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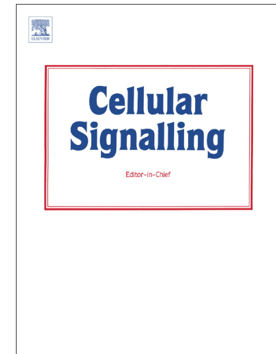

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Metabolic Reprogramming and the Role of Mitochondria in Polycystic Kidney Disease

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Abstract

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a slowly progressive disease characterized by the relentless growth of renal cysts throughout the life of affected individuals. Early evidence suggested that the epithelia lining the cysts share neoplastic features, leading to the definition of PKD as a "neoplasm in disguise". Recent work from our and other laboratories has identified a profound metabolic reprogramming in PKD, similar to the one reported in cancer and consistent with the reported increased proliferation. Multiple lines of evidence suggest that aerobic glycolysis (a Warburg-like effect) is present in the disease, along with other metabolic dysfunctions such as an increase in the pentose phosphate pathway, in glutamine anaplerosis and fatty acid biosynthesis, while fatty acid oxidation and oxidative phosphorylation (OXPHOS) are decreased. In addition to glutamine, other amino acid-related pathways appear altered, including asparagine and arginine. The precise origin of the metabolic alterations is not entirely clear, but two hypotheses can be formulated, not mutually exclusive. First, the polycystins have been recently shown to regulate directly mitochondrial function and structure either by regulating Ca^{2+} uptake in mitochondria at the Mitochondria Associated Membranes (MAMs) of the Endoplasmic Reticulum, or by a direct translocation of a small fragment of the protein into the matrix of mitochondria. One alternative possibility is that metabolic and mitochondrial dysfunctions in ADPKD are secondary to the de-regulation of proliferation, driven by the multiple signaling pathways identified in the disease, which include mTORC1 and AMPK among the most relevant. While the precise mechanisms underlying these novel alterations identified in ADPKD will need further investigation, it is evident that they offer a great opportunity for novel interventions in the disease.

Keywords: Polycystic Kidney Disease; Metabolism; Glucose; Lipids; OXPHOS; Mitochondria; Cell Signaling

Introduction

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is the most common monogenic disorder affecting humans and an important cause of end-stage renal disease (ESRD) (1, 2). Much

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progress has been made over the past few decades leading to a better understanding of the pathophysiology of the disease and to the design and approval of the first therapy able to retard disease progression (Tolvaptan) (1, 2).

While the molecular details driving cystogenesis are still under intense investigation, it is astonishing to notice that Dr. Jared Grantham in work from the early nineties (1) had already identified the three major pillars around which cystogenesis rotates: i) increased proliferation; ii) increased secretion; and iii) increased matrix deposition. In particular, PKD is characterized by an aberrant cell cycle progression and the marked biochemical similarities often observed in tumors have led Dr. Grantham to label PKD as a "neoplasia in disguise" (1).

In more recent years work from our and other laboratories revealed that proliferation is associated with a profound metabolic reprogramming in ADPKD through pathways that again resemble those found in cancer (3, 4). In particular, "the Warburg effect", a central metabolic alteration characterized by enhanced conversion of glucose to lactate rather than fully oxidizing it in the tricarboxylic acids (TCA) cycle in mitochondria, is recognized as a central feature of cancer metabolic reprogramming and was identified in ADPKD as well (3). Further to this, additional metabolic reprogramming routes occurring as a consequence or in parallel to glycolysis previously observed in cancer were also identified in PKD (4).

In this review, we try to concisely summarize our current knowledge of the metabolic and mitochondrial alterations observed in PKD cells and tissues. This is a rather new field of investigation in ADPKD that will certainly attract further interest in the future, both for the potential to explain the disease pathogenesis and for its amenability to specific interventions.

Metabolic Reprogramming in PKD

Metabolic reprogramming describes the rewiring of intracellular metabolic pathways occurring in response to specific needs of the cell in physiological or pathological conditions. In ADPKD a broad reprogramming of multiple metabolic pathways was described, mostly involved in central carbon metabolism, which culminates in the mitochondrial TCA cycle and oxidative phosphorylation (OXPHOS) regulation. We describe here the current knowledge on the topic following a logical rather than a chronological order of the findings in the field.

Glucose metabolism

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Warburg described reprogramming of energy metabolism in cancer cells that shift from oxidative phosphorylation to aerobic glycolysis, i.e. the conversion of pyruvate to lactate, a process yielding a low amount of ATP that usually occurs when oxygen is not available (and called anaerobic glycolysis in physiological conditions) (5-8). Work from our laboratory has uncovered that *Pkd1*-mutant cells, like cancer cells, depend on aerobic glycolysis for energy production (Fig.1). This renders the cells more susceptible to cell cycle arrest and cell death when they are glucose deprived (3, 4). The discovery that *Pkd1*$^{-/-}$ mouse embryonic fibroblasts (MEFs) have a shift towards aerobic glycolysis resulted from the evidence that they tend to consume more glucose from the medium and to produce/release more lactate than the matching controls, which eventually causes extracellular medium acidification (Fig.1) (3). In line with this, increased expression of glycolytic enzymes is observed in cells, murine, and human tissues derived from *Pkd1*/*PKD1* mutant kidneys, as well as in non-orthologous animal models of the disease (3, 4, 9, 10). Given the prominent Warburg effect observed, several investigators have used the glucose analogue 2-deoxy-D-Glucose (2DG) in murine orthologous models of PKD (*Pkd1*-mutant) (3, 9, 11), as well as in non-orthologous rat models (10) and in an orthologous mini-pig model (12), showing a very prominent retardation of disease progression. These data are very promising given the low toxicity of this compound (13). Furthermore, several diet interventions including fasting and a ketogenic diet, all meant to diminish the availability of glucose to the cystic epithelia showed a strong retardation of disease progression in multiple animal models, supporting the central role of glucose in ADPKD energy production (14-17).

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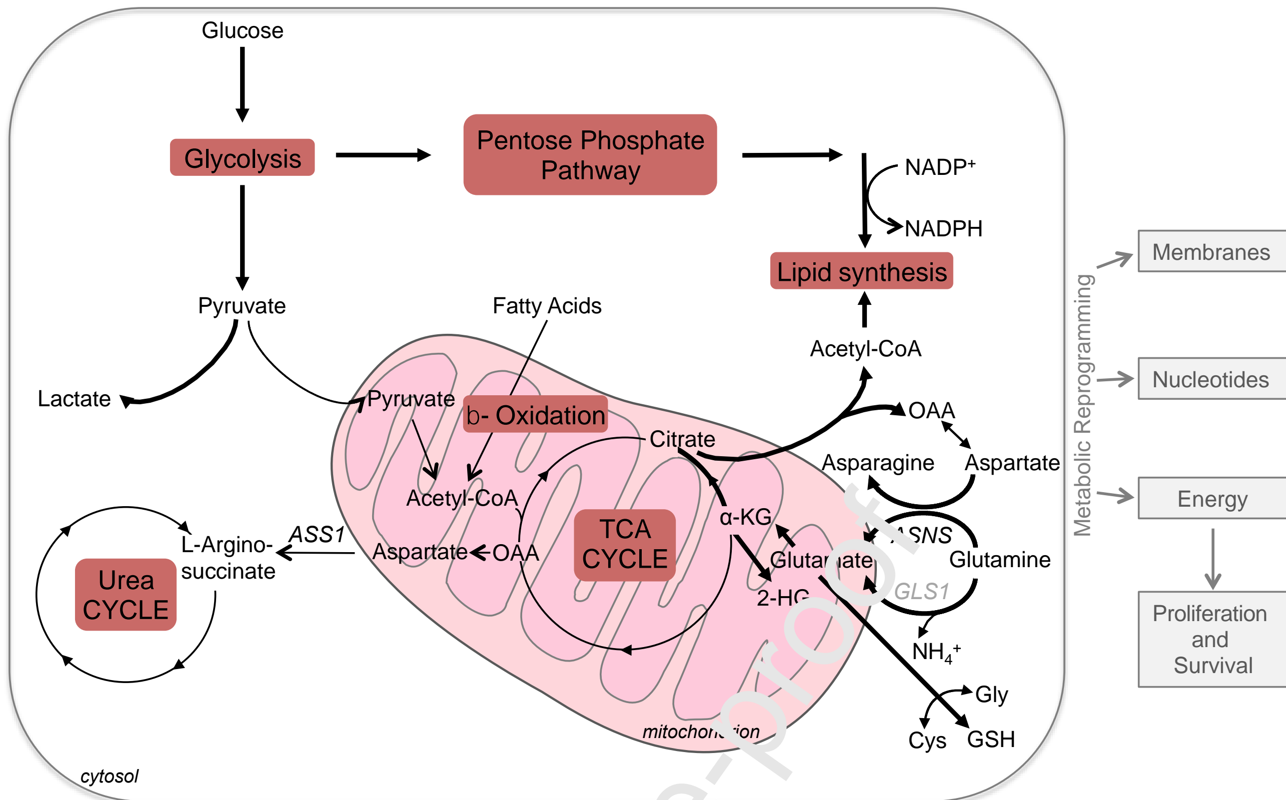

