The Crosstalk between Polycystin-2 and CFTR in Autosomal Dominant Polycystic Kidney Disease

Mónica Roxo-Rosa and Susana S. Lopes

CEDOC - Chronic Diseases Research Center, Nova Medical School | Faculdade de Ciências Médicas, Universidade Nova de Lisboa; Edifício CEDOC I, Rua do Instituto Bacteriológico nº 5, 5-A e 5-B, 1150-190 Lisboa, Portugal
monica.rosa@nms.unl.pt; susana.lopes@nms.unl.pt

1. BACKGROUND

Affecting as many as 1 in 400 - 1000 new-borns, **Autosomal Dominant Polycystic Kidney Disease** (**ADPKD**) is the most common genetic cause of renal failure and the fourth leading cause of Chronic Kidney Disease. It is clinically characterized by the development of massive kidney cysts that destroy the organ's function. With no biomarkers, nor effective therapies, the majority of patients require dialysis, and renal transplantation by age 60.

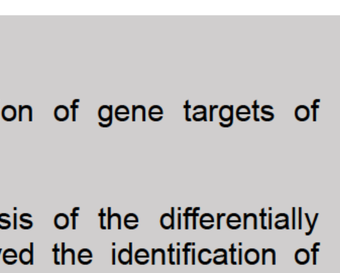
ADPKD is caused by mutations in *PKD1* and *PKD2* genes, encoding Polycystin-1 and 2, respectively. These assemble together, with Polycystin-1 acting as a mechanosensor and **Polycystin-2** functioning as a Ca^{2+}-conducting channel. At the cellular level, ADPKD is characterized by the lack of intracellular Ca^{2+} homeostasis which is thought to trigger cystogenesis. Afterwards, cyst inflation and continuous enlargement entail marked transepithelial ion and fluid secretion into the cyst lumen. This process is mediated by Cystic Fibrosis Transmembrane conductance Regulator (**CFTR**). Indeed, the inhibition or degradation of CFTR prevents the fluid accumulation within cysts. However, the molecular mechanisms involved in the activation of CFTR during kidney cyst inflation are still emerging.

Our aim is to understand the *in vivo* mechanisms by which the lack of Polycystin-2 leads to CFTR stimulation in ADPKD.

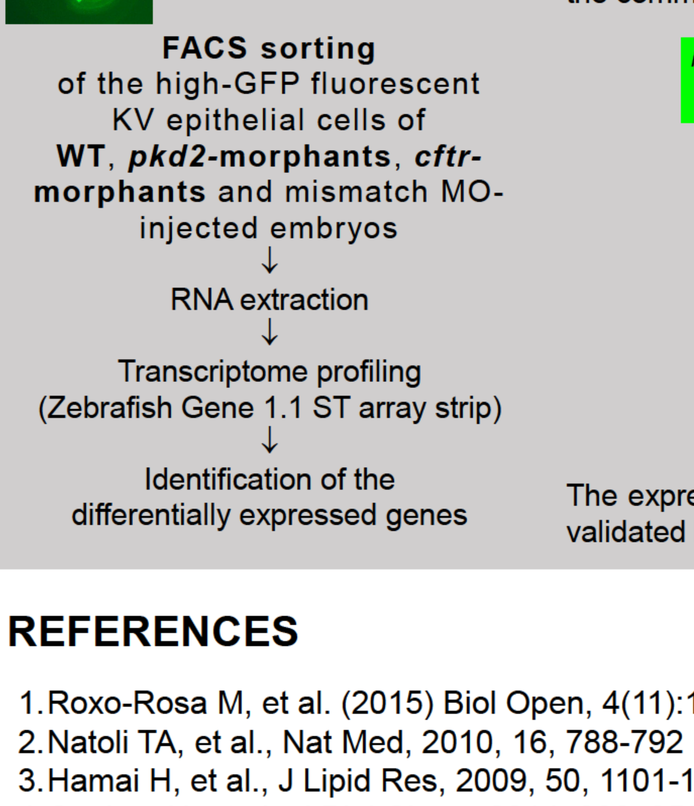
2. MODEL SYSTEM – the zebrafish Kupffer's Vesicle

The Kupffer's vesicle (KV) is the organ that defines the laterality axis during the embryogenesis of zebrafish. We have recently shown that, although not being a renal-related organ, the KV is a useful model organ to study the process of cyst inflation (1).

