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Apoptosis and autophagy in polycystic kidney disease (PKD)

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Abstract

Apoptosis in the cystic epithelium is observed in most rodent models of polycystic kidney disease (PKD) and in human autosomal dominant PKD (ADPKD). Apoptosis inhibition decreases cyst growth, whereas induction of apoptosis in the kidney of Bcl-2 deficient mice increases proliferation of the tubular epithelium and subsequent cyst formation. However, alternative evidence indicates that both induction of apoptosis as well as increased overall rates of apoptosis are associated with decreased cyst growth. Autophagic flux is suppressed in cell, zebra fish and mouse models of PKD and suppressed autophagy is known to be associated with increased apoptosis. There may be a link between apoptosis and autophagy in PKD. The mammalian target of rapamycin (mTOR), B-cell lymphoma 2 (Bcl-2) and caspase pathways that are known to be dysregulated in PKD, are also known to regulate both autophagy and apoptosis. Induction of autophagy in cell and zebrafish models of PKD results in suppression of apoptosis and reduced cyst growth supporting the hypothesis autophagy induction may have a therapeutic role in decreasing cyst growth, perhaps by decreasing apoptosis and proliferation in PKD. Future research is needed to evaluate the effects of direct autophagy inducers on apoptosis in rodent PKD models, as well as the cause and effect relationship between autophagy, apoptosis and cyst growth in PKD.

Keywords

Apoptosis; Autophagy; Polycystic kidney disease

1. Introduction

ADPKD is the commonest life threatening hereditary disease often resulting in end stage kidney disease requiring dialysis and kidney transplantation [1]. Most ADPKD is caused by a mutation in either the Pkd1 gene (85% of cases) or the Pkd2 gene [2]. There are a multitude of signaling pathways that are either increased or decreased in PKD [3] [4] [5] [6]. Abnormal cross-talk between intracellular calcium and cAMP signaling is likely one of the first effects of PKD mutations and can result in increased proliferation of the tubular cells lining the cyst [3]. Protein kinase A-induced phosphorylation of cystic fibrosis transmembrane conductance regulator (CFTR) allows chloride and fluid secretion into the cysts and increases cyst growth [3]. Mammalian target of rapamycin (mTOR), that plays a role in both autophagy and apoptosis signaling, is increased in PKD kidneys [7]. mTOR

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inhibitors that protect against cyst growth in animal models [7] [8] are known to affect both autophagy and apoptosis. The Bcl-2 family of proteins that is dysregulated in PKD [9] [10] [11] also plays an important role in both apoptosis and autophagy signaling. Caspases, the major mediators of apoptosis that can also affect autophagy are increased in PKD kidneys [12] [9] [13] [14]. Studies in *Pkd1*^{-/-} cells [15], *Pkd1*^{-/-} zebrafish [16] and mouse models of PKD [17] [18] [19] suggest that there may be a disturbance in the balance between tubular cell proliferation, apoptosis, and autophagy in PKD. This review will focus on the role of tubular cell apoptosis and autophagy in cyst growth in PKD and explore the possible interactions between apoptosis and autophagy in PKD.

2. Apoptosis

Apoptosis is a process of programmed cell death characterized by volume reduction, cell surface blebbing, chromatin condensation, internucleosomal cleavage of DNA, and formation of apoptotic bodies [20] [21] [22]. Apoptotic cells are quickly phagocytosed by macrophages to prevent the release of intracellular components and inflammatory factors, resulting in “clean” cell death. Lockshin & Williams [23] originally defined programmed cell death in the context of insect development. Subsequently, Kerr et al. noticed by ultrastructural analysis two morphologically different types of cell death in humans: apoptosis and necrosis [24]. In necrosis, the cells swell, plasma membranes rupture, and cellular components are released. In apoptosis, the cells shrink with intact plasma membranes, and nuclei are condensed and fragmented. As apoptosis was discovered to be mediated by gene products, it was termed as being programmed. Thus, the term programmed cell death has been used in the context of apoptosis. It should be noted that cell death with a necrotic morphology that occurs during inflammation or infection can also be programmed or regulated by gene products, and is categorized as necroptosis and pyroptosis [25] [26].

3. Apoptosis in PKD

Apoptosis in the tubular epithelial cells lining the cyst and/or noncystic tubular epithelium in PKD kidneys is observed in most rodent models of PKD, regardless of the genetic defect (Table 1).

Apoptosis has also been detected in human ADPKD kidneys compared to normal kidneys, regardless of renal function [27] (Table 1). Specifically, apoptosis is evident in normal non cystic tubular epithelium in human polycystic kidneys from non-uremic ADPKD patients [27].

As apoptosis in renal tubular epithelium is detected in most models of PKD resulting from differing gene defects, it is unlikely that the *Pkd1* or *Pkd2* gene defect directly causes apoptosis.

mTOR, which exists in association with two different complexes, mTORC1 and mTORC2, may provide the link between cyst growth and apoptosis in diverse models of PKD [28] [29]. mTORC1 consists of mTOR and regulatory associated protein of mTOR (Raptor), while mTORC2 consists of mTOR and rapamycin-independent companion of mTOR (Rictor) [29].

mTOR is known to regulate apoptosis and proliferation [30]. Increased mTOR signaling is an almost universal phenomenon in PKD kidneys from different gene defects. Increased mTORC1 signaling has been observed in many rodent models of PKD [12] [31] [32] [8] [33] and in human ARPKD kidneys [34] [35]. Both mTORC1 and 2 signaling are increased in mouse models of PKD [36] [37]. The effects of mTORC $\frac{1}{2}$ inhibition on apoptosis in PKD models will be discussed later in this review.

4. Pathways of caspase-mediated apoptosis in PKD

A family of cysteine proteases, known as caspases, are the major mediators of apoptosis. The caspase pathways that are centrally important in apoptosis involve the “initiator” caspases-8 and -9 and the “executioner” caspases-3 and -7 [38] [39] [40]. Caspase-3 plays a crucial and extensively studied role in the promotion of apoptosis [41] [42] [43]. Caspase-3 terminates contacts with surrounding cells, re-organizes the cytoskeleton, shuts down DNA replication, interrupts splicing, destroys DNA, disrupts the nuclear structure, marks the cell for phagocytosis, and disintegrates the cell into apoptotic bodies [44] [45].