Figure 1. Metabolic reprogramming in PKD. A schematic overview of the metabolic alterations observed in PKD is presented. In cells and tissues carrying *Pkd1* disease mutations there is an increase in the flux of both glucose and glutamine metabolism. The thickness of the arrows indicates relative flux compared to a control condition (increased, thick arrow). These nutrients are used to fuel the TCA cycle and oxidative phosphorylation as well as the Pentose Phosphate Pathway and fatty acids biosynthesis (lipids). Together, these pathways generate sufficient levels of cellular components to support cell proliferation. Following glycolysis, pyruvate is converted into lactate. Furthermore, glycolytic intermediates are also being used for PPP. Glutamine is an important carbon source, as the amount of acetyl-CoA that is generated from pyruvate is rather small, in this context glutamine is used for the replenishment of TCA cycle intermediates. Glutamine uptake can be driven by glutaminase enzyme, releasing ammonia, or through asparagine synthetase (ASNS), which converts glutamine to glutamate by generating asparagine from aspartate. Glutamate is next imported into the mitochondria and converted into α-KG and can be fully oxidized to maintain the TCA cycle but also can be reductively carboxylated to generate citrate (reverse arrow), used for lipid synthesis. Citrate is converted into OAA and acetyl-CoA. Acetyl-CoA allows initiation of fatty acid synthesis, which requires NADPH, provided by the PPP. OAA in turn generates Aspartate. Glutamine also contributes to the generation of the tripeptide glutathione (composed of glutamate, cysteine, and glycine). The first step in glutathione synthesis is the condensation of glutamate and cysteine through glutamate–cysteine ligase (GCL; not shown in the figure). Glutamine input contributes directly to the availability of cysteine (Cys) and glycine (Gly) for production of glutathione (GSH). Glutamine derived α-KG can also produce 2-HG. Glutamine can also feed the Urea cycle. ASS1, which is reduced in PKD, encodes the enzyme that catalyzes arginosuccinate formation, the rate-limiting step of *de novo* arginine synthesis. PPP, pentose phosphate pathway; GLS1, glutaminase1 (in grey to indicate variability among different studies); α-KG, α-ketoglutarate; 2-HG, 2-hydroxyglutarate, OAA, oxaloacetate; ASNS, asparagine synthetase; ASS1, arginosuccinate synthase1.

The increased glycolytic activity is also associated with an increased intracellular ATP content (3). These findings are very similar to those reported in cells and tissues lacking the tuberous sclerosis complex 1 or 2 (*Tsc1* or *Tsc2*) genes and characterized by a constitutive activation of the

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mechanistic target of rapamycin, mTORC1 (18, 19). Indeed, the increased glycolytic rates observed in *Pkd1* mutant tissues is dependent on the mTORC1 pathway and they were somehow expected based on the activation of this cascade (3, 19, 20). Several studies have confirmed the increased glucose uptake and lactate production in cells and tissues mutant for the *Pkd1* gene (Fig.1) (3, 4, 9). It should be noted, however, that this evidence is controversial and two studies reported lack of increased glycolytic rates in *Pkd1* mutant cells (14, 21). Measurement of aerobic glycolysis can be challenging. Indeed, for long time it has been debated whether or not renal carcinoma cells and tissues do present with a Warburg effect or not (5). Recent work has solved the controversy, showing that *in vivo* tracing of ^{13}C-glucose in patients undergoing surgery for renal cancer indeed revealed a strong Warburg effect in this tumor, i.e. increased glycolysis and reduced glucose oxidation in the TCA cycle, as opposed to brain and lung cancers that have a much lower aerobic glycolysis (22). This study is also the first definitive demonstration that the Warburg effect takes place *in vivo* in a tumor setting (22). Therefore when considering the controversial interpretation of data related to analysis of aerobic glycolysis in ADPKD it should be noted that tracing analysis using ^{13}C-glucose *in vivo* in murine models of PKD has already been performed and showed evidence of increased glucose uptake and lactate production in three distinct experimental settings characterized by an increasingly severe disease manifestation (3, 4, 9). This status was also associated with a transcriptional de-regulation of key glycolytic enzymes in mouse and human (3, 4, 23), although a definitive evidence using isotope tracing in humans is still lacking.

In addition to be used in aerobic glycolysis, the increased uptake of glucose is also used in *Pkd1* knock-out cells and kidneys to sustain the Pentose Phosphate Pathway (PPP) (Fig.1) (4, 24). This is an essential metabolic pathway which branches from glycolysis right at the first step of commitment of glucose after hexokinase generates Glucose-6-Phosphate (24). The PPP is fundamental during anabolic processes as it is required for the synthesis of ribonucleotides needed for both DNA and RNA synthesis (24). Furthermore, the PPP is the major cellular source of NADPH, providing the needed reducing power for the biosynthesis of fatty acids, required for membrane generation in any dividing cell (24) (Fig.1). Finally, the PPP also plays a pivotal role in scavenging reactive oxygen species (ROS). The enhanced usage of the PPP in *Pkd1* mutant cells and tissues is therefore in line both with the proliferative potential of these tissues and with the increased glycolysis and fatty acid biosynthesis (see below) (24).

Glutamine and amino acid metabolism

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One important open question is why cancer cells and more in general proliferating cells tend to use lactate production as an energy source rather than fueling pyruvate into mitochondria where its conversion into acetyl-CoA and full oxidation to CO_2 yields a much higher ATP production. One possible explanation is that the process of proliferation requires a net increase in biomass more than it needs the generation of energy (5). Independently of the type of proliferating cell, cell division requires *de novo* synthesis of nucleic acids, lipids, and proteins to increase cellular mass. Therefore, the metabolic needs inevitably shift from a catabolic to an anabolic mode.

In this context, glutamine is an essential carbon source for proliferating cells (5, 25, 26). Indeed, glutamine is catabolized into an intermediate of the TCA cycle, α-ketoglutarate (α-KG) through a process called glutamine anaplerosis (Fig.1) (26, 27). This phenomenon is made of two steps, the first one is the conversion from glutamine into glutamate, whereas the second one is the conversion from glutamate to α-KG. Canonically, produced α-KG enters the TCA and replenishes it to fuel the electron transport chain and maintain the mitochondrial electrochemical membrane potential when pyruvate import is reduced (27). Further to this, α-KG can be carboxylated and lead to the production of citrate, pushing the TCA cycle in the opposite direction (26) (Fig.1). Citrate is a critical metabolite as it is the most important source of acetyl-CoA in mammalian cells, which serves as a substrate both to achieve acetylation of proteins, but also for *de novo* fatty acids biosynthesis (26). The use of isotope labeling in *Pkd1* mutant cells has revealed that this is exactly the fate of glucose and glutamine in these cells (Fig. 1). In line with the proliferative potential of PKD, the cells divert glucose into glycolysis and PPP, and compensate with–glutamine usage in the TCA cycle and to generate citrate for lipid production (Fig.1).