- The KV is a fluid-filled enclosed cavity lined by monociliated cells that endogenously express Polycystin-2 and CFTR;



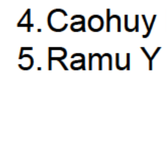
Live z-scans of zebrafish KVs



- Its inflation depends on CFTR (Fig. A);
- Mimicking kidney cysts, the knockdown of Polycystin-2 results in larger KVs through CFTR overstimulation (Fig. C)
- Indeed, the volume of the *pkd2*-morphant KVs was rescued by inhibiting CFTR and was synergistically enlarged by its stimulation (Fig. D, E).

3. Microarray analysis

Microarray analysis of KV sorted cells allowed the identification of gene targets of Polycystin-2 and CFTR specific knockdown.



foxj1a:GFP
trangenic embryos

FACS sorting
of the high-GFP fluorescent KV epithelial cells of **WT**, ***pkd2*-morphants**, ***cftr*-morphants** and mismatch MO-injected embryos
↓
RNA extraction
↓
Transcriptome profiling
(Zebrafish Gene 1.1 ST array strip)
↓
Identification of the differentially expressed genes

The comparative analysis of the differentially expressed genes, allowed the identification of the common targets of these two proteins.

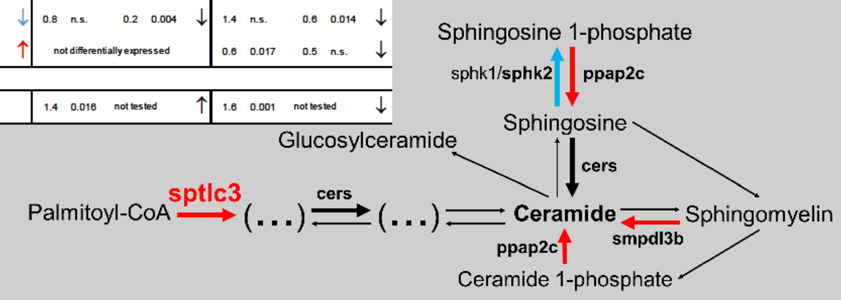
The expression of several genes of interest was validated by qPCR.

4. Differentially expressed genes

Among our targets, we found genes encoding enzymes of the **Sphingolipid Metabolism**. Our data suggests that the lack of Polycystin-2 alters the cellular sphingolipid homeostasis. It is our goal to understand the extent of this change.

Gene ID	Gene description	Fold Change: *pkd2*-morphant vs WT array *p*	*pkd2*-morphant vs WT qPCR *p*	*cftr*-morphant vs WT array *p*	*cftr*-morphant vs WT qPCR *p*	*cftr*-morphant vs *pkd2*-morphant array *p*	*cftr*-morphant vs *pkd2*-morphant qPCR *p*
PKD2 related							
b3galt2	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 2	1.9 0.001	not differentially expressed ↑	1.4 0.019	0.5 <0.0001 ↓	0.8 0.046	0.5 0.009 ↓
cers2b	ceramide synthase 2-like	1.4 0.006	not differentially expressed ↑	not differentially expressed		0.8 0.015	0.7 0.037 ↓
cers3b	ceramide synthase 3b	0.7 n.s.	0.4 0.047 ↓	not differentially expressed		1.5 0.011	8.0 0.05 ↑
ppapc	phosphatidic acid phosphatase type 2C	1.6 0.013	1.2 n.s. ↑	not differentially expressed		0.6 n.s.	0.7 n.s. ↓
smpdl3b	acid sphingomyelinase-like phosphodiesterase 3b-like	1.7 0.001	not tested ↑	not differentially expressed	not tested ↑	0.7 0.005	not tested ↓
sphk2	sphingosine kinase 2	0.6 0.04	0.3 0.005 ↓	0.8 n.s.	0.2 0.004 ↓	1.4 n.s.	0.6 0.014 ↓
sptlc3	serine palmitoyltransferase, long chain base subunit 3	1.4 n.s.	3.0 0.042 ↑	not differentially expressed		0.6 0.017	0.5 n.s. ↓
CFTR related							
GAL3ST1	galactose-3-O-sulfotransferase 3-like	not differentially expressed	not tested	1.4 0.016	not tested ↑	1.6 0.001	not tested ↓