There are two major pathways of caspase-mediated apoptosis [39]²⁰. In the mitochondrial or “intrinsic” pathway, stress-induced signals act via Bcl-2 proteins to promote cytochrome c release from mitochondria [46] [47]. Cytochrome c binds to the cytosolic protein Apaf-1 to facilitate the formation of an apoptosome. Once formed, the apoptosome can then recruit and activate caspase-9, that can in turn activate caspase-3. In support of a role of the Bcl-2 protein family in PKD, Bcl-2 deficient mice have increased apoptosis and die from severe PKD [48] [49] [50]. Activation of caspase-3 and dysregulation of the balance between pro- and anti-apoptotic Bcl-2 family members, specifically a downregulation of anti-apoptotic Bcl-X_L, correlates with increased apoptosis in polycystic Han:SPRD rat kidneys [9]. In Han:SPRD rat kidneys, the pro form of caspase-9, cytochrome c release into the cytosol, and caspase-2 protein and activity are increased, demonstrating involvement of the intrinsic pathway [51]. In the “extrinsic” pathway, the binding of a ligand to its death receptor recruits an adaptor protein that in turn recruits and activates procaspase-8, which subsequently activates caspase-3 [52]. The proform of caspase-8 is also increased in PKD, demonstrating involvement of the extrinsic pathway [51]. No differences in Fas ligand (FasL) mRNA are observed, suggesting that the extrinsic pathway is independent of the death receptor ligand, FasL [51].

5. Relationship between apoptosis and cyst growth in PKD

Apoptosis may be causally linked to the development of renal cystic disease. For example, apoptosis is essential for Madin-Darby canine kidney (MDCK) cell cyst cavitation in collagen type 1 matrix. Cystogenesis in this system is inhibited by overexpression of the anti-apoptotic gene, Bcl-2 [53]. In a novel cell culture system for studying how PKD1 regulates apoptosis, proliferation and cyst formation, expression of human PKD1 in MDCK cells slows their growth and protects them from apoptosis [54]. MDCK cells expressing PKD1 also spontaneously form branching tubules, while control cells form simple cysts. Thus, PKD1 may function to regulate both apoptosis and proliferation pathways, allowing cells to enter a differentiation pathway that results in tubule formation. Collectively, this

provides evidence that poly-cystin-1 (PC-1) may inhibit apoptosis, and confirms that increased apoptosis is associated with cyst formation in MDCK cells. Similarly, inhibition of fibrocystin (the gene product of PKHD1 that is responsible for autosomal recessive PKD) by short hairpin RNA inhibition in inner medullary collecting duct cells disrupts normal tubulomorphogenesis and results in increased apoptosis and proliferation [55].

Y528C and R1942H are two missense mutations that have been identified from a PKD1-affected individual. MDCK cells expressing the Y528C variant form cysts in culture and display increased rates of growth and apoptosis compared to MDCK cells stably expressing the wild-type [56]. The protein product of PKD1 (PC-1) is a large transmembrane protein with a short intracellular C terminus that interacts with numerous signaling molecules, including Galpha(12). G12/G13 alpha subunits are alpha subunits of heterotrimeric G proteins. MDCK cells can increase apoptosis via Galpha(12) stimulation of JNK and degradation of the anti-apoptotic protein Bcl-2, with PC1 expression levels determining the activity of the JNK/Bcl-2 apoptosis pathway [57]. Thus, there appears to be a causal relationship between apoptosis, proliferation and cyst formation. In summary, most (but not all) *in vitro* studies in PKD cells support that increased levels of apoptosis are associated with formation and growth of cysts. Next, the effect of therapies that decrease cyst growth *in vivo* on apoptosis will be discussed.

6. The effect of therapies that decrease cyst growth on apoptosis in vivo (Table 2)

Therapies that decrease cyst growth in PKD have variable effects on apoptosis. Some therapies that decrease cyst growth also decrease apoptosis. The cell cycle inhibitor roscovitine results in long-lasting arrest of cystogenesis, along with decreased apoptosis [58] [59]. Erb-b2 receptor tyrosine kinase 4 (ErbB4) is a receptor tyrosine kinase and member of the epidermal growth factor receptor family that is highly expressed in cystic kidneys. In cpk mice, ErbB4 deletion results in accelerated cyst progression, renal function deterioration, increased cell proliferation in the cyst-lining epithelial cells, and significantly more apoptotic cells. This is associated with decreased levels of cyclin D1, increased levels of p21, p27, and cleaved caspase 3, suggesting that decreased cell cycle progression may contribute to apoptosis [60]. Similarly, in Han:SPRD rats treated with 2-hydroxyestradiol (2-OHE), the resultant decrease in cyst growth and preservation of kidney function is associated with suppression of proliferation, apoptosis, and markers of angiogenesis [61]. Sphingolipids and glycosphingolipids are emerging as major regulators of proliferation, apoptosis, and activation of growth regulatory pathways. Blockade of glucosylceramide (GlcCer; a glyco-sphingolipid) accumulation with the GlcCer synthase inhibitor Genz-123346 effectively inhibits cystogenesis in Pkd1 conditional knockout mice and jck and pcy mouse models of nephronophthisis [62]. Mechanism-of-action studies suggest that GlcCer synthase inhibition results in effective cell cycle arrest and inhibition of the Akt-mTOR pathway, ultimately leading to decreased apoptosis and mitogenic signaling [62]. Of note, enrollment in a phase II/III trial in adults with ADPKD evaluating the efficacy of Venglustat, which reduces accumulation of GlcCer, is ongoing (NCT03523728).

In contrast, some therapies that decrease cyst growth can increase apoptosis; likewise, worsening ADPKD may associate with reduced apoptosis. Immunodepletion of CD8+ T cells from one to three months in C57Bl/6 Pkd1^{RC/RC} mice results in worsening of ADPKD pathology, decreased apoptosis, and increased proliferation compared to IgG-control, consistent with a reno-protective role of CD8+ T cells [63]. microRNAs (miRNAs) are short noncoding RNAs that function as sequence-specific inhibitors of gene expression [64]. The miR-17 family and miR-21 are both upregulated in kidney cysts and promote ADPKD progression in mouse models. miR-21 represses proapoptotic genes and thus inhibits cyst apoptosis and promotes PKD progression [65]. Overexpression of exogenous kidney-specific neutrophil gelatinase-associated lipocalin (Ngal) attenuates progressive cyst development and prolongs lifespan in a murine model of PKD, which is associated with reductions in interstitial fibrosis and proliferation and augmented apoptosis [66]. Macrophage migration inhibitory factor (MIF) is another important regulator of cyst growth in ADPKD. MIF is upregulated in cyst-lining epithelial cells in PC-1-deficient murine kidneys and accumulates in cyst fluid of human ADPKD kidneys [67]. MIF regulates cystic renal epithelial cell apoptosis through p53-dependent signaling. MIF deletion or pharmacologic inhibition of MIF delays cyst growth and increases p53-dependent apoptosis in multiple murine ADPKD models [67].

