RGLS4326 confers efficacy and modulate aberrant signaling and metabolic pathways in PKD mouse models

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Abstract

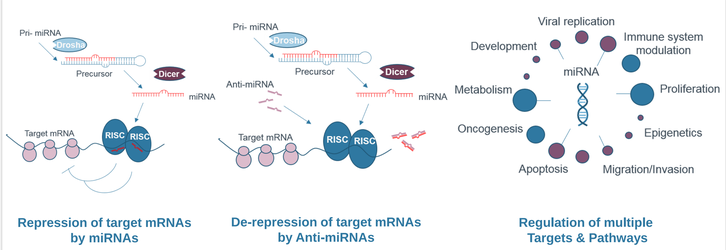
Background:
Autosomal dominant polycystic kidney disease (*ADPKD)* is caused by mutations in either *PKD1* or *PKD2* genes, where expansion of fluid-filled cysts and renal fibrosis often leads to end-stage renal disease. MicroRNAs are short non-coding RNAs that modulate several biological processes. We have previously shown that aberrant expression of miR-17 family of microRNAs is involved in human ADPKD pathogenesis. RGLS4326 is a chemically-modified oligonucleotide designed to sterically inhibit miR-17 functions and has been shown to reduce cyst growth *in vitro* and *in vivo*. The goal of this study was to determine the signaling pathways modulated by RGLS4326 treatment in PKD mouse models

Methods:
RGLS4326 is efficacious in the *Pkd2KO* and *Pcy*/CD-1 mouse models of PKD. We performed RNA sequencing and metabolite profiling using kidney samples from both mouse models following RGLS4326 treatment. Ingenuity Pathway Analysis was used to provide novel insights into signaling pathways modulated by RGLS4326 treatment

Results:
Through RNA sequencing, we identified >1000 differentially expressed genes in the PKD kidney samples compared to their age- and strain-matched normal controls. Comparative pathway analysis identified several dysregulated signaling pathways in the two PKD mouse models, including the Pparα, WNT/ β-catenin and PCP signaling, that were in turn modulated following RGLS4326 treatment. Next, we performed kidney global metabolite profiling comparing *Pkd2KO* and normal kidneys, and identified several biochemical alterations in the *Pkd2KO* model, including substantial changes in lipid metabolism. In particular, decrease in β-fatty acid oxidation pathway was observed in the *Pkd2KO* kidneys, which corroborates with previously observed Pparα-dysregulation in PKD mouse models

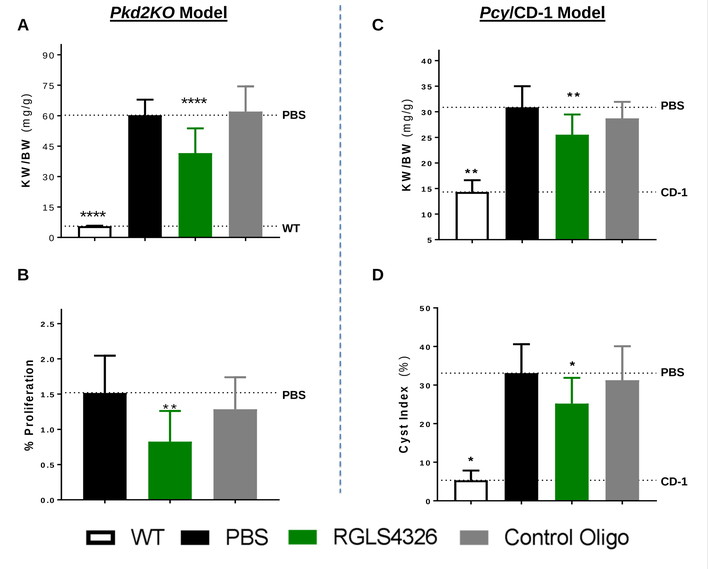
Conclusion: Our results indicate that RGLS4326 confers efficacy and modulates aberrant PKD signaling and metabolic pathways. These results support the clinical development of RGLS4326 for the treatment of ADPKD

miRNA targets multiple signaling pathways



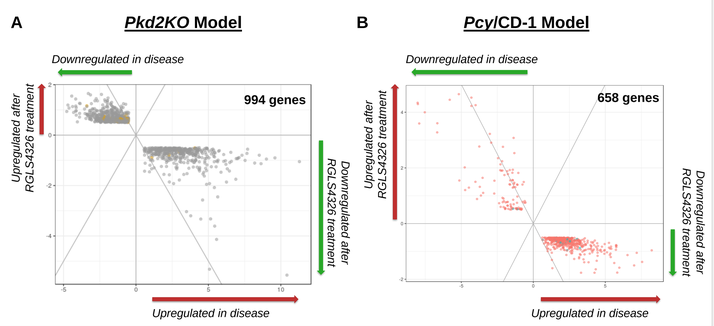
Repression of target mRNAs by miRNAs | **De-repression of target mRNAs by Anti-miRNAs** | **Regulation of multiple Targets & Pathways**