Of interest, the first description of a striking parallel between PKD and cancer in glutamine addiction was reported by Hwang *et al.* 2015 (28). In this study, a non-targeted metabolomic approach in recessive PKD kidneys was taken, which lead to the identification of metabolic reprogramming as to necessitate glutamine exogenously for the production of glutamine-sourced 2-hydroxyglutarate (2-HG). 2HG, an oncometabolite, was found accumulated likely due to glutamine-sourced α-KG (28) (Fig.1).

A subsequent study demonstrated that genetic ablation of the Liver kinase B1 (*Lkb1*) gene in the developing ureteric bud had no evident effect, but combination with mutation in *Tsc1* gene resulted in a very aggressive PKD in the mouse (29). The authors demonstrated that this is driven by a glutamine dependence, caused by ablation of *Lkb1*. Furthermore, they demonstrated that loss of

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Pkd1 during developmental stages renders cells dependent on glutamine for their growth, due to the contribution of this non-essential amino acid to glutathione (GSH) metabolism. Based on these studies, they inhibited the Glutaminase 1 (GLS1) enzyme using Bis-2-(5-phenylacetamido-1,3,4-thiadiazol-2-yl)ethyl sulphide (BPTES) and demonstrated that this was sufficient to retard PKD progression in both *Lkb1/Tsc1* double mutants and in *Pkd1* mutants (29).

Further to this study, inhibiting the metabolism of glutamine by a different GLS1 inhibitor CB-839 was also shown to retard disease progression in a mild cystic disease model (*Aqp2-Cre;Pkd1$^{fl/fl}$* mice), but not in the aggressive *Pkhd1-Cre;Pkd1$^{fl/fl}$* cystic model (30). The reasons for these differences are not apparent, but the investigators proposed that mTORC1 upregulation might drive an adaptive metabolic pathway able to bypass GLS1 inhibition (30). One important consideration is that inhibition of glutaminase (GLS1) tends to be toxic, both to cells and to animals. In neither of the two studies reporting GLS inhibition, a thorough analysis of the toxicity was not shown (29, 30). CB-839 in particular is a very effective compound developed for the use in renal cancer, a medical condition in which a low safety profile might be well justified based on a cost/benefit consideration (31). For ADPKD, on the contrary, a safety and tolerability profile of any compound to be used in humans will be at least as relevant as its efficacy for it to be realistically considered for the use in the clinic.

In line with the above findings in a more recent study we have also found that glutamine usage in PKD is indeed remarkably high (Fig.1) (4) due to the need of the cell to maintain the TCA cycle active and to avoid losing mitochondrial membrane potential, which would inevitably lead to apoptosis. Thus, the finding it is not *per se* surprising. However, during tracing studies of $^{13}C_6$-Glucose and $^{15}N_2$-$^{13}C_5$-Glutamine we demonstrated that glutamine is converted into glutamate through an unusual mechanism, i.e. rather than utilizing GLS1 that we did not find upregulated in ADPKD (Fig.1 in grey), cells lacking the *Pkd1* gene utilize the enzyme asparagine synthase (ASNS) (4). This enzyme is a transamidase that converts aspartate into asparagine while deamidating glutamine to form glutamate (32) (Fig.1). As a result, glutamate can be imported into mitochondria and be converted into α-KG (32). In normal conditions, the amino acid asparagine, an important regulator of amino acid homeostasis, can be synthesized by ASNS and its expression levels are low, but in response to limitation of a single amino acid, it can be rapidly induced (33). Indeed, in our study (4) we found that silencing of ASNS in *Pkd1* mutant cells completely abolished the glutamine contribution to α-KG generation. This is suggestive that glutamine uptake and conversion to glutamate relies on asparagine synthesis. A second important finding resulting from

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the tracing studies with $^{13}C_5$-Glutamine was the observation that not only glutamine-derived α-KG can be fully oxidized to maintain the TCA cycle and to avoid collapse of the electron transport chain activity, but also α-KG is reductively carboxylated in *Pkd1* mutant cells to generate citrate (4). Citrate exported from mitochondria is next broken into Acetyl-CoA and Oxalacetate (OAA). The first is used by the cell for the synthesis of fatty acids (lipids, Fig.1). The second is converted into aspartate, thus replenishing this amino acid as a substrate of ASNS for an efficient import of glutamine into the cells and TCA cycle (4).

Finally, Trott *et al.* 2018, investigated an alternative glutamine pathway, via the arginine synthetic enzyme arginosuccinate synthase 1 (ASS1) (34). The authors demonstrate that arginine, a nonessential amino acid, regulates ASS1 levels and is required for cytogenesis in PKD cells and in an *ex vivo* cystogenesis model (Fig.1). Given the connection between amino acid homeostasis and glutamine, this might be relevant for synthesis of glutathione, an anti-oxidant that prevents cellular damage from ROS and controls the production rates of non-essential amino acids including glutamate, which is the precursor of glutamine.

Therefore, the high glutamine dependence identified in PKD, along with the glucose dependence represents an essential part of the metabolic reprogramming occurring in this disease and might offer several interesting opportunities for therapy (4, 28-30, 34) . Which of the critical nodes in this complicated rewiring is the best target(s) for therapy remains to be established, and will inevitably need to be confronted with the requirement for a safe, chronic therapy in the case of ADPKD.

Lipid Metabolism

Among the first reported alterations in the metabolic landscape of ADPKD there is evidence of alterations in lipid metabolism (35). Indeed, acetylcarnitine was identified in the urine of *Pkd1* mutant mice through a metabolomic study revealing that its presence in the urine correlates with disease progression (35). Acetylcarnitine is an important metabolite involved in the regulation of β-oxidation in the mitochondrion. Thus, analysis of this evidence at the light of our current knowledge of the cellular metabolic alterations likely occurring in PKD suggests that perhaps early metabolic alterations can be captured in the urine and used as potential biomarkers. In the same study transcriptional profiling revealed alterations in the regulation of additional metabolic pathways including oxidative phosphorylation, purine and tyrosine metabolism (35). The same group has subsequently reported a profound defect in fatty acid oxidation in *Pkd1* mutant kidneys and cells in the absence of any increase in glycolysis or any other compensatory metabolic reprogramming (21).

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While the data appear interesting, the authors did not provide an explanation of how cells presenting with such a phenotype would be able to proliferate/survive and form a cyst. Further work is warranted to better understand the details of the model proposed and likely to reconcile the findings with those of other groups (3, 4).

Indeed, the alteration in cellular metabolism, including changes not only restricted to glucose metabolism but also changes in fatty acids is emerging as an important modifier of disease progression in ADPKD (21, 36, 37). Alterations in diet, specifically with a low-fat diet in an orthologous mouse model of ADPKD reduced cystic progression (21). Also, feeding a low-calorie diet showed reduced disease progression (14, 16, 17). Even though not much is known about the metabolism of renal epithelial cells, renal tubular epithelial cells (especially proximal) have high levels of baseline energy consumption and supply a copious amount to the mitochondria (38). Fatty acid oxidation (FAO) is the preferred energy source for highly metabolic cells, as oxidation of fatty acids generates more ATP per molecule than does oxidation of glucose. Indeed, reduced FAO was elegantly demonstrated to be a hallmark of chronic kidney disease progression (CKD) in the mouse and in humans (38). In a recent study, Hajarnis *et al* has shown that the reduced FAO is secondary to the upregulation of microRNA-17, which in turn downregulates the expression levels of PPARα, ultimately reducing FAO (39). The deletion of the miR-17 cluster reduced cyst burden in four orthologous mouse models. Likewise, a more recent study showed that anti-miR-17 oligonucleotides effectively retard disease progression (40). The investigators showed that the primary cellular consequence of the upregulation of anti-miR-17 treatment was improvement of the expression of metabolic-related gene networks including peroxisome proliferator-activated receptor alpha (*Pparα*) and –gamma coactivator 1-alpha (*Pgc-1α*) genes (41). Of interest, the authors were able to rescue the phenotype of *Pkd1* mutant mice by using fenofibrate, an agonist of Pparα, resulting in an increased input of β-oxidation (39, 41). Furthermore, the authors showed that the oncogene c-Myc is the main driver of miRNA17 expression (39). c-Myc is considered a master regulator of metabolism in several types of cancer, with glycolysis and glutaminolysis both being centrally regulated by this oncogene (42).