Sirolimus is an autophagy inducer, but its effects on apoptosis in PKD are complex [68]. The effect of sirolimus on apoptosis in PKD may be determined by the dose. High-dose sirolimus increases apoptosis of cyst lining epithelium in PKD [8] [69] [70]. However, low-dose sirolimus may also result in effective blockade of PKD without an effect on apoptosis [33]. Thus, an appropriate therapeutic dose of sirolimus may not have an effect on apoptosis in PKD. Furthermore, the effect of mTOR inhibitors on apoptosis may depend on whether there is dual blockade of both mTORC1 and 2. The mTOR kinase inhibitor, torin-2, decreases PKD severity and improves kidney function in the Pkd1^{RC/RC} mouse model of PKD [37]. Torin-2 decreases proliferation and increases apoptosis in cells lining the cysts in these mice [37]. However, in Han:SPRD rats with PKD, the mTOR kinase inhibitor PP242, which inhibits both mTORC1 and 2, has no effect on caspase-3 activity, TUNEL positive or active caspase-3-positive tubular cells, despite significantly reducing kidney enlargement, cyst density, blood urea nitrogen, and proliferation in cells lining the cysts and non-cystic tubules [71]. Finally, an mTOR anti-sense oligonucleotide (ASO) that blocks both mTORC1 and 2, decreases PKD severity in mice with a targeted mutation in Pkd2, which is associated with a significant decrease in proliferation and apoptosis of tubular epithelial cells [36]. Thus, blockade of both mTORC1 and 2 does not have a consistent effect on apoptosis in the cells lining the cysts in the PKD kidney.

Differences in apoptosis observed in *in vivo* studies may be due to different methods used to detect apoptosis *in vivo*. Numerous studies simply count TUNEL-positive cells in an automated fashion. However, the gold-standard is to count cells that have morphologic criteria of apoptosis that include cellular rounding and shrinkage, nuclear chromatin compaction, and formation of apoptotic bodies [72]. Morphology is the gold-standard for detection of apoptosis and TUNEL staining fails to discriminate between proximal tubule apoptosis and necrosis, particularly *in vivo* in the kidney, and it grossly overestimates proximal tubule apoptosis in the kidney [73] [72] [74] [75] [76]. This is especially true in

cystic tubular epithelial cells that may have injury that can cause necrosis [77]. For these reasons, the number of TUNEL-stained nuclei that also demonstrate the morphological features of apoptosis should be also be quantitated.

With this background, studies have been performed to determine the effect of direct apoptosis inhibition or apoptosis induction on tubular cell proliferation, cyst formation, kidney size, and kidney function in PKD.

7. Caspase inhibition in PKD

Caspases are attractive potential targets for treatment of diseases because of their central role in apoptosis and the appealing prospect of small-molecule inhibitor therapy [78]. In animal models, caspase inhibitors decrease ischemia-perfusion injury in heart [79] [80], liver [81] and kidney [82] [83]. The pan-caspase inhibitor IDN-1965 improves cardiac function and decreases mortality in mouse cardiomyopathy [84] and reduces apoptosis of sinusoidal endothelial cells during liver preservation injury [85]. The pan-caspase inhibitor IDN-6556 decreases liver enzyme elevations in patients with hepatic dysfunction [86]. Of note, the orally active caspase inhibitor, Emricasan, is currently being tested in multiple clinical trials conducted in patients with liver disease [87] (see clinicaltrials.gov).

Early evidence in rodents suggests that caspase inhibitors may also be an attractive therapy to slow PKD progression. Three-week old heterozygous and littermate control Han:SPRD male rats treated with the caspase inhibitor, IDN-8050 (10 mg/kg/d) via a minipump for 5 weeks, have a 44% reduction in kidney enlargement, a 29% reduction in cyst volume density, and attenuated increase in BUN, as compared to vehicle [12]. IDN-8050-treatment additionally reduces the number of proliferating cell nuclear antigen (PCNA) positive tubular cells and apoptotic tubular cells in non-cystic and cystic tubules. Collectively, this indicates that in a rat model of PKD, caspase-inhibition with IDN-8050: 1) decreases apoptosis and proliferation in cystic and non-cystic tubules, 2) inhibits renal enlargement and cystogenesis, and 3) attenuates the loss of kidney function. These findings will require confirmation in other animal models of PKD prior to translation to clinical trials in humans with ADPKD.

Mice with caspase-3 gene deletion have been crossed with mice harboring the congenital polycystic kidney (cpk) mutation to generate double-mutant mice [14]. Homozygous caspase-3 (cpk;casp3^{-/-} mice) live nearly four-times longer than littermate control cpk mice, and heterozygous (cpk;casp3^{+/-} mice) live significantly longer than controls. In addition, kidney weight, relative to body weight, is significantly lower in the cpk;casp3^{-/-} mice than in the cpk and cpk;casp3^{+/-} mice. However, despite deletion of caspase-3, apoptosis occurs and cysts form; therefore, alternative pathways of apoptosis in cystic kidneys have been investigated. Caspase-7 is up-regulated and the anti-apoptotic protein Bcl-2 is down-regulated in cpk, cpk;casp3^{+/-}, and cpk;casp3^{-/-} mice compared with wild-type controls. In summary, homozygous deletion of caspase-3 markedly prolongs survival of cpk mice, but a caspase-7-mediated pathway may compensate for the deficiency of functional caspase-3 [14]. These findings suggest that pan-caspase inhibition may have a greater therapeutic effect than selective caspase inhibition in PKD.

8. Apoptosis induction in PKD

The mitochondrial protein Smac promotes caspase activation in the cytochrome c/Apaf-1/caspase-9 pathway by binding to inhibitor of apoptosis proteins and removing their inhibitory activity [88]. While Smac is normally a mitochondrial protein, it is released into the cytosol when cells undergo apoptosis [89]. Overexpression of Smac increases cells' sensitivity to apoptotic stimuli. *In vitro*, a Smac-mimetic and co-treatment with tumor necrosis factor- α (TNF- α) augments the formation and activation of the receptor-interacting serine/threonine-protein kinase 1-dependent death complex and the degradation and cleavage of the caspase-8 inhibitor FLIP. This results in death in Pkd1 mutant epithelial cells, with no effect on normal renal epithelial cells [90]. *In vitro*, a Smac-mimetic selectively induces TNF- α -dependent cystic renal epithelial cell death and delays cyst formation [90]. Similarly, treatment with a Smac-mimetic slows cyst growth and kidney enlargement, as well as preserves renal function in two genetic strains of mice with Pkd1 mutations, without affecting proliferation.

How do we reconcile the seeming contradiction that both apoptosis inhibition and apoptosis induction can lessen cyst growth? Whether apoptosis promotes or retards cyst growth is confounded by factors such as differing rodent models of PKD with differing mutations, early-versus late-disease, and apoptosis in cysts versus apoptosis limited to non-cystic tubules and interstitial cells [91]. It is possible that apoptosis inhibition and apoptosis induction decrease cyst growth by very different mechanisms that can act independently. Under certain conditions, enhanced apoptosis may preserve renal structure by eliminating mural cells from cysts that otherwise would expand endlessly without having an effect on proliferation [90]. Likewise, apoptosis inhibition can decrease proliferation of cystic epithelial cells that is crucial for growth of the cyst [12].