RGLS4326 is efficacious in *Pkd2KO* and *Pcy*/CD-1 mouse models



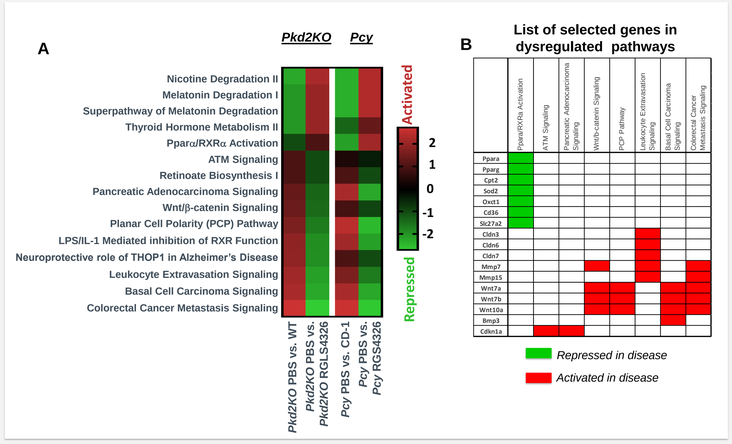
(A) Decrease in Kidney-Weight-to-Body-Weight (KW/BW) in the *Pkd2KO* efficacy model by subcutaneous administration of RGLS4326 at 20 mg/kg on days P11, P12 and P13 and P19; Kidneys were collected at P28. **(B)** Reduction in proliferation after RGLS4326 treatment as shown by phospho-histone3 staining in the *Pkd2KO* model. **(C)** Decrease in KW/BW in the *Pcy*/CD-1 efficacy model following Q4W dose of RGLS4326 at 25 mg/kg from 5 to 30 weeks of age. **(D)** Reduction of cyst index after RGLS4326 treatment in the *Pcy*/CD-1 efficacy model

RNA Seq differential expression analysis in efficacy models



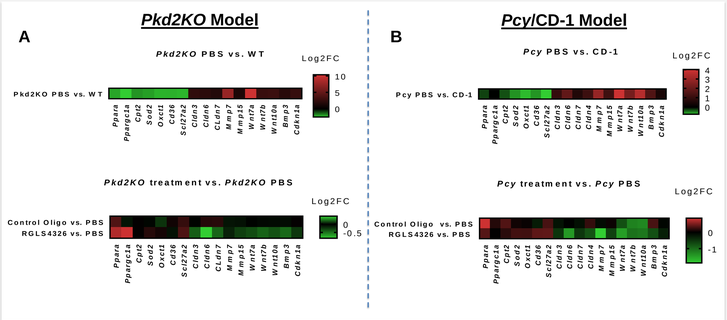
RNAseq differential expression (DE) analysis of *Pkd2KO* and *Pcy*/CD-1 efficacy models. DE genes upregulated by disease and downregulated by RGLS4326 treatment and vice-versa for the *Pkd2KO* **(A)** and *Pcy*/CD-1 **(B)** efficacy models. Criteria FDR<0.05, Log2FC>0.5 in disease and <-0.5 in RGLS4326 vs PBS or Log2FC<-0.5 in disease and >0.5 in RGLS4326 vs PBS.

Comparative pathway analysis –*Pkd2KO* and *Pcy* efficacy models



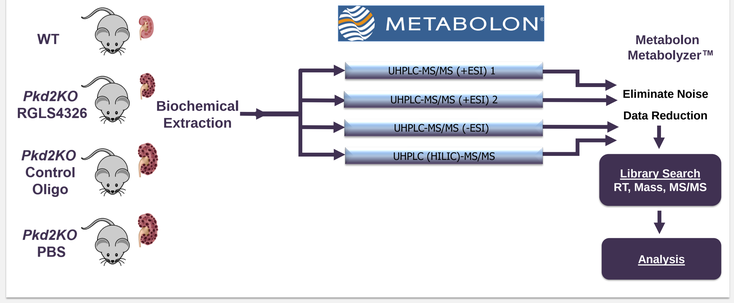
Ingenuity pathway analysis of the DE genes from *Pkd2KO* and *Pcy*/CD-1 efficacy models
(A) Comparative pathway analysis revealed that dysregulated pathways in the disease models (when compared to age matched WT controls) are restored upon RGLS4326 treatment in both models
(B) Genes from selected dysregulated pathways identified in IPA comparative analysis were assessed by qPCR in both models

