PROJECT

Supervisor:	LAURA CASSINA
Title:	RECESSIVE POLYCYSTIC KIDNEY DISEASE: MECHANISTIC INSIGHTS ON MITOCHONDRIAL DYSNFUNCTION
Curriculum:	CELLULAR AND MOLECULAR BIOLOGY
Link to the personal page of the University or relevant hospital site website:	https://research.hsr.it/en/divisions/genetics-and-cell-biology/cystic-kidney-disorders.html

Description of the Project (max 3,000 characters including spaces)

Background/gap of knowledge

Polycystic kidney Disease (PKD) is a genetic disorder mainly characterized by bilateral renal cysts formation, but other organs may be affected. There are two main forms of monogenic PKD, autosomal dominant and autosomal recessive (ADPKD and ARPKD, respectively) and they are both cilia-related disorders. ADPKD is characterised by a well-established increase in cell proliferation driven by metabolic reprogramming and activation of several proliferative signalling pathways. On the contrary, little is known about the cellular alterations consequent to the lack of expression of the *Pkhd1* gene, which in renal epithelial cells leads to ARPKD, a rare severe genetic disease usually present perinatally or in early childhood.

Rationale and hypothesis

Mouse models lacking *Pkhd1* gene expression have been developed and they develop liver cysts, but the kidney phenotype is uncommon. Accordingly, cellular models are becoming a valuable tool to study the molecular mechanisms underlying the cellular dysfunction in ARPKD. We generated *Pkhd1* KO mouse inner medullary collecting duct cells (the main kidney cell lines expressing this gene), lacking expression of the encoded protein fibrocystin/polyductin (FPC). We demonstrated that mitochondrial respiration is reduced in *Pkhd1* KO cells as compared to controls. In addition, we showed that mitochondrial OXPHOS reduction can be rescue by expression of the mitochondria-targeted C-terminal fragment of FPC.

These data shows that the lack of expression of *Pkhd1* gene induces a mitochondrial dysfunction. Nevertheless, the molecular bases of these mitochondrial defects have not been elucidated yet, and these can represent a possible target for an improvement of the cellular fitness.

Objectives and specific aims

The project aims to identify the mitochondria alterations, which lead to the observed OXPHOS defect. We aim to: 1, complete the characterization of the mitochondrial respiratory defects by identifying the mitochondrial parameters affected by the lack of FPC expression, such as mitochondrial respiration, mitochondrial network morphology, mass, membrane potential and ROS production; 2, define the mitochondrial proteome profile by proximity labelling in the absence of FPC protein; 3, assess cell growth in 2D and 3D cultures, and in different metabolic conditions; 4, leverage the identification of mitochondrial vulnerabilities as possible targets to improve the mitochondrial function, thus developing targeted rescue strategies.

Expected outcomes

We expect to get mechanistic insights into the mitochondrial defects in the ARPKD cellular models, thus identifying possible novel molecular players which can participate to the cellular alterations in ARPKD.

Skills that the student should acquire (max. 600 characters including spaces):

During the three-year PhD training the student will develop the ability to design experiments with appropriate controls, identify and overcome experimental failures, will acquire high-level technical expertise and will master project literature review. The student will also acquire ability in presenting research findings clearly in lab meetings, seminars, and conferences. The student will learn to utilize standard molecular and cell biology procedures, to perform mitochondrial assays including the use of Seahorse technology, and to set up and analyse proteome profiling by proximity labelling.

References (max. 15)

1. Bergmann C, Guay-Woodford LM, Harris PC, Horie S, Peters DJM, Torres VE. Polycystic kidney disease. Nat Rev Dis Primers. 2018 Dec 6;4(1):50. doi: 10.1038/s41572-018-0047-y.
2. Ma M. Cilia and polycystic kidney disease. Semin Cell Dev Biol. 2021 Feb;110:139-148. doi: 10.1016/j.semcdb.2020.05.003.
3. Walker RV, Yao Q, Xu H, Maranto A, Swaney KF, Ramachandran S, Li R, Cassina L, Polster BM, Outeda P, Boletta A, Watnick T, Qian F. Fibrocystin/Polyductin releases a C-terminal fragment that translocates into mitochondria and suppresses cystogenesis. Nat Commun. 2023 Oct 16;14(1):6513. doi: 10.1038/s41467-023-42196-4. PMID: 37845212.