9. Apoptosis may be causally linked to proliferation and the development of cysts

Abnormal proliferation in tubular epithelial cells plays a crucial role in cyst development and/or growth in PKD [92] [2,93] and apoptosis may be causally linked to these processes. Convincing evidence indicates that increased tubular cell proliferation is accompanied by tubular apoptosis in PKD, and apoptosis and proliferation are directly related [94] [95] [50] [96] [97]. A massive increase in both apoptosis and proliferation is observed in both SBM mice that overexpress c-myc and Bcl-2 deficient mouse models of cystic disease [95] [50]. In fact, increased apoptosis and proliferation occur early in the course of the disease and precede cystogenesis in SBM mice [95]. In Han:SPRD rats fed soy protein, the improved renal function and decreased cyst formation is accompanied by decreases in both tubular cell proliferation and apoptosis [97]³⁴. Also, mice deficient in the pro-apoptotic Bcl-2 gene have hyperproliferation as well as apoptosis that accompanies renal cysts [50,98] [48]. Kidneys from patients with ADPKD have high levels of apoptosis as well as cellular proliferation [27] [99]. Thus, epithelial cell apoptosis and proliferation are dysregulated in ADPKD and may represent a general mechanism for cyst growth and tissue remodeling [94] [2] [100].

The precise pathways that link apoptosis and proliferation in PKD remain to be determined. However, a common pathway of apoptosis and proliferation may involve adhesion-dependent control of apoptosis and overexpression of the proto-oncogene c-myc. Changes in cell shape and loss of cell to cell adhesion during apoptosis may stimulate surrounding cells to proliferate [101] [102]. Theoretically, in PKD loss of tubular cells by apoptosis may initiate proliferation of neighboring tubular cells. In this case, both apoptosis and proliferation would be observed in the same cyst and caspase inhibition would attenuate both of these processes. Overexpression of c-myc is thought to play a role in the dysregulation of both proliferation and apoptosis in ADPKD. Mice overexpressing c-myc (also known as SBM mice) develop PKD concomitant with massively increased apoptosis and proliferation [103,104]. Renal c-myc expression is also increased in the Han:SPRD rat model of ADPKD [105]. The pathway of c-myc-induced apoptosis is thought to be mediated by the “initiator” caspase-9 [106] and the “executioner” caspase-3 [107] [108]. A novel Ste20-related kinase (SLK) is cleaved and activated by caspase-3 during c-myc-induced apoptosis [109]. In human ADPKD, a large increase in c-myc expression is associated with both tubular cell proliferation and apoptosis [96] [110] [111] [94]. Thus, caspase inhibition may have therapeutic relevance by impeding a common pathway for both apoptosis and proliferation, resulting in reduced cyst formation.

With caspase inhibition, it is important to consider that apoptosis in ADPKD may be a double-edged sword [112]. While increased apoptosis may result in increased proliferation, cyst growth, and deterioration of renal function in PKD, apoptosis may also be a defense against oxidative or other forms of DNA damage and thus reduce the risk of neoplastic transformation. In this regard, the simultaneous induction of cell proliferation and apoptosis has been regarded as a safeguard against neoplastic transformation. However, cancer-prone mice treated with the caspase inhibitor IDN-8050 (Emricasan) from a young age have no detectable deleterious effects, including no evidence of treatment-related tumor formation or carcinogenicity [113]. Emricasan does not affect apoptosis in normal healthy cells, and as noted previously, it is currently being tested in multiple Phase 3 clinical trials, without reports of carcinogenicity (see clinicaltrials.gov). So it is unlikely that caspase inhibition would result in tumor development in PKD kidneys.

10. Summary on apoptosis

There is abundant evidence that apoptosis plays a causal role in cyst formation: 1) induction of apoptosis in tubular cells in culture results in cyst formation; 2) tubular epithelial cell apoptosis occurs in most animal models of PKD and in kidneys from humans with ADPKD; 3) both apoptosis and proliferation occur in non-cystic as well as cystic epithelial cells early in the course of PKD; 4) caspase inhibition results in less apoptosis and proliferation in the tubular epithelium, with attenuation of cyst formation and kidney failure.

11. Autophagy

Autophagy is a process that occurs in all eukaryotic cells to keeps cells alive under stressful conditions [114]. In autophagy, damaged organelles are sequestered into double-membraned autophagosomes that subsequently fuse with lysosomes to deliver their cargoes for

degradation and recycling. Autophagy received prominence in 2016 when the Nobel Prize in Physiology or Medicine was awarded to Dr. Yoshinori Ohsumi for “discoveries of the mechanisms for autophagy.” It is now known that there are 3 major forms of autophagy: macroautophagy, microautophagy and chaperone—mediated autophagy (CMA) [26]: 1) Macroautophagy is the best characterized variant of autophagy, that occurs in all eukaryotic cells and involves the sequestration of cytoplasmic components in double-membraned autophagosomes that subsequently fuse with lysosomes, where the cargo e.g. damaged organelles is delivered for degradation and recycling; 2) Microautophagy, or endosomal microautophagy, is a form of autophagy where cytoplasmic cargo destined for degradation is taken up by the vacuole via direct membrane invagination; 3) Chaperone-mediated autophagy (CMA) involves the direct delivery of cytosolic proteins targeted for degradation to the lysosome. The characteristic feature of CMA is that neither vesicles nor membrane invaginations are required for substrate delivery to lysosomes, and substrates reach the lysosomal lumen via a proteintranslocation complex at the lysosomal membrane. Other forms of autophagy target mitochondria (mitophagy), peroxisomes (pexophagy), endoplasmic reticulum (reticulophagy), protein aggregates (aggrephagy), lipid droplets (lipophagy), and inactive proteasomes (proteaphagy).

Autophagic flux is the most well accepted measure of the state of autophagy. For example, increased autophagosomes may be used to characterize systems of either increased autophagosome production or decreased autophagosome clearance by the lysosome, two dissimilar cellular responses [115,116]. According to the 2016 Guidelines for the use and interpretation of assays for monitoring autophagy, if the basal increase in microtubule-associate protein 1A/1B-light chain 3-II (LC3-II; a central protein in the autophagy pathway) is due to increased autophagosome production, then it is expected that lysosomal inhibition will further increase LC3-II [116]. Alternatively, if the increase in LC3-II is due to a block in autophagosome-lysosome fusion or a defect in lysosome function, then lysosomal inhibition would not affect LC3-II expression. The measurement of autophagic flux requires the measurement of autophagosomes (e.g., LC3-II, a marker of autophagosomes), in the presence and absence of lysosomal inhibition. Autophagic flux, measured as described above, is decreased in the cpk mouse model of ARPKD [17].