Reduced FAO is also often accompanied by an aberrant lipid metabolism in the form of increased *de novo* fatty acid synthesis (FASN). Indeed, rapidly proliferating cells must be able to synthesize more fatty acids for membrane production, rather than consuming them for energy purposes. As a result, in highly proliferating cells FAO can be shut off by the cells in favor of FASN, through a feedback loop whereby the increased synthesis inhibits the carnitine palmitoyltransferase 1 (CPT1)

and CPT2 transporters into mitochondria and therefore FAO (43). A similar mechanism was proposed to occur in *Pkd1* mutant cells (4) (Fig.2). It should be noted that mutations in the CPT2 transporter was found associated with a severe case of infantile Polycystic Kidney Disease associated with a severe lactic acidosis (44). This raises the possibility that reduced FAO might itself be sufficient to drive PKD and possibly aerobic glycolysis, independently of FASN.

Epigenetics Cross-talk

Cells maintain a metabolic balancing by integrating the control of gene expression with metabolism and by compartmentalization between mitochondria, for energy generation, the cytoplasm, and the nucleus for gene expression. Moreover, different cell types vary in their metabolic and gene expression requirements. Of potential interest to PKD, Wellen *et al* provide evidence for a mechanism in mammalian cells that links the production of acetyl-CoA from citrate to the regulation of metabolic genes through alterations in histone acetylation (45). In fact, it was shown that a regulatory loop connects citrate generated by mitochondria to histone acetylation resulting in the expression of genes required for glycolysis and other metabolic pathways (45). As mentioned above, citrate is the main contributor to cellular acetyl-CoA. The reaction of citrate scission into acetyl-CoA and OAA could take place directly in the nucleus where the ATP-citrate lyase (ACLY) was reported to translocate (45). This regulatory mechanism might function in cellular homeostasis contributing to the transcriptional regulation of metabolic reprogramming (45). Evidence indicates that at least one of the epigenetic modifier histone deacytylase 6, (HDAC6) is relevant for ciliogenesis and preventing cyst formation (46). Indeed, HDAC6 inhibitors slowed cystic growth in ADPKD cells reducing cAMP and protein expression of adenylyl cyclase 6 (47).

Mitochondrial alterations in PKD

The metabolic reprogramming observed in PKD has been recently explained by possible alterations in mitochondrial function. The mitochondrion is a pivotal organelle in mammalian cells. It is the central hub for metabolic pathways such as the TCA cycle, and OXPHOS takes place in the matrix of the mitochondrion. This results in generation of an electrochemical gradient across the inner mitochondrial membrane (the mitochondrial membrane potential, $\Delta\psi_m$) essential to drive ATP synthesis via the ATP synthase (Fig.2). The mitochondrion is also a key regulator of cell survival, mediating the intrinsic apoptotic program characterized by cytochrome *c* release when the $\Delta\psi_m$ is lost (48). Finally, the mitochondrion is the central buffering organelle for Ca^{2+}, along with the Endoplasmic Reticulum (ER), in mammalian cells (49).

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Pathways regulating mitochondrial biogenesis, metabolism, and structure have recently been investigated in ADPKD and will possibly identify novel molecular mechanisms of pathogenesis and relevant targets for therapy. We are here summarizing the recent experimental findings that indicate the presence of altered mitochondrial structure and energy-related functions in PKD.

Oxidative phosphorylation

Mitochondria are the major site of energy production in the cell through the metabolic pathway of oxidative phosphorylation. The first piece of evidence of OXPHOS impairment in ADPKD came from the transcriptional analysis of cells derived from cysts of patients carrying a *PKD1* mutation (23) and subsequently transcriptional profiling of kidneys carrying *Pkd1* ablation (35). In both studies the investigators had identified reduced OXPHOS as enriched pathway. Maybe because this alteration was not the main focus of these two studies, reduction in OXPHOS did not attract much attention, until a metabolomics analysis performed in control and *Pkd1*$^{-/-}$ MEFs revealed higher aerobic glycolysis in *Pkd1*$^{-/-}$ cells in comparison to controls, as mentioned above (3). In this study, we showed that the mitochondrial membrane potential ($\Delta\psi_m$) was preserved in *Pkd1*$^{-/-}$ MEFs as compared to controls, yet inhibition of mitochondrial ATP production by oligomycin A, a selective inhibitor of the mitochondrial ATP synthase, lowered the total ATP content only in wild-type cells, and not in *Pkd1*$^{-/-}$ cells (3) (Fig.2). This indicated a weak mitochondrial contribution of the OXPHOS to the total ATP production (3).

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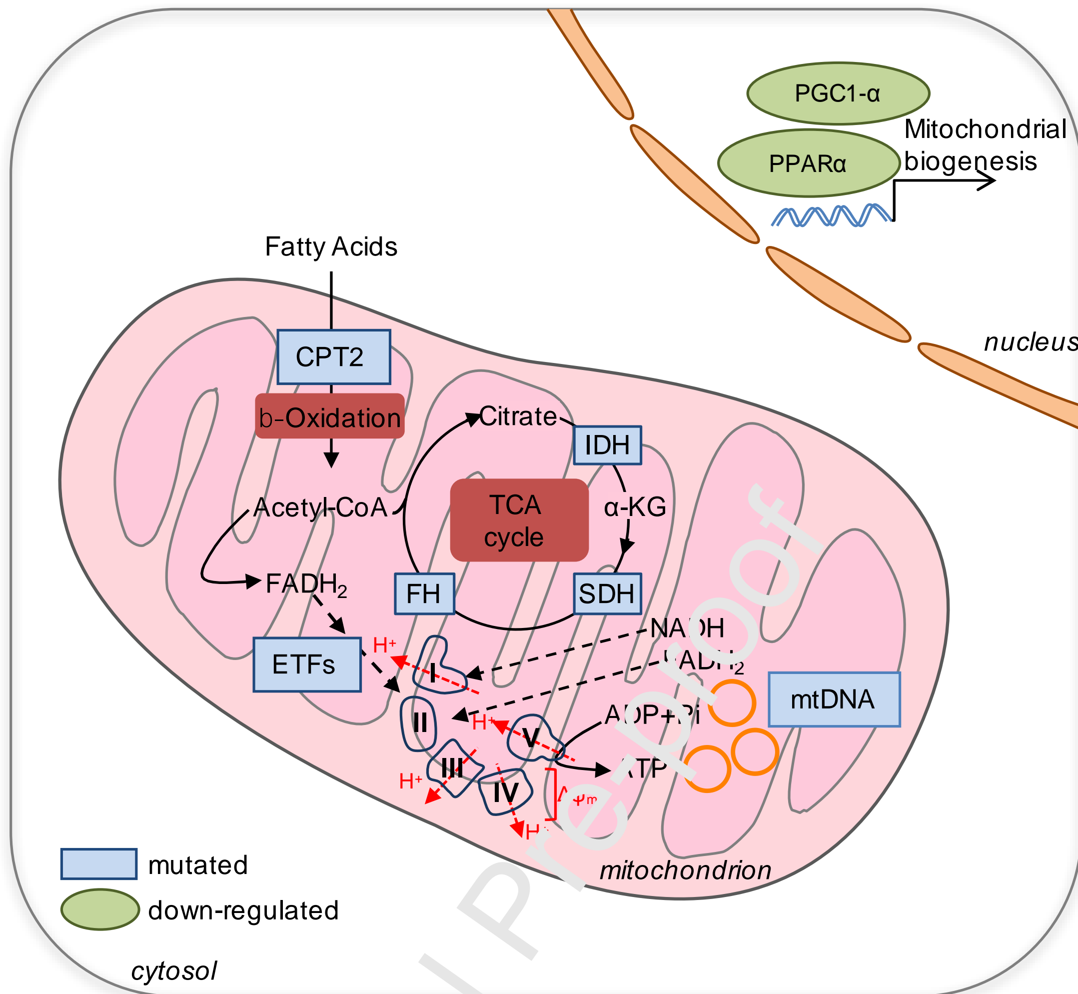

Figure 2. Mitochondria alterations in PKD. Nuclear encoded mitochondrial proteins that once mutated can drive cystogenesis in the kidney are presented. The key metabolic enzymes of the TCA cycle, fumarate hydratase (FH), succinate dehydrogenase (SDH), and isocitrate dehydrogenase (IDH); the carnitine palmitoyltransferase II, CPT2, involved in the import of fatty acids into mitochondria; the electron transfer flavoproteins (ETFs) that transfer electrons from fatty acid oxidation to the respiratory chain complexes (I, II, III, IV, and V, respectively). In addition, deletions in the mitochondrial DNA (mtDNA) in more than 80% of the copies of the heteroplasmic mtDNA in the kidney have been reported to result in cystic kidneys. A general decrease in mitochondrial biogenesis in PKD has also been reported, secondary to down-regulation of PPARα and PGC1α via miRNA-17 expression.

