Deciphering the impact of RAC1-SPTAN1 in ARPKD cystogenesis using multifaceted models

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Introduction… Autosomal recessive polycystic kidney disease (ARPKD) is a devastating ciliopathy characterized by the formation of severe renal cysts and a progressive decline in kidney function. The causative gene for most ARPKD cases is Polycystic Kidney and Hepatic Disease 1 (*PKHD1*). In a recent study, we developed an organoid-on-chip model with *PKHD1-mutants* and identified RAC1/c-FOS as potential therapeutic targets for ARPKD patients. However, the precise mechanism through which RAC1 influences cyst formation in ARPKD remains elusive.

Methods… We analyzed RNA-seq data derived from human kidney organoids generated from pluripotent stem cells (PSCs) carrying *PKHD1* heterozygous and homozygous mutations, cultured in organoid-on-chip models. These models induced cyst formation in CDH1+ distal nephrons, mimicking the pathophysiology observed in ARPKD patients. Differentially expressed gene analyses were performed to compare *PKHD1* heterozygous and homozygous mutants. To validate our findings from our initial organoid-on-chip model, we employed various approaches, including an improved perfusion system employing microfluidic chips and examinations of patient kidney samples.

Results... Our analysis identified 27 RAC1 effectors that exhibited significant alterations in *PKHD1*-/- mutants when compared to *PKHD1*+/- organoids. Notably, the majority of these effectors were downregulated. In-depth exploration of these 27 effectors, combined with data from existing literature and human kidney databases, revealed only one effector, SPTAN1, potentially associated with cystic biological processes and distal nephrons. Immunostaining of kidney organoids mirrored the expression pattern of SPTAN1 in the human kidney, demonstrating reduced SPTAN1 expression in *PKHD1*-/- ARPKD organoids. This downregulation was confirmed quantitatively by both mRNA and protein levels, underscoring the significance of SPTAN1 reduction. Furthermore, analysis of SPTAN1 expression in ARPKD patient kidneys through immunohistochemistry revealed suppressed expression in the cystic epithelium, consistent with our observations in ARPKD organoids.

Conclusions... These findings suggest that RAC1 activation in ARPKD is driven by the reduced expression of its effector, SPTAN1, thereby contributing to the development of pathological cystic processes. Ongoing investigations include the confirmation of this phenotype using SPTAN1 knockout PSCs and mouse transgenic models characterized by renal enlargement. These results may offer novel insights into the pathogenesis of ARPKD, potentially leading to novel therapeutic avenues for ARPKD patients.

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