Autophagy was originally characterized as a hormonal and starvation response. It is now known that autophagy has a broad role in biology, including organelle remodeling, protein and organelle quality control, prevention of genotoxic stress, tumor suppression, pathogen elimination, regulation of immunity and inflammation, maternal DNA inheritance, metabolism, and cellular survival [117]. While autophagy is usually a degradative pathway, it also plays a role in biosynthetic and secretory processes. As autophagy is critical to many essential cellular functions, it is not surprising that defects in autophagy have been implicated in a variety of diseases [16,17,19,118–121]. Autophagy is crucial in maintaining cell and organ homeostasis, protecting against disease, and promoting recovery after injury [122], particularly in the kidneys. There is accumulating evidence that autophagy may be dysregulated in kidney disease and injury. Acute kidney injury, induced by either cisplatin or ischemia, has been hypothesized to upregulate autophagy as a recovery mechanism [123–126]. Autophagy enhancement benefits mice with cisplatin-induced acute kidney injury, and autophagy inhibition exacerbates injury in this model [124]. Further, autophagy is important

for maintaining podocyte proteostasis in aging mice [127]. Autophagy also has implications as a stress response during kidney transplant injury, with opposing effects resulting from immunosuppressive drugs or ischemic stresses [119].

12. Autophagy in PKD

Autophagy research in PKD is in its infancy, but there are reasons to believe that autophagy may be dysregulated in the PKD kidney and specifically that autophagy may be suppressed in PKD. Autophagic flux is suppressed in ADPKD as evidenced by studies in PKD1^{-/-} cells, Pkd1^{-/-} zebrafish and rodent models of PKD.

12.1. In vitro models

In human Pkd1^{-/-} cells, there is an attenuated increase in LC3-II expression after bafilomycin A1 (BafA1) treatment demonstrating decreased autophagic flux [16]. Additionally, by immunofluorescence, the number of both autophagosomes and autolysosomes in PKD1^{-/-} cells is significantly lower, and the number of autophagosomes fails to be up-regulated in response to BafA1 treatment, suggesting inadequate autophagosome formation and diminished autophagosome-lysosome fusion. Autophagy impairment accounts for a weakened ability to remove protein aggregates, as PKD1^{-/-} cells accumulate more aggregates in the absence of any stress and also break down MG132-induced protein aggregation less efficiently. Mouse Pkd1^{-/-} cells show a similar deficiency in the clearance of protein aggregates [16]. Zebrafish mutants for pkd1a develop mTOR activation, impaired autophagic flux and cystic kidneys [16].

12.2. In vivo models

Dysregulated autophagy has also been described in rodent models of PKD. Autophagic vacuoles in the cells surrounding cysts have been described in polycystic kidneys in the Han:SPRD rat [17]. By electron microscopy, features suggestive of autophagy-like autophagosomes, mitophagy, and autolysosomes are observed in both wild-type and PKD kidneys [17]. Specific to the Han:SPRD rat, autophagosomes are found by electron microscopy in the tubular cells lining the cysts. LC3 staining by immunofluorescence is also present in the tubular epithelial cells lining the cysts. Autophagic flux is also dysregulated in the cpk mouse model of ARPKD [17]. LC3-II expression is increased in PKD kidneys of cpk mice as compared to wild-type kidneys. Additionally, *in vivo* treatment with the lysosomal inhibitor BafA1 increases LC3-II expression in the kidneys of wild-type mice. In contrast, BafA1 has no effect on LC3-II in the polycystic kidneys of cpk mice, suggesting a defect in autophagic flux in PKD resulting from impaired autophagosome-lysosome fusion and degradation. In a Pkd1-hypomorphic mouse model (Pkdd1 miRNA transgenic mice) there is a significantly lower renal mRNA expression of autophagy-related genes, including Atg12, Atg3, beclin1, and p62, compared with wild-type control mice [18]. Collectively, these results suggest a modifying effect of ADPKD on autophagy and establish autophagy activation as a potential novel therapy for ADPKD [128].

13. Interactions between apoptosis and autophagy in PKD

There is cross talk between mTOR pathway of autophagy and the apoptosis pathways [129] (Figure 1). The mTOR pathway that inhibits autophagy is activated in PKD [28]. The intrinsic and extrinsic pathways of apoptosis are activated in PKD [51]. Many of the signals that regulate apoptosis in PKD also regulate autophagy, for example, the Bcl-2 family of proteins and caspases [130] [131] [132] [133] [134]. The relationship between apoptosis and autophagy is complex, depending on the type of cell and the nature and timing of the injury [133]. For example, increased autophagy may result in a delay of apoptosis [135] [136] [137] and genetic deletion of crucial autophagy proteins increases apoptotic cell death [138]. Autophagy is closely related to apoptosis, a process that is dysregulated in PKD. PKD kidneys have a significantly lower renal expression of autophagy-related genes, including Atg12, Atg3, beclin1, and p62, compared with wild-type control mice [166]. Inhibition of autophagy by knocking down the essential autophagy protein Atg5 promotes cystogenesis and specific induction of autophagy inhibits apoptosis [16]. Thus, it could be hypothesized that suppressed autophagy in PKD may be associated with increased apoptosis and cyst growth.

The hypothesis that autophagy suppression may contribute to cyst formation and growth is based on studies that many of the agents that protect against PKD, such as mTOR inhibitors, cyclin-dependent kinase inhibitors, caspase inhibitors, tyrosine kinase inhibitors, metformin, curcumin, and triptolide, affect both autophagy and apoptosis. Discussion of the effect of these agents on autophagy and apoptosis will be divided into agents that have been used in human studies, agents that have shown positive effects on PKD in animals and represent potential new therapies for PKD and in vitro studies.

13.1. Human studies

The mTOR inhibitors sirolimus and everolimus have been used in patients with ADPKD [139] [140]. The effect of mTOR inhibitors on PKD in humans was disappointing most likely related factors like dosage, side effects and penetration of mTOR inhibitors into the polycystic kidney [141]. mTOR is the major regulator of autophagy. mTOR activation inhibits autophagy, while mTOR inhibitors induce autophagy. With present technology, the effect of mTOR inhibitors on autophagy in human kidneys would be near impossible to determine.

13.2. Rodent studies

The effects of treatments on apoptosis/autophagy and PKD in *in vivo* models of PKD is shown in Table 2. There are autophagy inducers that have shown positive effects on PKD in animals and represent potential new therapies for PKD. mTOR inhibitors that induce autophagy can protect against PKD in rat and mouse models with variable effects to either increase or decrease apoptosis depending on the dose of mTOR inhibitor and PKD model [7] [69]. The traditional Chinese medicine, triptolide, which affects Ca^{2+} signaling as well as autophagy and apoptosis, protects against PKD [142] [143]. Curcumin, a hydrophobic polyphenol compound extracted from the spice turmeric, is an autophagy inducer [144]. Curcumin inhibits cystogenesis in Pkd-1 knockout mice by inhibiting signal transducers and

activator of transcription 3 (STAT3), which play a major role in the regulation of autophagy [145] [146] [147] [148] [149]. Of note, an ongoing randomized controlled trial is evaluating the efficacy of curcumin supplementation in children and young adults with ADPKD ([NCT02494141](#)). The cyclin-dependent kinase inhibitor, Roscovitine [59], a dual Src and tyrosine kinase inhibitor, and epidermal growth factor receptor (EGFR) tyrosine kinase inhibition, all known autophagy inducers, also ameliorates PKD in rodent models [150] [151]. While roscovitine decreased apoptosis in PKD, the effect on autophagy was not determined [59]. The adenosine monophosphate-activated protein kinase (AMPK) inhibitor, metformin, a known autophagy inducer, also slows cyst growth in mouse models of PKD [152] [153,154]. However, the effects of metformin on autophagic flux, mitophagy, or apoptosis in PKD were not described.