Further experimental evidence of reduced OXPHOS came from two studies reporting reduced Oxygen consumption rates (OCR) in cells derived from *Pkd1* mutant animals (21, 50). The first study by Menezes *et al.* reported the reduction in OXPHOS and mitochondrial FAO. OCR was reduced in immortalized proximal or distal tubule cell lines from *Pkd1* conditional mice, and in *Pkd1* knock-down mouse cortical collecting duct cell line (mCCD) in comparison to control, in the presence of palmitate as an energy source (21). In the second study, Padovano *et al.* reported for the first time a more general reduction of OCR independent of the substrate provided to the cells, in *Pkd1*$^{-/-}$ mouse renal proximal tubule cell lines, as compared to control heterozygous *Pkd1*$^{fl/-}$ cells (50). Our group also subsequently showed reduced OCR in immortalized or primary *Pkd1*$^{-/-}$ MEFs compared to controls, and in murine Inner Medullary Collecting Duct (mIMCD3) cells, after

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silencing of the *Pkd1* gene expression (4). Finally, alterations in mitochondrial OXPHOS were shown in ADPKD-cyst-derived cells with a homozygous *PKD1* mutation. Basal respiration, ATP production, maximal respiration, spare capacity, and proton leakage were decreased in human tubular cells with a homozygous *PKD1* mutation compared with those of the control (51) (Fig.2).

The alterations in OXPHOS in PKD cellular and mouse models correlate with down-regulation of gene expression of nuclear encoded mitochondrial genes and regulatory proteins for mitochondrial biogenesis and metabolic pathways. Gene expression profiling by cDNA microarray on human *PKD1* cysts identified pathways associated with renal cyst growth (23). The authors showed that oxidative phosphorylation was among the top 50 most down-regulated gene sets in *PKD1* renal cysts. The down-regulation of OXPHOS genes was subsequently associated to the reduced PPARα and of its co-activator, both modulators of the energy metabolism promoting FAO and OXPHOS (39, 51, 52). In particular, PPARα gene expression was inhibited by the expression of the miR-17 in ADPKD models and in human ADPKD cysts. Measurements of OCR in cells over-expressing miR-17, PPARα, or both in kidney cells indicate that miR-17 expression decreased OXPHOS activity, which can be recovered by high levels of PPARα (39). In line with these experimental findings, PGC-1α expression was reduced specifically in the kidney cyst-lining cells of ADPKD animals compared to the non-cystic tubules of control animals (51).

Therefore, the reduction in mitochondrial OXPHOS activity appears to be the most robust and less controversial aspect of all metabolic alterations reported in ADPKD to date, and it appears to be shared by multiple different cell types (4, 21, 39, 50-52) perhaps suggesting that this represents a very strong signature of the disease.

Mitochondria structure and morphology

OXPHOS alterations in PKD correlate with mitochondrial structural abnormalities. Studies on the structure of mitochondria in the cyst-lining cells in kidneys of ADPKD mouse models and patients identified mitochondria structure abnormalities, including swollen mitochondria with abnormal cristae, and a decrease in the mtDNA copy number, indicating a general reduction of the mitochondrial mass in the renal cystic epithelia of the *Ksp-Cre;Pkd1*$^{fl/fl}$ mouse and the heterozygous Han:SPRD Cy (*Cy/+*) rat (51). These results are in line with a decrease in mitochondrial biogenesis, consequent to the down-regulation of PPARα and PGC-1α (39, 51).

Furthermore, analysis of the morphology of the mitochondrial network in isolated renal epithelial cells also indicated that control cells have mitochondria more elongated and interconnected than

mutant *Pkd1* cells (53). Ultra-structural analyses of murine and human renal epithelial cells by transmission electron microscopy (TEM) indicate that mitochondria in mouse and human PKD cells and tissues are less elongated in comparison to their genetic controls (53). The morphology of the mitochondrial network was also investigated in renal epithelial cells carrying *Pkd2* knockdown showing that in this case as well mitochondria have a larger area and a more circular morphology in comparison to control cells, indicative of an increased fragmentation (54).

In summary, parallel analyses on mitochondrial structure and morphology point to reduced fusion of mitochondria, with altered cristae and swollen organelles, as a feature generally observed by all investigators.

Modulation of mitochondrial functions by the polycystins

The outstanding question that derives from the numerous descriptive studies above is what is the connection among metabolic reprogramming, mitochondrial alterations and the genes mutated in ADPKD? Three recent studies have proposed that both Polycystin-1 (PC-1) and Polycystin-2 (PC-2) might play a direct role in regulating mitochondrial physiology(50, 53, 54). Padovano and colleagues (50) demonstrated that O_2 levels regulate the subcellular localization and channel activity of the polycystins complex through its interaction with the oxygen-sensing prolyl hydroxylase Egl-9 family hypoxia inducible factor 3 (EGLN3), which hydroxylates PC-1. In addition, they showed that both PC-1 and PC-2 are localized at the mitochondria-associated ER membranes (MAMs) (50). These are the sites where Ca^{2+} is transferred from the ER to the mitochondrial matrix to positively regulate mitochondrial metabolism (55). Matrix Ca^{2+} indeed modulates pyruvate dehydrogenase phosphatase (PDP), isocitrate dehydrogenase (IDH), oxoglutarate dehydrogenase (OGDH), and ATP synthase activity, increasing NADH and $FADH_2$ production and sustaining electron transport chain (Fig.3) (56). Reduction of the PC channel activity during hypoxia results in decreased Ca^{2+} dependent OXPHOS activity of mitochondria (50). Thus, the investigators proposed that loss of PC-1 reduces calcium intake into mitochondria and this may explain the altered mitochondrial metabolism, the reduction in OXPHOS and mitochondrial ATP production, and the glycolytic switch in PKD (Fig.3) (50). A role for the PC complex at the MAMs has also recently been supported by evidence of Kuo *et al.* (54), showing that the reduction of PC-2 expression affects mitochondria calcium buffering and increases the fragmentation of the mitochondrial network. Of note, the authors proposed that PC-2 functions to reduce mitochondria-ER contacts. Accordingly, the lack of PC-2 enhances the uptake of Ca^{2+} into the mitochondrial matrix, increasing mitochondrial biogenesis and metabolism (54). These

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experimental findings indicate the PC complex can participate to the regulation of mitochondrial metabolism at the ER-mitochondria boundaries; further investigation will shed light on the reported profound differences between cells lacking PC-1 and PC-2 proteins. Of note, PC-2 has also been shown to regulate the Ca^{2+}-activated mitochondrial ATP-Mg/Pi solute carrier 25 A 25 (SLC25A25), a carrier family involved in modulating the adenine nucleotide pool in the mitochondrial matrix in response to changes in energy demands (57).