Kidney expression of selected genes following RLGS4326 treatment in *Pkd2KO* and *Pcy* efficacy models

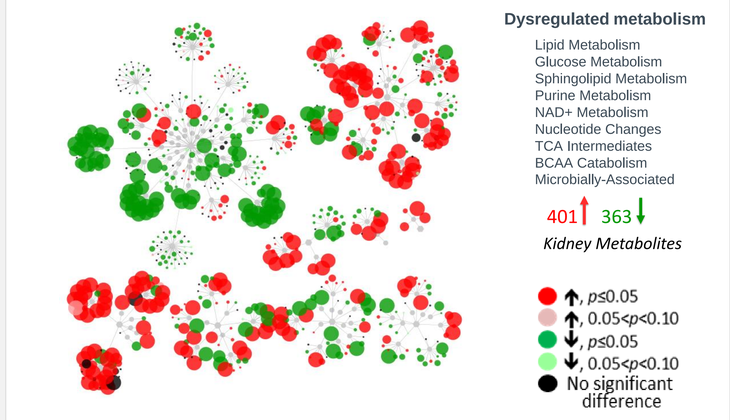


- Selected genes identified key PKD-disease related pathways in *Pkd2KO* **(A)** and *Pcy*/CD-1 **(B)** and their modulation after RGLS4326 treatment
- Upregulation of Pparα upon RGLS4326 treatment in the *Pkd2KO* model corroborates with previous findings where genetic deletion of miR17-92 cluster in the *Pkd2KO* kidney restores the de-repressed Pparα pathway (Hajarnis *et al*. 2017)
- Overexpression of claudins in ADPKD patients compared to normal tissue has been reported previously (Almeida *et al*. 2016). Consistently we detected elevated levels of claudins in disease kidney and downregulation following RGLS4326 treatment in both efficacy models
- Aberrant regulation of PCP and Wnt signaling in Pkd1 Knockout cystic kidneys has been already described (Luyten *et al*. 2010; Quin *et al*. 2012). In agreement with this, we also observed the overexpression of Wnt7a and Wnt7b in *Pkd2KO* and *Pcy* mouse models, where their levels were significantly downregulated after RGLS4326 treatment

Kidney global metabolite profiling

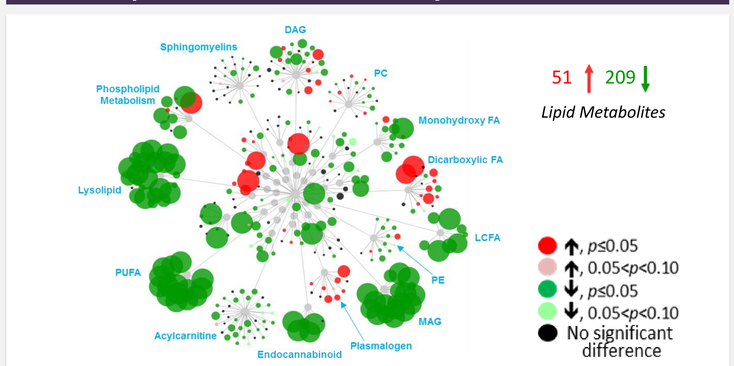


Global metabolic profile: *Pkd2KO* vs WT



Metabolic profiles between disease *Pkd2KO* and healthy WT kidney displayed significant differences in metabolites (401 upregulated and 363 downregulated) across all biochemical nodes, indicating a broadly altered metabolism in the disease kidney such as glucose, lipid, sphingolipids, Purine, NAD+ and amino acids metabolism

Lipid metabolism in the kidney: *Pkd2KO* vs WT



Altered lipid metabolism noted in *Pkd2KO* kidney reflected the differential uptake, synthesis or utilization of lipids. Several lipid classes were significantly decreased in *Pkd2KO* vs WT including monoacylglyerols (MAG), long chain fatty acids (LCFA), polyunsaturated fatty acids (PUFA), endocannabinoids and lysolipids. Preliminary data demonstrated that treatment with RGLS4326 modulated some dysregulated metabolites.

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