Trehalose is a natural, nonreducing disaccharide that has been shown to enhance autophagy independent of mTOR [155]. In a *Pkd1*-hypomorphic mouse model, renal mRNA expression of autophagy-related genes, including *atg5*, *atg12*, *ULK1*, *beclin1*, and *p62* are reduced and there is positive staining for the p62 protein in cystic lining cells, indicating impaired degradation by the autophagy-lysosome pathway [18]. However, trehalose treatment does not affect autophagy signaling, nor does it reduce kidney cysts or improve kidney function *in this model*, suggesting that trehalose supplementation is not a candidate to slow PKD progression.

Lifestyle interventions may also modulate autophagy in PKD. Fasting inhibits the mTOR pathway and stimulates autophagy to remove damaged molecules and organelles, including in the kidney [156]. Thirty-percent caloric restriction for two weeks prior to ischemia reperfusion injury in a rodent model improves renal function, which may be mediated by increased autophagy [157]. More recently, the effects of caloric restriction and agents targeting metabolic pathways on autophagy have been evaluated in rodent models of PKD. Mild-to-moderate caloric restriction resulted in a decrease in cleaved caspase-3, a marker of apoptosis, but autophagy was not measured [158]. Mild food restriction decreased PKD associated with no change in LC3-II, a marker of autophagosomes, but not a marker of true autophagic flux [159]. In this study, apoptosis was not determined. The caloric restriction mimetic, 2-deoxy-glucose (2DG) [160] [161] [15] also protects against PKD, however the effect of 2DG on apoptosis or autophagy was not determined in the PKD kidney.

Notably, overweight, and particularly obesity, are strong independent predictors of more rapid kidney growth, as well as kidney function decline in adults with early-stage ADPKD [162]. Of relevance to this observation, mouse models of deficient autophagic competence exhibit significantly greater weight gain in response to metabolic challenge, suggesting an intimate link between autophagy and obesity [163]. An ongoing clinical trial ([NCT03342742](#)) is evaluating the feasibility of two weight loss interventions in adults with ADPKD who are overweight or obese (daily caloric restriction and intermittent fasting); these diets may influence disease progression via weight loss and/or periods of fasting, both of which are tied to autophagy.

In summary, while many potential autophagy inducers are potential therapies for PKD, the role of autophagy induction in the protection against PKD is not known. mTOR inhibitors that protect against PKD have variable effects on apoptosis, but autophagy was not determined in mTOR inhibition studies (Table 2). The effect of potential autophagy inducers on both apoptosis and autophagy has not been systematically studied in preclinical studies of PKD.

13.3. In vitro studies

The effects of treatments on apoptosis/autophagy and PKD in *in vitro* models of PKD is shown in Table 2. Saikosaponin-d (SSd), a sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase pump (SERCA) inhibitor, may suppress proliferation in ADPKD cells by up-regulating autophagy [164]. SSd results in the accumulation of intracellular calcium, activation of the calcium/calmodulin-dependent protein kinase β (CaMKK β)-AMPK signaling cascade, inhibition of mTOR signaling, and induction of autophagy. Notably, treatment with an autophagy inhibitor (3-methyladenine), AMPK inhibitor (Compound C), CaMKK β inhibitor (STO-609), or an intracellular calcium chelator (BAPTA/AM) reduces autophagy puncta formation mediated by SSd. Thus, SERCA may represent a new autophagy target in ADPKD. Likewise, histone deacetylase inhibitors (HDACi) have therapeutic effects in *in vitro* models of ADPKD. Treatment with trichostatin A, a specific HDACi, prevents cyst formation in *Pkd1*^{-/-} cells and also stimulates autophagy, suggesting a role for autophagy in slowing cyst growth with HDACi [165]. However, in these two studies, the effects of SSd or trichostatin on apoptosis was not determined.

Studies in *Pkd1*^{-/-} zebrafish and *Pkd1*^{-/-} cell models have shown consistent relationship between apoptosis and autophagy. Evidence from a zebrafish model of PKD implicates autophagy in cystogenesis [16]. Zebrafish mutants for *pkd1a* develop cystic kidneys and mTOR activation, suggesting a conserved ADPKD model. Further assessment of the *pkd1a* mutants reveals impaired autophagic flux and increased apoptosis. Inhibition of autophagy by knocking down the core autophagy protein Atg5 promotes cystogenesis. Activation of autophagy using a specific inducer Beclin-1 peptide results in a decrease in apoptosis and ameliorates cysts in this model. Additionally, treatment with both mTOR-dependent (sirolimus) and mTOR-independent (carbamazepine and minoxidil) autophagy activators, markedly attenuate cyst formation.

There appears to be an important connection between the *Pkd1* gene, apoptosis, and autophagy. *Pkd1*^{+/+} cells deprived of glucose activate cell autophagy to survive; however, two different *Pkd1*^{-/-} cell lines fail to activate autophagy, but instead increase apoptotic rates [15]. The effect is in part dependent on mTORC1, as treatment with sirolimus partially restores autophagy and decreases apoptosis in *Pkd1*^{-/-} cells. This is the first direct evidence of a connection between the *Pkd1* gene, apoptosis, and autophagy.

Metformin also reduces cyst formation in a zebrafish model of polycystin-2 deficiency, in part via modulation of autophagy [118]. In this model, metformin inhibits pronephric cyst formation by 42–61% compared to untreated controls. Metformin also reduces the number of proliferating cells in the pronephric ducts, increases the phosphorylation of adenosine monophosphate-activated protein kinase (AMPK), and enhances autophagy in the

pronephros. In this study, the effect of autophagy induction with metformin on apoptosis was not determined.

In summary, the lack of autophagy and increased apoptosis in *Pkd1*^{-/-} zebrafish and *Pkd1*^{-/-} cells and the effect of autophagy inducers to increase autophagy and decrease apoptosis in these cells supports the hypothesis that a lack of autophagy and increased apoptosis are central features of PKD and may explain why drugs or lifestyle interventions that induce autophagy and inhibit apoptosis may have therapeutic value in PKD.

