Chapter 9

Apoptosis in Polycystic Kidney Disease: From Pathogenesis to Treatment

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Abstract

Apoptosis plays an important role in many developmental processes and contributes to cell and tissue homeostasis. Induction of apoptosis can involve the "intrinsic pathway", which is activated by diverse stress signals, and the "extrinsic pathway", which is activated by proapoptotic receptor signals at the cell surface. Excessive or aberrant apoptosis is a crucial factor in many human disorders, including polycystic kidney disease (PKD). Renal cyst formation is caused by dysregulation of cell proliferation, involving diverse and poorly understood molecular mechanisms. Elevated apoptosis of tubular epithelial cells has been

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described in autosomal dominant PKD (ADPKD) and autosomal recessive PKD (ARPKD), as well as in animal models of PKD. It has been suggested that the dysregulation of apoptosis contributes to cystogenesis of PKD and is associated with the progressive loss of normal nephrons. Inhibition of apoptosis has been shown to delay renal cyst growth in some animal models of PKD. However, increased apoptosis is not a feature in cystic kidneys from Pkd1 mutant mice and inducing apoptosis of the cystic epithelial cells by activation of intrinsic or extrinsic signaling pathways has been shown to slow disease progression with or without inhibition of proliferation. In this chapter, we discuss the positive and negative roles of apoptosis in PKD and the associated molecular mechanisms in regulating cystic renal epithelial cell apoptosis during cyst development.

Key words: Apoptosis; Caspases; Cystogenesis; Mitochondrial pathway; Polycystic kidney disease; Proliferation

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common genetic kidney disease with the prevalence between 1:400 and 1:1000 worldwide and is characterized by the progressive development of renal cysts that replace normal kidney tissue, resulting in kidney expansion and the decline in renal function, often requiring dialysis or kidney transplantation (1). ADPKD is caused by the mutations in PKD1 (85% of cases) or PKD2 (15% of cases), which encode polycystin 1 (PC1) and polycystin 2 (PC2), respectively. PC1 and PC2 are large transmembrane proteins localized at primary cilia and plasma membranes, and regulate multiple intracellular signaling pathways (2).

Autosomal recessive polycystic kidney disease (ARPKD) is less common than ADPKD with a prevalence of 1:20,000 live births. ARPKD is caused by mutations in the PKHD1 gene, which encodes fibrocystin, a large type I transmembrane protein localized to the apical membrane, the primary cilium/basal body and the mitotic spindle (3). Abnormalities in the renal epithelial cells, including dedifferentiation, dysregulated cell proliferation, abnormal fluid secretion, abnormalities of cell-matrix interactions, loss of planar cell polarity and abnormal ciliary function have been found to contribute to cystogenesis in genetic forms of PKD. Currently, the role of apoptosis in normal kidney development (4) and cyst growth (5) remain elusive. This chapter summarizes the current knowledge on the roles and mechanism of apoptosis in PKD and how components of apoptotic signaling can be used as therapeutic target in PKD.

Apoptosis

Apoptosis is a term that originates from the Greek word for "dropping off" and refers to the Autumnal falling of leaves from trees as described by Kerr et al. in 1972 (6). It is an orchestrated event in which cells are programmed to die upon receiving certain physiological and pathological stimuli (6), whereas not all cells will necessarily die in response to the same stimulus. As one of the most investigated processes in biological research, apoptosis occurs normally to maintain tissue homeostasis or as a defense when cells are damaged by disease or noxious agents (7). Abnormalities in apoptosis can be one of the significant features of diseases, including the insufficient apoptosis in cancers and excessive apoptosis in autoimmune and neurodegenerative diseases, and ischemia-associated injury (8).

Morphological and biochemical changes in apoptotic cells

Apoptosis is accomplished by a series of energy-requiring biochemical events which lead to the characterized morphologic changes. The classic morphological hallmarks of apoptosis during the early process, that is distinct from necrosis, include chromatin condensation, nuclear fragmentation, cellular volume reduction (pyknosis), and all of these morphological changes are enclosed within an intact plasma membrane. The morphological features of apoptosis during the late process include blebbing of plasma membrane, ultrastructural modification of cytoplasmic organelles including the swelling of mitochondria and loss of membrane integrity before the phagocytosis (8). During apoptosis, a process called "budding" occurs whereby extensive plasma membrane blebbing and separation of cell fragments into apoptotic bodies occur. The apoptotic bodies consist of cytoplasm with tightly packed organelles with or without nuclear fragments. They are subsequently phagocytosed by macrophages, parenchymal cells, or neoplastic cells and degraded within phagolysosomes (9-12).