This is consistent with the work of Natoli *et al.* showing that ADPKD patient tissues accumulate glucosylceramide and that inhibition of its synthesis rescues the cystic phenotype in mice (2).

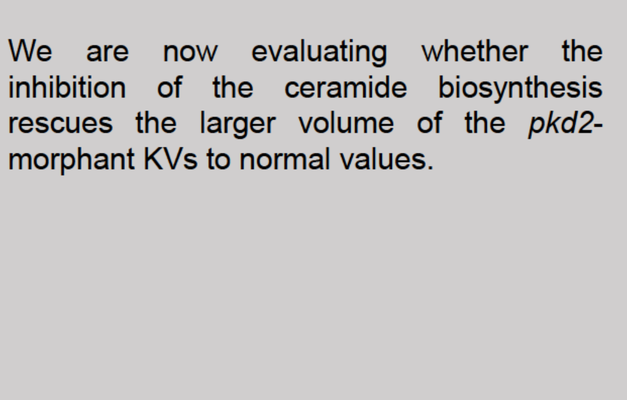


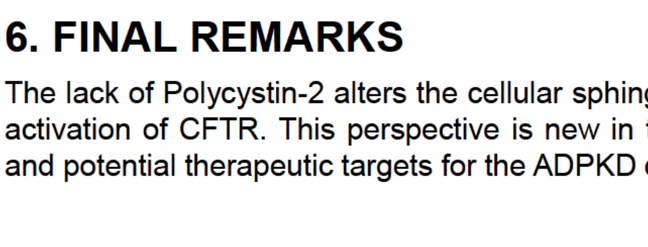
5. Is the lack of sphingolipid homeostasis correlated with the activation of CFTR in ADPKD?

It has been showed that ceramide interferes with the maturation, stability and activity of CFTR (3-5).

Thus, we postulate that, in the absence of Polycystin-2, changes in the sphingolipid homeostasis may underlie the activation of CFTR.

To test our hypothesis, we treated embryos from 6 ss (10 hpf) onwards with 20 μM Myriocin, an inhibitor of the **serine palmitoyltransferase** (**sptlc**), the first step in ceramide biosynthesis.





- Blockage of the ceramide biosynthesis has no effect in the Polycystin-2 expression pattern in KV cells.

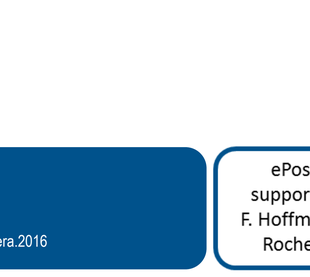
WT

Confocal live-microscopy scans of the whole KV of 10-11 ss *ras*:GFP transgenic embryos.
Average KV volumes in picoL.

V=59×10³ ± 24×10³ μm³ ≈ 59 picoL

- However, it causes a significant reduction in the KV volume of WT treated embryos. This suggests an inhibition of the CFTR activity.

20 μM Myriocin



V=38×10³ ± 26×10³ μm³ ≈ 38 picoL

We are now evaluating whether the inhibition of the ceramide biosynthesis rescues the larger volume of the *pkd2*-morphant KVs to normal values.

6. FINAL REMARKS

The lack of Polycystin-2 alters the cellular sphingolipid homeostasis and this may underlie the activation of CFTR. This perspective is new in the field and may bring important biomarkers and potential therapeutic targets for the ADPKD cystogenesis.

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