Charles L Edelstein[1]

[1]Univ Colorado Denver;

INTRO

Unchecked proliferation of cystic epithelial cells is a major contributor to cyst growth in PKD. The 4E-BP1 pathway is a crucial checkpoint in translation initiation and cellular proliferation. The aim of this study was to determine 1) whether PKD patient and animal model kidney tissues have dysregulated phospho (p4E-BP1) species, 2) the effect of a phosphorylation insensitive 4E-BP1 (F113A) on p4E-BP1 species distribution, cap dependent protein translation, and proliferation in renal epithelial cells and 3) the safety of in vivo administration of F113A adeno-associated virus (AAV) in neonatal and adult mice.

METHODS

Immunofluorescence staining of phospho 4E-BP1 species (T70, T37/47, S65) was performed on patient ADPKD biopsies, Han:SPRD (Cy) rat, Pkd1-/-, Pkd2 -/-, and cpk mouse kidneys. Western blot analysis, Cyquant cellular proliferation, and Firefly-renilla assays were performed on human primary epithelial cells from normal renal cortical tubular epithelium ($PKD1^{+/+}$) and ADPKD cyst-lining epithelium ($PKD1^{-/-}$) transfected with control or pCAG-F113A or transduced with control or F113A lentivectors. AAV9 F113A vectors were prepared and administered at D3 and aged to D17 or D120, and aged to D150.

RESULTS

p4E-BP1 species were present in patient ADPKD and murine PKD model renal tissues. In vitro, phosphorylation insensitive 4E-BP1 (F113A) resulted in substantially reduced p4E-BP1 T37/46(0.89±0.08 vs 0.012±0.004DU, $p<0.01$) and S65 (0.63±0.04 vs 0.003±0.001DU, $p<0.01$), reduced cap-dependent protein translation (37%, $p<0.01$), and reduced 72hr proliferation (250±4 vs 180±5 480/528nm O.D, $p<0.0001$) in $PKD1^{-/-}$ cells. Surprisingly, in $PKD1^{+/+}$ cells, F113A resulted in no p4E-BP1 reduction, reduced cap-dependent protein translation (32%, $p<0.01$), and marginally reduced proliferation (375±5 vs 314±4 480/528nm O.D, $p<0.0001$). Acute stimulation with insulin resulted in maintained S65 suppression with F113A transfection in $PKD1^{-/-}$ (2.1±0.3 vs 0.2±0.1AU, *$p<0.0001$). In neonatal and adult mice, administration of AAV-F113A was both well tolerated, and resulted in detectable F113A expression.

CONCLUSIONS

F113A overexpression results in a shift towards hypophosphorylated 4E-BP1 species, reduced cap dependent protein translation, and reduced proliferation, with more aggressive effects in $PKD1^{-/-}$ vs $PKD1^{+/+}$ cells. F113A gene therapy to counter dysregulated 4E- BP1 in a murine model of PKD, is the next step in addressing a pathway seemingly integral to the pathobiology of PKD.