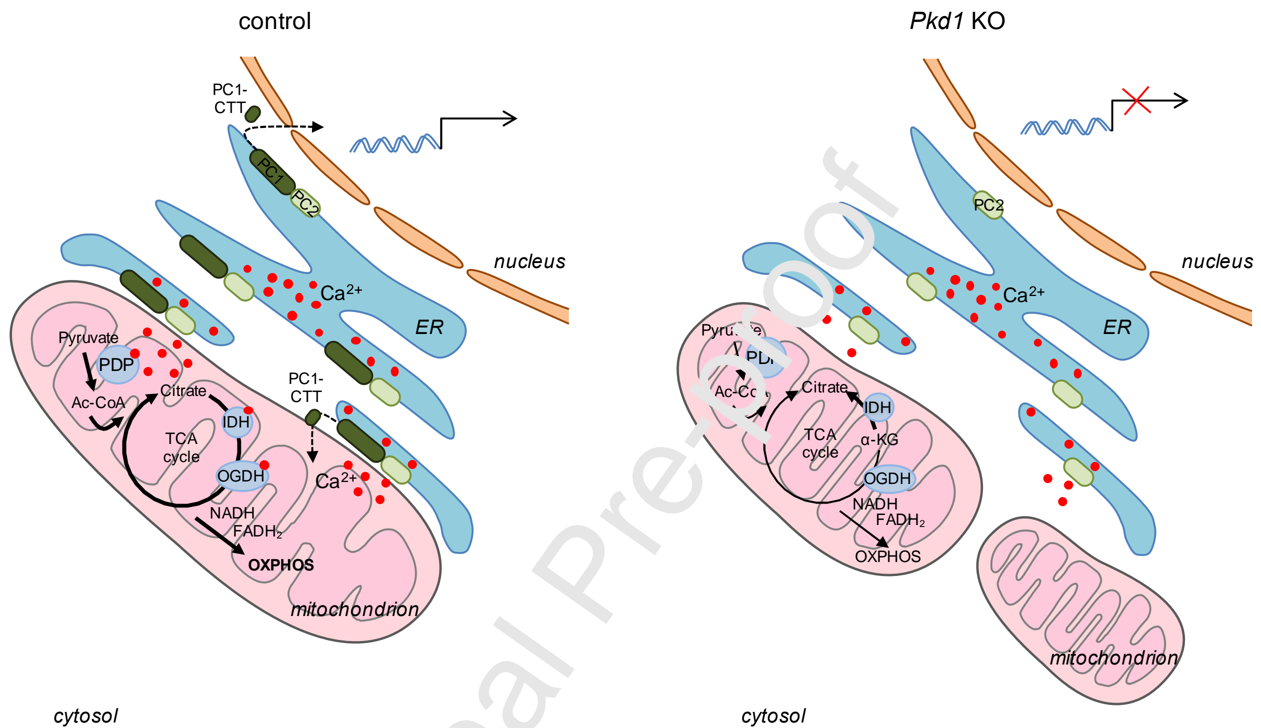

Figure 3. Modulation of mitochondrial functions by the polycystins. Polycystins can localize to the MAMs favoring the intake of Ca^{2+} from the ER to mitochondria (left panel). The increase in mitochondrial matrix Ca^{2+} concentration stimulates the activity of pyruvate dehydrogenase phosphatase (PDP), isocitrate dehydrogenase (IDH), oxoglutarate dehydrogenase (OGDH), sustaining OXPHOS and ATP synthesis. The CTT of PC1 can translocate to the nucleus to modulate gene expression, but it might also translocate to the mitochondrial matrix to directly regulate mitochondria signaling pathways. The absence of PC1 (right panel) results in decreased Ca^{2+} dependent OXPHOS activity of mitochondria and increased mitochondrial fragmentation. This could favour glycolysis for ATP production and the reductive carboxylation of α-KG to isocitrate and then citrate for lipid biosynthesis. The thickness of the arrows indicates relative flux (increased, thick arrow). ER: endoplasmic reticulum

Finally, an additional piece of evidence has been reported by Lin and colleagues, pointing to a direct role of PC-1 in the regulation of mitochondrial function (53). The authors proposed that a C-terminal cleavage product of polycystin-1, the C-terminal tail (CTT), which has been extensively reported to localize to the nucleus and to be transcriptionally active (58), could also translocate to the mitochondria matrix, directly influencing mitochondrial function (53) (Fig.3). These data are particularly intriguing as PC-1 might exert a direct role in regulation of mitochondrial metabolism, by physically interacting with mitochondrial proteins which will need to be identified (53). It should be considered, however, that the protein fragment corresponding to the endogenous CTT of the PC-

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1 protein has not been reported to date, and that all studies related to this fragment have made use of overexpression systems, either in cells or in transgenic mice (53, 58).

Mitochondrial alterations and pseudohypoxia can per se drive cystogenesis in the kidney

A comprehensive view of the metabolic reprogramming and the mitochondria function involvement in PKD is still lacking and will require further investigations. Nevertheless, it is intriguing that several lines of evidence point to the involvement of these cellular programs in renal cystogenesis.

The production of ATP by glycolysis and the consequent increase in lactate production are the key features of the Warburg effect, which sustains proliferation of cancer cells as mentioned. A similar metabolic switch is observed as a consequence of mutation in key TCA cycle metabolic enzymes, such as fumarate hydratase (FH), succinate dehydrogenase (SDH) and isocitrate dehydrogenase (IDH). FH and SDH are two important tumor suppressors, whereas IDH is an oncogene (Fig.2). Mutations in each of these genes cause tumorigenesis in the kidney and result in reduced TCA cycle, reduced OXPHOS, and a glycolytic switch along with additional metabolic reprogramming features (59-61) (Fig.2). Of great interest, while in humans *FH* mutations cause Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC), in the mouse inactivation of the *Fh1* gene result in death of the animals by PKD (62), suggesting an important role of the TCA cycle in normal tubular structure maintenance. Similarly, mutations in the different subunits of Succinate dehydrogenase (SDH) are associated with different types of cancer, including renal cancer (63). Of interest, inactivation of the SDHB subunit using a kidney-specific Cre line also causes massive Polycystic Kidney Disease and death of the animals by renal failure (Gottlieb *et al*, personal communication).

Among the genes possibly driving cystogenesis is the *CPT2* gene, which encodes carnitine palmitoyltransferase II, an enzyme located in the inner mitochondrial membrane. This enzyme is part of the carnitine shuttle that imports fatty acids into mitochondria and thus regulates their oxidation (Fig.2). Mutations in *CPT2* cause neonatal or infantile Carnitine Palmitoyltransferase II Deficiency (OMIM #600650) characterized by acute metabolic decompensation associated with hypoketotic hypoglycemia, and intermittent myopathic symptoms, including cardiomyopathy (64, 65). A recent study showed that one patient with a homozygous P227L mutation also manifested polycystic kidneys, and died at age 14 days of severe lactic acidosis (44).

Additional examples of mutations driving cystogenesis are those in the mitochondrial electron transfer flavoproteins, which cause Multiple Acyl-Coa Dehydrogenase Deficiency (MADD) also called Glutaric aciduria II (GA2) (OMIM #231680), an autosomal recessively inherited disorder of

fatty acid, amino acid, and choline metabolism. GA2 results from deficiency of any of these three molecules: the alpha and beta subunits of electron transfer flavoprotein (ETFA and ETFB, respectively), and the electron transfer flavoprotein dehydrogenase (ETFDH) (Fig.2). The heterogeneous clinical features of patients also include polycystic kidney in some cases (44, 66).

One additional piece of evidence that deserves careful attention is the potential direct role of mitochondrial DNA (mtDNA) in preserving normal tubular structure. Mammalian cells contain a range of hundreds to thousands of copies of mtDNA. Mutations in the mtDNA can be inherited or accumulated resulting in a mixture of mutant and wild-type genomes (mtDNA heteroplasmy) that can vary among tissues and change over time as mitochondria replicate and cells age. These mutations cause a clinically diverse group of disorders, often referred to as mitochondrial encephalomyopathies (for a review see (67)). To model these disorders, a mouse carrying a large deletion in the mtDNA was generated, the so-called mito-mouse (68), lacking 6 tRNA genes and 7 structural genes, encoding subunits of the respiratory chain complexes. Interestingly, the mito-mouse dies of renal failure (68). The presence of >60% of mutant mtDNA in the kidney results in grossly enlarged kidneys with a pale appearance and dilatation of both proximal and distal tubules with intraluminal casts accumulation (68). Cytochrome *c* oxidase (COX, respiratory chain complex IV) staining confirmed defects in COX activity in these renal tubules (69, 70) (Fig. 2). Similar renal tubule dilatations and renal dysfunction are not occurring in mice carrying somatic mutations in the mtDNA, in the so-called mtDNA mutator mice, (71, 72), indicating that a minimal alteration in mtDNA is not sufficient to drive this phenotype. These studies indicate that the impairment of mitochondrial OXPHOS might *per se* be causative, or involved in the cystic phenotype in the kidney.