14. Autophagy inhibition in the polycystic liver

Contrary to the hypothesis that increasing autophagy will slow PKD, autophagy appears to be increased in polycystic liver disease (PLD) cholangiocytes and contribute to hepatic cystogenesis [166]. PLD cholangiocytes have increased number and size of autophagosomes, lysosomes, and autolysosomes both *in vitro* and *in vivo*, overexpress autophagy-related proteins (Atg5, Beclin1, Atg7, and LC3), and have enhanced autophagic flux. Molecular and pharmacologic interventions to inhibit autophagy with ATG7 small interfering RNA, BafA1, or hydroxychloroquine, reduce proliferation of PLD cholangiocytes and growth of hepatic cysts. Hydroxychloroquine also efficiently inhibits hepatic cystogenesis in the pck rat. In contrast, autophagy inhibition using siRNA against LC3 and the inhibitor 3-methyladenine significantly increase the cell proliferative activity of pck rat cholangiocytes treated with NVP-BEZ235, a combined PI3K, mTORC_{1/2} inhibitor [167]. *In vivo*, NVP-BEZ235 treatment attenuates cystic dilatation of the intrahepatic bile ducts, without affecting renal cyst development. Finally, knockdown of hepatocystin, a gene implicated in autosomal dominant PLD, results in an autophagy defect that can be rescued by ectopic expression of wild-type hepatocystin [168]. The pathogenesis of PLD is different from PKD [4]; for example, growth of liver compared to kidney cysts is very estrogen dependent [4], and this may influence the role of autophagy in the polycystic liver versus the polycystic kidney. However, in pursuing autophagy inducers as a potential therapeutic option to treat PKD, it will be important to also determine whether there is an adverse effect on PLD.

15. Summary

Apoptosis is present in the cells lining cysts in most rodent models of PKD and in human PKD kidneys. Inhibition of apoptosis using caspase inhibitors, knockout of apoptosis in PKD mice, as well as apoptosis induction using a SMAC-mimetic can all protect against PKD, highlighting the complex nature of the role of apoptosis in cyst growth. Increased proliferation of the cells lining the cyst is a major factor in cyst growth. Highlighting the connection between apoptosis and proliferation in PKD, both proliferation and apoptosis are hugely increased in the SBM mouse, a unique transgenic model of PKD induced by the dysregulated expression of c-myc in renal tissue. Apoptosis is likely closely related to dysregulated autophagy in PKD. *Pkd1*^{-/-} zebrafish, *Pkd1*^{-/-} cells, and some PKD mouse models demonstrate both increased apoptosis and suppressed autophagy in the kidney. Autophagy induction directly leads to decreased apoptosis and protection against PKD in zebrafish models. Autophagy induction directly leads to decreased apoptosis in *Pkd1*^{-/-} cells suggesting a direct connection between apoptosis and autophagy in PKD. However, mTOR

inhibitors, that are known autophagy inducers, have a variable effect on apoptosis depending on dose and PKD model. In conclusion, autophagy is suppressed in *in vitro* and *in vivo* PKD models and autophagy induction may have a therapeutic role in decreasing cyst growth, perhaps by decreasing apoptosis and proliferation. In the future, direct autophagy inducers should be evaluated in rodent PKD models, and the cause and effect relationship between autophagy, apoptosis and cyst growth in PKD should be tested via autophagy knockout in PKD kidneys.

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Credit Author Statement

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Abbreviations:

ADPKD	autosomal dominant polycystic kidney disease
AMPK	adenosine monophosphate-activated protein kinase
ARPKD	autosomal recessive polycystic kidney disease
BafA1	bafilomycin A1
ErbB4	Erb-b2 receptor tyrosine kinase 4
HDACi	histone deacetylase inhibitors
LC3-II	microtubule-associate protein 1A/1B-light chain 3-II
MDCK	Madin-Darby canine kidney
mTOR	mammalian target of rapamycin
Ngal	neutrophil gelatinase-associated lipocalin
PKD	polycystic kidney disease
PC-1	polycystin-1
PC-2	polycystin-2; TNF- α , tumor necrosis factor- α

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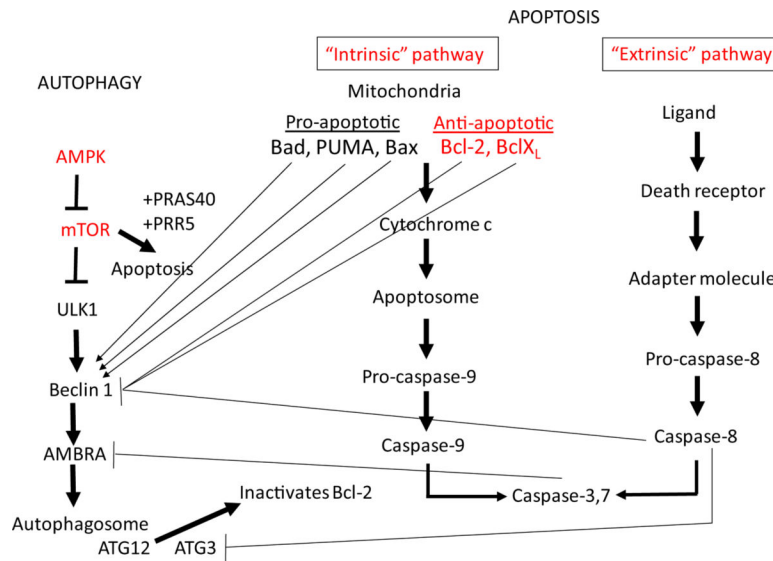


Figure 1.

Molecular pathways involved in PKD, autophagy and apoptosis. Autophagy and apoptosis are intimately related. Pathways known to be activated in PKD are shown in red. The mTOR pathway that inhibits autophagy is activated in PKD [28]. There is cross talk between mTOR pathway of autophagy and the apoptosis pathways [129]. Bcl-2 is a central regulator of autophagy and apoptosis and functions by interacting with Beclin-1 and inhibiting autophagy. The pro-apoptotic mitochondrial protein, Bad, disrupts the interaction between Bcl-2 and Beclin-1 to induce autophagy. The pro-apoptotic protein, PUMA, a p53-inducible BH3-only protein, triggers mitochondrial-specific autophagy. Activation of caspase-3 and dysregulation of the balance between pro- and anti-apoptotic Bcl-2 family members, specifically a down-regulation of anti-apoptotic Bcl-xL, has been shown in PKD [9] and Bcl-2 downregulation worsens PKD [10]. Caspases activated during apoptosis can cleave and inactivate Beclin-1. Ambra-1, a key molecule that promotes the initial steps of autophagy, is irreversibly cleaved by both calpains and caspases. During apoptosis, Atg3 is cleaved by caspase 8 and cleaved Atg3 inhibits autophagy. Autophagy is inhibited by caspase-8-mediated cleavage of Beclin-1 [174]. The intrinsic and extrinsic pathways of caspase activation in apoptosis are activated in PKD [51]. In addition to the role that apoptosis-related proteins play in modulating autophagy, many autophagic proteins can induce apoptosis. mTOR forms a complex with PRAS40 and PRR5-like proteins to induce apoptosis. Activation of AMPK is known to inhibit mTOR and induce autophagy. AMPK activation with metformin is protective in PKD [152] but the effect of metformin on autophagy and apoptosis in PKD is not known. Atg12 increases mitochondrial apoptosis by directly binding to and inactivating Bcl-2. PKD mice have a significantly lower renal mRNA expression of Atg12 and other autophagy-related genes, Atg3, beclin1 and p62, [18]. Abbreviations: B-cell lymphoma 2 (Bcl-2), B-cell lymphoma-extra large (Bcl-xL), p53 upregulated modulator of apoptosis (PUMA), proline-rich Akt substrate of 40 kDa (PRAS40), PRoline-Rich protein 5 (PRR5), activating molecule in Beclin1-regulated autophagy protein-1 (Ambra-1), 5' AMP-activated protein kinase (AMPK).

