Autoimmune Abnormalities Ameliorate Polycystic Kidney Disease

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Background: We have published that there is a decrease in autophagy proteins in Pkd1ΔRGC mouse kidneys. Our study aimed to determine the mechanistic role of suppressed autophagy in causing cyst growth using pharmacological and genetic autophagy inhibition.

Methods: Male Pkd1ΔRGC (RC) mice were treated with 2-Deoxyglucose (2DG) or Chloroquine (CHLQ) from 50-120 d of age. Kidney specific Pkd1 Atg7 double knockout mice were generated by Kesp1-Cre-lox recombinative. Relative densitometry units (RDU) were determined on immunoblot. Autophagic flux was measured by the change in LC3-II (autophagosomes)/+/- Baflofinycin (Baf).

Results: Autophagic flux was present in wild type (WT) and 120 d old RC but suppressed in 150 d old RC kidneys. LC3-II (RDU) +/- Baf was 0.1 vs 0.7 in WT (p=0.01), 0.6 vs 1.0 in 120 d old RC (p=0.05) and 2.4 vs 2.1 (NS) in 150 d old RC. 2DG resulted in a decrease in ATG12-5 complex and suppressed autophagic flux in RC kidneys. LC3-II (RDU) +/- CHLQ was 0.5 vs 0.8 in VEH (p=0.05), 0.7 vs 0.7 in 2DG (NS). 2DG significantly reduced cyst growth and improved kidney function. Cystic index (%), count +/- 2DG; 7.7 vs 3.7 (p=0.01), 211 vs 161 (p=0.05). BUN (mg/dL) +/- 2DG: 35 vs 27 (p=0.01). Next, RC mice were treated with CHLQ, a specific autophagy inhibitor. CHLQ resulted in suppressed autophagic flux, less PKD and improved kidney function in RC mice. LC3-II (RDU) +/- Baf was 1.2 vs 1.2 (NS) in CHLQ treated kidneys. Cyst index (%), Cyst no +/- CHLQ in RC mice was 15.5 vs 7 (p=0.07), 231 vs 105 (p=0.05). BUN and creatinine (by HPLC) (mg/dL) +/- CHLQ: 41 vs 26 (p=0.05), 2.9 vs 2.3 mg/dL (p=0.05). Next, autophagy was inhibited in PKD kidneys by generating double Pkd1 Atg7 KO mice. The 2 kidney/BW (%) was improved in Pkd1 Atg7 KO vs. single Pkd1 KO mice (p<0.05). Next, autophagy was inhibited in PKD kidneys by generating double Pkd1 Atg7 KO mice. Mutation-specific agonist mechanism may also contribute to renal cystogenesis. Better understanding of EVs was achieved by tunable resistive pulse sensing and dynamic light scattering, electron microscopy, and western blot analyses.

Results: Physical characterization of EVs revealing similar average sizes and zeta potentials (at pH 7.4) for EVs from mIMCD3 (123.5 ± 5.7 mV and –16.3 ± 2.1 mV), T1G cells (131.5 ± 8.3 mV and –19.8 ± 2.7 mV), and T2J cells (127.3 ± 4.9 mV and –20.2 ± 2.1 mV). EVs derived from parental mIMCD3 cells and both mutated cell lines were heterogeneous (>90% of EVs < 150 nm) in nature. Immunoblotting detected cilial HEDGEhog signaling protein Arl13b; intercellular proteins TSG101 and Alix; and transmembrane proteins CD63, ED9, and CD81. Compared to T2J deletion, T1G deletion cells had reduced EV production and release rates. EVs from T1c1 mutant cells altered mTORC1, autophagy, and β-catenin pathways differently than EVs from Tsc2-mutated cells. Quantitative PCR analysis revealed the down regulation of mir-212a-3p and mir-99a-5p in EVs from Tsc2-mutated cells compared to EVs from T1c1-mutant cells.

Conclusions: EV-derived mir-212-3p and mir-99a-5p axes may represent therapeutic targets or biomarkers for TSC disease.

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ADPKD Mutations in the Stalk/Tethered Agonist of Polycystin-1 CTF

Arl13b Negatively Regulates Kidney Cysts from Within Cilia

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Background: Like Adhesion G protein-coupled receptors (aGPCRs), the N-terminal ectodomain of polycystin-1 (PC1) contains a membrane-proximal GAIN domain that catalyzes self-cleavage at its embedded GPCR proteolysis site (GPS), dividing these proteins into extracellular N-terminal (NTF) and membrane-embedded C-terminal (CTF) fragments. CTF depends on a novel mechanism and undergoes a conformational change that eliminates the N-terminal GPCR G-αiβγ complex. PC1 stalk has more than one region important in regulation of CTF activation; deletion of the stalk dramatically inhibited signaling, while synthetic stalk-derived peptides could rescue signaling by the stalkless CTF and utilized an aGPCR-like tethered agonist mechanism to activate G protein signaling to its proper orientation. This study utilizes an aGPCR-like tethered agonist mechanism to activate G-protein signaling to an NFF reporter, which involves the short, N-terminal, extracellular stalk of the CTF serving as the tethered agonist. Deletion of the stalk dramatically inhibited signaling, while synthetic stalk-derived peptides could rescue signaling by the stalkless CTF and inhibit cystogenesis in metastatic organ cultures of hypomorphic Pkd1ΔRGC kidneys. Here we assess the effect of stalk-localized ADPKD missense mutations on signaling by the dissociated CTF subunit and on GPS cleavage of full-length (FL) PC1 to gain additional insight for this regulatory mechanism.

Methods: Wild type (WT) or mutant, FL or CTF forms of PC1 were expressed in HEK293T cells and compared for activation of an NFAT promoter-luciferase reporter, levels of total and cell surface expression and GPS cleavage. Homology modeling of the PC1 (A2) domain was also performed.

Results: Of 11 substitutions throughout the stalk, 6 significantly reduced, 2 increased, and 3 had no effect on signaling by PC1 CTF. Total and surface expression levels of the CTF mutants ranged from 62-125% of WT CTF. Most mutations had no effect on GPS cleavage by FL-PC1. Mutations that inhibited signaling or GPS cleavage mapped to the N-terminal portion of the stalk, while the 2 mutations that increased signaling were in the latter half.

Conclusions: PC1 stalk has more than one region important in regulation of CTF signaling. Perhaps as part of the agonistic sequence or for its proper orientation. This study underscores the importance of PC1 GPS cleavage and suggests disruption of a tethered agonist mechanism may also contribute to renal cystogenesis. Better understanding of the tethered agonist mechanism is necessary for development of PKD1 mutation-specific therapies.

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Cystic Kidney Disease - I

Polygenic Kidney Disease - II