One final consideration goes to the experimental findings present in the literature suggesting that a state of hypoxia or pseudohypoxia might be important in modulating renal cystogenesis. Indeed, several studies have reported that mutants in ciliary proteins or mice carrying a late inactivation of the *Pkd1* gene, which is associated with a mild disease progression, are all exacerbated when ischemia is applied (73). Among other effects, ischemia is a potent inducer of hypoxia causing stabilization of the hypoxia-inducible factor 1 subunit alpha (HIF1α), a strong driver of the transcription of glycolytic genes (74). The increased transcription of glycolytic genes in the PKD kidneys *in vivo* was shown not to be associated to the hypoxic state that might occur in late PKD. Nevertheless, the stabilization of HIF1α, which can also be a consequence of mTORC1 activation in the absence of hypoxia, has been observed and it is likely responsible of enhanced transcription

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of glycolytic genes (3). Furthermore, inactivation of the von Hippel-Lindau (VHL) tumor suppressor, the ubiquitin ligase responsible for degradation of HIF1α in the presence of oxygen, was shown to equally exacerbate the renal cystic phenotype associated with inactivation of kinesin family member 3A (KIF3A) (75), suggesting that HIF1α could act as a modifier of the cystic phenotype. Finally, an elegant study has worked out the mechanism of regulation of the transcription factor HIF2α in the kidney (76). The investigators have discovered that while the levels of HIF2α are never detectable in the kidney, this transcription factor becomes visible in tumor lesions of VHL patients. Indeed, they further demonstrated that VHL inactivation in the kidney results in stabilization of HIF2α and mild tubular dilatation (76). Of great interest, the investigators also showed that driving continuous transgenic expression of HIF2α using a kidney-specific promoter was sufficient to drive renal fibrosis associated with multiple renal cysts (76).

All these data taken together might suggest that the presence of a pseudohypoxic state (i.e. activation of pathways normally activated by hypoxia, even in the presence of oxygen) might play an important role and be involved in initiation of cystogenesis.

ADPKD signaling pathways involved in metabolic reprogramming and potential for therapy

It is noteworthy that in addition to a potential direct role of PC-1 in regulation of the mitochondrial activity, several of the signaling pathways found de-regulated in PKD and considered to be directly or indirectly regulated by the polycystins might in principle explain the metabolic reprogramming observed (Fig.4). Indeed, some of the aberrant metabolic alterations in ADPKD cysts are likely consequent to pro-tumorigenic pathways. Multiple lines of evidence have shown that mTORC1 is constitutively activated in ADPKD (77, 78). mTORC1 regulates energy metabolism, particularly glucose and glutamine rewiring (19, 79, 80). Furthermore, downstream of mTORC1 sterol regulator element-binding proteins (SREBPs) are major transcriptional factors driving fatty acids synthesis (19). The enhanced *de novo* synthesis of lipids, as was shown by increased expression of *Fasn* in *Pkd1*$^{-/-}$ mutant cells (4), may be the result of SREBP-dependent induction of the relevant enzymes involved in lipogenesis downstream of mTORC1 (19) (Fig.4). Furthermore, there is growing evidence showing that mTORC1 activation suppresses β-oxidation of fatty acids for energy production, likely due to its activity in promoting lipid biosynthesis ((reviewed in (81)). Fatty acids are also essential for the synthesis of glycerolphospholipid membrane and membrane signaling molecules. Early studies have reported increased levels of glucosylceramide-based glycolipid formation in PKD (82). Subsequently, sphingolipid analysis was performed in human PKD samples, nephronophthisis (juvenile cystic kidneys jck and polycystic kidney disease pcy mice) and in *Pkd1* conditional mouse models. Increased expression of glucosylceramide was reported and

inhibition glucosyl-transferase decreased cystogenesis in both orthologous and non-orthologous models of PKD (83) leading to a phase II/III clinical trial with a specific inhibitor (for a table of all compounds under development please see (84)). mTORC1 is also involved in regulation of both PGC-1α and PPARα whose relevance in PKD has been described above. Based on this one could hypothesize that mTORC1 serves a central role in regulation of metabolic rewiring in PKD, and indeed experimental data also support this notion (3). Further to this, several lines of evidence show that some of the metabolic alterations might also be dependent on c-Myc, an oncogene demonstrated to be involved in PKD a long time ago and more recently re-discovered (39, 85, 86) (Fig.4).