Table 1

Apoptosis of tubular cells lining the cyst is seen in most rodent models of PKD and in human PKD kidneys. Oak Ridge polycystic kidney (orpK), post natal (PN), Not applicable (NA)

Model	Inheritance	Gene	Characteristics	Ref
Male Han:SPRD rat	ADPKD	<i>Anks6</i>	Doubling kidney size and kidney failure by 8 wks of age. Apoptosis in cystic and non cystic tubules. Hypertension, anemia, ESRD	[12] [51]
pck rat	ADPKD	<i>Pkhd1</i>	Cysts in kidney and liver at 1 yr of age. Apoptosis in cystic and non cystic tubules.	[169]
C57BL6 PKD1 ^{RCRC}	ADPKD	<i>Pkd1</i>	Hypomorphic PKD1 gene knockout matching a human disease variant. Renal failure at 70 d of age. Apoptosis in cystic and non cystic tubules.	[37]
<i>Pkd1^{pl/nt}</i> mice	ADPKD	<i>Pkd1</i>	Apoptosis induced in cystic epithelium by smac-mimetic	[90]
PKD2 ^{WS25} - mice	ADPKD	<i>Pkd2</i>	PKD and renal failure at 16 wks of age. Apoptosis in cystic epithelium and caspase-3 activity not affected by sirolimus.	[33]
orpK mouse	ARPKD	<i>Ift88^{1g737Rpw}</i>	Ciliary defect model of ARPKD. Sirolimus increases apoptosis in cystic epithelial cells.	[8]
C57BL6 jck mouse	ARPKD	<i>Nphp9</i>	Mutated gene product in primary cilia affects normal expression of PC1/2. High rate of epithelial cell proliferation and apoptosis in cystic epithelium	[170]
cpk mouse	ARPKD	<i>Cys1</i>	Lifespan 4–6 weeks. Apoptosis of tubular and interstitial cells. Deletion of caspase-3 gene increases lifespan.	[14]
pcy mouse	ADPKD	<i>Nphp3</i>	Orthologous to adolescent nephronophthisis. Apoptotic DNA fragmentation evident in preuremic kidneys.	[27]
SBM mice	ADPKD	<i>c-myc</i>	Over expression of c-myc. Tubular apoptosis increased 3–9 -fold over controls.	[95] [104] [96]
AP-2 $\beta^{-/-}$ knockout mice	NA	<i>AP-2 β</i>	Mice lack transcription factor AP-2 β die at PN day 1–2 from PKD. Enhanced apoptotic cell death of renal epithelial cells.	[171]
Bcl-2 ^{-/-} mice	NA	<i>Bcl-2</i>	Knockout of anti-apoptotic Bcl-2. Fulminant lymphoid apoptosis, polycystic kidneys, and hypopigmented hair. Apoptosis in kidney interstitium.	[50]
Dysplastic human renal disease	NA	NA	Cell death prominent in undifferentiated cells around dysplastic tubules and occasionally cystic epithelia	[172]
Human kidneys	ADPKD	<i>Pkd1/2</i>	Apoptotic cells in glomeruli, cyst walls, cystic and noncystic tubules regardless of renal function	[27]
Human kidneys	ARPKD	<i>Pkhd1</i>	Apoptosis more prevalent in human ARPKD kidneys than in age-matched normal kidneys	[91] [173]

Table 2

Effects of treatments on apoptosis/autophagy and PKD.

Treatment	In vivo model	Effect on apoptosis	Effect on autophagy	Effect on PKD	Ref
Cell cycle inhibitor, roscovitine	jck, cpk mice	Decreased	ND	Long-lasting arrest of cystogenesis	[58] [59].
Hydroxyestradiol (2-OHE)	Han: SPRD rat	Decreased	ND	Decreased cyst growth	[61].
GlcCer synthase inhibitor, Genz-123346	jck and pcy mice	Decreased	ND.	Decreased cyst growth	[62]
Immunodepletion of CD8 + T cells	C57BL/6 Pkd1 ^{RC/RC} mice	Decreased	ND	Worsening	[63]
Caspase inhibitor, IDN-8050	Han:SPRD rat	Decreased apoptosis and proliferation	ND	Decreased cyst growth	[12]
MIF inhibitor ISO-1	Pkd1 ^{-/-} mice	Increased	ND	Delays cyst growth	[67]
High dose sirolimus	Pkd1 ^{-/-} , orpk mice	Increased	ND	Decreased cyst growth	[8] [69] [70]
Low dose sirolimus	Pkd2 ^{WS25/-} mice	No effect	ND	Decreased cyst growth	[33]
mTOR kinase inhibitor, torin-2	Pkd1 ^{RC/RC} mice	Increased	ND	Decreased cyst growth	[37]
mTOR kinase inhibitor, PP242	Han:SPRD rat	No effect	ND	Decreased cyst growth	[71]
mTOR ASO	Pkd2 ^{WS25/-} mice	Decreased	ND	Decreased cyst growth	[36].
Smae-mimetic	Pkd1 ^{-/-} mice	Increased TNF- α -dependent apoptosis	ND	Delays cyst growth	[90]
Bafilomycin	cpk mice	ND	Decreased	ND	[17]
Autophagy inducer: Trehalose	Hypomorphic Pkd1 ^{-/-} mice	ND	No effect on suppressed autophagy	No effect	[18]
Caloric restriction mimetic, 2DG	Pkd1 ^{-/-} mice	ND	ND	Delays cyst growth	[160] [161]
Saikosapomin-d (SSd), a SERCA inhibitor	UCL93, 0X161 ADPKD cells, MDCK cells	ND	Increased	Decreased	[164]
Glucose deprivation	Pkd1 ^{-/-} cells	Increased	Decreased. Rescued by sirolimus	ND	[15]
Autophagy activators: Beclin-1, sirolimus, carbamazepine and minoxidil	Zebrafish pkd1a mutants	Decreased	Increased	Decreased cysts	[16]
Metformin	Zebrafish pkd2 mutants	ND	Increased	Decreased cysts	[118]
HDAci, Trichostatin A	Pkd1 ^{-/-} cells	ND	Increased	Prevents cyst formation	[165]

Not determined (ND), glucosylceramine (GlcCer), antisense oligonucleotide (ASO), macrophage migration inhibitory factor (MIF-1), 2-deoxy-glucose (2DG), second mitochondria-derived activator of caspase (Smac), Madin-Darby canine kidney cells (MDCK), Sarcoplasmic/endoplasmic reticulum Ca²⁺ + ATPase pump (SERCA).