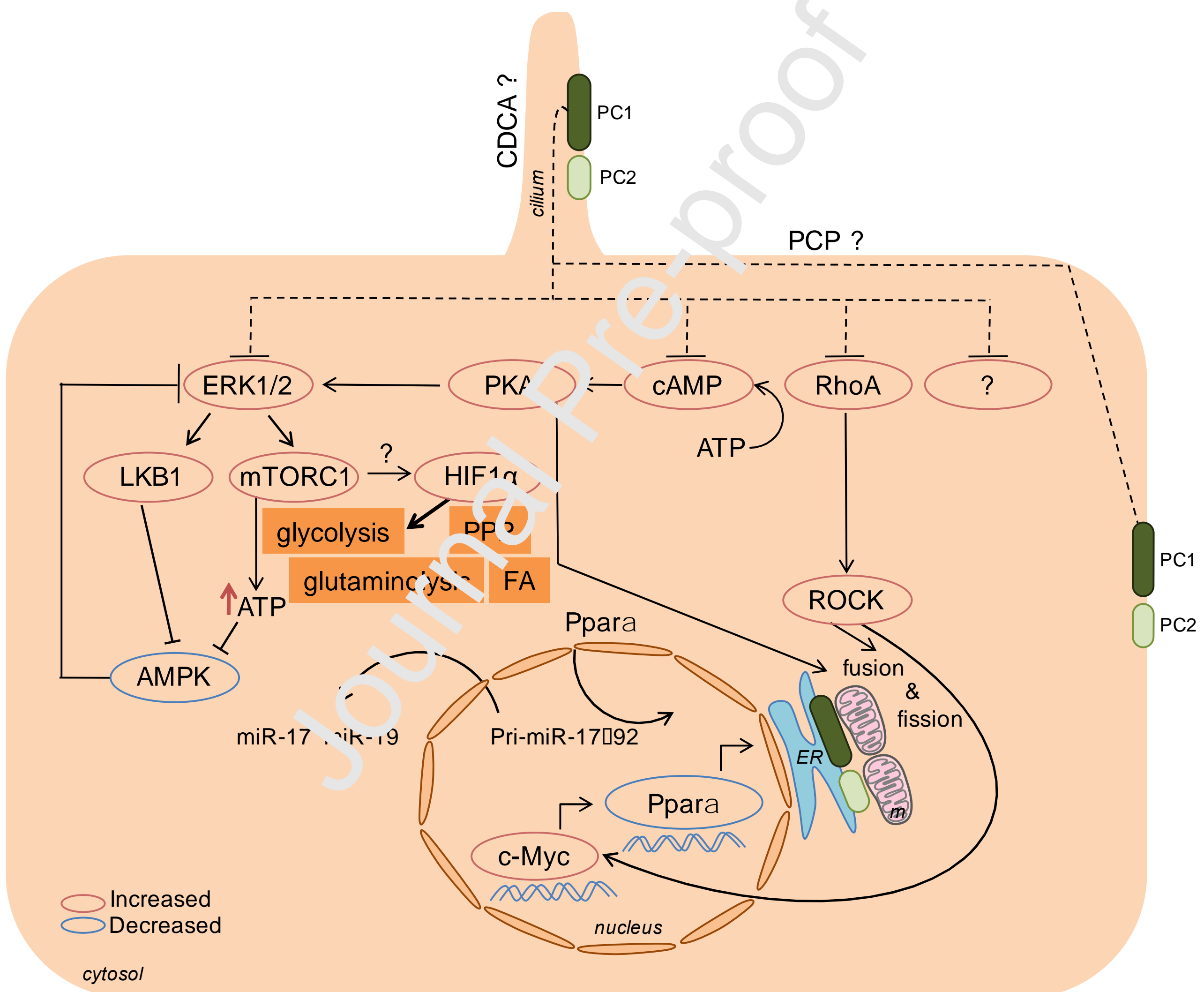

Figure 4. Diagram depicting pathways up- or down-regulated in PKD and impacting on metabolism. The absence of PC-1 results in the upregulation of several signaling pathways including the ERKs responsible for the regulation of LKB1 causing inhibition of ATP production and on the other hand, they affect mTORC1 activity which activate glycolysis, PPP, glutaminolysis and fatty acids increasing the amounts of ATP and further inhibiting AMPK. cAMP signaling increases PKA which enhances ERKs. *Pkd1* inactivation further activates RhoA which activates ROCK a molecule that could be directly involved in regulation of mitochondrial fusion and fission. Furthermore, ROCK increases the transcriptional activity of c-Myc-mediated expression of miR-17-92 cluster. The mature miRNA in the cytoplasm bind to *Pparα* 3'-UTR. PPARα regulates the expression of key genes involved in mitochondrial OXPHOS pathways. PPP, pentose phosphate pathway; FA, fatty acid biosynthesis. The direct involvement of PC-1/2 in regulation of all these pathways is less clear. Defects in polycystins might result in release of a cilia-dependent cyst activating

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(CDCA) still to be identified. The PC-1/2 complex at cell-cell/matrix interface and their role in regulation of planar cell polarity (PCP) might also be involved. ER: endoplasmic reticulum; m: mitochondrion.

Finally, a central cascade involved in regulation of metabolic rewiring in ADPKD is the one controlled by AMP-activated protein kinase (AMPK), a major cellular regulator of metabolism. AMPK is a highly conserved metabolic sensor of intracellular adenosine nucleotide levels; it is activated in the presence of low energy (high AMP levels) and it normally inhibits mTORC1 (87) (Fig.4). Indeed, forced activation of AMPK by metformin was shown to have a dual effect on proliferation and on fluid secretion by acting on the cystic fibrosis transmembrane conductance regulator (CFTR) and improving disease progression (87, 88) and it is currently employed in a phase II clinical trial (89). Of relevance, a hypomorphic orthologous *Pkd1* mouse model exposed to reduced calorie intake was shown to result in a very important improvement of disease progression, due to a strong restoring effect on mTORC1 and AMPK (87, 90). The induction of AMPK activity involves the activation of various protein kinases including the tumor suppressor LKB1. In our studies we showed that reduced activation of AMPK in PKD models is secondary to the alterations in metabolism and in addition are due to a direct regulation of the LKB1/AMPK axis by the extracellular signal–regulated kinases (ERKs) (3, 10) (Fig.4). The dual role of these cascades in driving cystogenesis is further supported by genetic studies demonstrating that an *Lkb1/Tsc1* double mutant presents with a very severe PKD phenotype as described above (29).

Finally, it should be mentioned here that a central regulator of cystogenesis in PKD is cAMP, which has been implicated in almost all aspects of the disease (2). cAMP promotes cyst enlargement and its production upregulates protein kinase A (PKA) activity which in turn activates the ERKs (91) (Fig.4).

Of note, both PKA and AMPK are among the signalling cascades that can participate in the regulation and balance of mitochondrial fusion and fission events (for an overview see review by (92)). Mitochondria are indeed highly dynamic organelles that undergo fusion, fission, transport, and degradation. In addition to PKA and AMPK, activation of Ras homolog family member A (RhoA) in a Rho-associated coiled-coil protein kinase (ROCK)-dependent manner can affect mitochondrial morphology (86). All these signalling pathways were found differentially regulated in PKD and could in principle drive the observed alterations in mitochondria (Fig.4) (93).

Concluding remarks

In this review, we have tried to provide an overview of how the metabolic alterations observed in PKD can be part of a broad reprogramming that likely involves the many pathways described to

date and additional ones. In-depth understanding of the derangement will be fundamental both to better understand the pathophysiology of the disease, but also to exploit the unique opportunity for therapy that metabolic intervention can offer. Indeed, the high interconnection of metabolic pathways and the propensity of the cells to adapt to any transient or constitutive alteration offers the possibility to interfere with "savage pathways" and to cause a collapse in the cellular capability to grow (94). Indeed, the few interventions directly interfering with the capability of cells to consume metabolites have proven very efficacious (3, 9, 10, 12).

One important consideration that rises from all the recent findings on metabolism is that the similarities to cancer metabolic reprogramming are intriguing, but also puzzling (20). It is unclear why major metabolic alterations that are considered central in the initiation or progression of cancer should be present in PKD tissues given that cysts do not show a strong propensity to transform into tumors (95). One possibility is that these metabolic alterations are not *per se* sufficient to drive transformation. A second possibility is that the metabolic reprogramming occurs at a much lower grade in PKD than in cancer. Finally, a third possibility is that PKD tissues are somehow protected from further transformation and they are arrested at the neoplastic stage. Whatever the reason for this discrepancy, the parallels with cancer have prompted investigators to propose that a program of cancer drug repurposing might be helpful in identifying a therapy in PKD (96). The same consideration could apply to the many metabolic interventions that are being developed for cancer (20, 94, 96). While this reasoning lies certainly on strong bases, it should be considered that due to the relatively mild condition and slow progression of PKD, safety and tolerability of a given drug are going to constitute the predominant rationale for designing a therapy in PKD. All the compounds designed for cancer treatment were not based on a similar logic and most of them are indeed extremely toxic. Therefore, cancer drugs repurposing in ADPKD might actually fail because of this. Perhaps rather than concentrating on the cancer-promising therapies, we should focus more on those compounds that were abandoned in cancer because not sufficiently effective in arresting the growth of the cancerous cells, but presenting with a mild to low toxicity.

One essential question that will need to be answered in the future is where do the multiple metabolic alterations observed in PKD originate from. One intriguing possibility that has been brought forward is that PC-1 and 2 might play a direct role in mitochondrial regulation and this might in principle be sufficient to cause a metabolic rewiring when the functional polycystins are absent (50, 53). One additional point that will need to be addressed is whether or not these alterations are primary, i.e. directly linked to the function of the polycystins and primary drivers of the disease, or secondary, i.e. they all occur as a consequence of cyst formation an event that

originates, by definition, from the absence of functional polycystins, perhaps elsewhere. Indeed, we should not forget that a central role for ciliary function in preventing cystogenesis has been demonstrated convincingly by a large body of work and future studies should try to address the question whether cilia can regulate some of the metabolic pathways affected in PKD (97, 98). Therefore, if the cilia play a central role in regulating a primary pathway that is involved in cystogenesis and is inhibited by the polycystins (the proposed cilia-dependent cyst activating pathway, CDCA (99), Fig. 4) the metabolic reprogramming and mitochondrial dysfunction might be secondary to other pathways and to ciliary dysfunction. The polycystins localized at site other than the cilia might contribute via other pathways as well, but which ones are the more relevant remain an open essential question.

Future studies should aim at pin-pointing the precise mechanism underlying the alterations observed in metabolic and mitochondrial biology in PKD and most importantly in linking this to the primary function of the Polycystins.

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Author contributions

CP, LC and AB wrote the manuscript. CP and LC generated the figures. All authors reviewed and edited the manuscript before submission.

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Highlights:

- Metabolic reprogramming is now recognised as a major feature in ADPKD
- Multiple metabolic pathways are dysregulated in ADPKD including increased glycolysis and glutamine metabolism, defective TCA cycle in mitochondria, reduced fatty acids oxidation and enhanced fatty acids synthesis
- The Polycystins might directly or indirectly impact on regulation of mitochondrial function and OXPHOS regulation
- The several metabolic alterations and mitochondrial dysfunctions in ADPKD might provide novel insights into the pathogenesis and allow for identification of innovative therapies

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