The characterized biochemical events of apoptosis include cleavage and activation of caspases, breakdown of DNA and proteins, and modification of the membrane for phagocytic cell recognition (13). Caspases are a group of enzymes belonging to the cysteine protease family, and are widely expressed in an inactive proenzyme form in most cells. The "c" of "caspase" refers to a cysteine protease, while the "aspase" refers to the enzyme's unique property cleave proteins at aspartic acid residues. Caspases have been broadly categorized into apoptotic caspases, including initiators (caspase-2,-8,-9,-10) and executioners (caspase-3,-6,-7) (14, 15), and inflammatory caspases (caspase -1,-4,-5,-11,-12) (14, 15). Once caspases are initially activated, initiator caspases cleave inactive forms of executing caspases, thereby activating them. Executing caspases cleave other vital cellular

proteins, break up the nuclear scaffold and cytoskeleton, and activate DNAase to degrade nuclear DNA, which triggers the morphological characteristics of the apoptotic process (16). Another feature of apoptosis is the "flip out" of phosphatidylserine from the inner layers to the outer layers of the cell membrane due to the loss of ATP. This allows the early recognition of dead cells by macrophages, resulting in phagocytosis without the release of pro-inflammatory cellular components (17). Apoptosis can occur without oligonucleosomal DNA fragmentation and can be caspase-independent (18), therefore we cannot always define apoptosis by using biochemical analyses of DNA fragmentation or caspase activation.

Methodologies and assays for detecting apoptosis

Apoptosis can be detected by multiple approaches since it occurs via a complex signaling cascade and has many specific features. Apoptotic assays can be classified into six groups (8), including: 1) cytomorphological alterations detected by light microscopy and electron microscopy, which is the gold standard for apoptosis identification; 2) DNA fragmentation detected by staining with a cell-permeable, DNA-binding fluorochrome in the terminal transferase-mediated dUTP nick end-labeling (TUNEL) assay (19); 3) detection of caspases, cleaved substrates, regulators and inhibitors such as cleaved caspase 3, and cleaved PARP by Western immunoblots or immunohistochemistry (20, 21); 4) membrane alterations detected by staining with FITC-labeled Annexin V that recognizes the phosphatidylserine (22); 5) detection of apoptosis in whole mounts by using dyes such as acridine orange (AO), Nile blue sulfate (NBS), and neutral red (NR) (23); and 6) mitochondrial assays to detect the cytochrome c release (24), the apoptotic or anti-apoptotic regulator proteins such as Bax, Bid and Bcl-2 (25), the mitochondrial membrane potential, calcium fluxes and mitochondrial redox status (26). Each assay has its advantages and disadvantages (27, 28). In addition, because many features of apoptosis and necrosis overlap, it is necessary to determine that apoptosis has occurred in cells, tissues or organs using two or more distinct assays based on the understanding of the principles of each methodology.

Mechanisms of apoptosis

Understanding the mechanisms of apoptosis helps in the development of drugs that target certain apoptotic genes or pathways. There are two commonly-described apoptotic pathways: the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway (Figure 1). Different caspases are activated in each pathway and molecules in one pathway can influence the other (29). There are two less well-known pathways, including the pathway that involves T-cell mediated cytotoxicity (30) and the intrinsic endoplasmic reticulum (ER) pathway (31), which will not be discussed in this chapter.

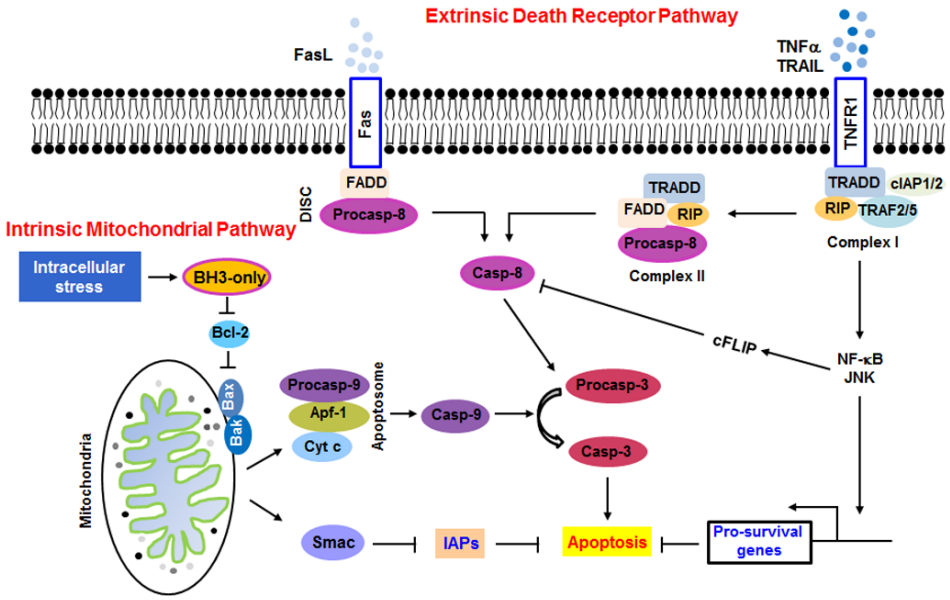

Figure 1. The intrinsic and extrinsic pathways of apoptosis. The two major apoptotic pathways are illustrated. The extrinsic death receptor pathway is activated by death receptor ligands, including FasL, TNF-α or TRAIL, etc. The binding of FasL to Fas initiates the recruitment of FADD and caspase-8 to form the DISC complex, which in turn activates caspase-8 and downstream executing caspases. The binding of TNF-α to TNFR1 initiates the recruitment of TRADD, RIP, TRAF2/5 and cIAP1/2 to form complex I, which activates NF-κB and JNK pathways and increases the transcription of pro-survival genes. However, the modification of RIP or degradation of cIAP1/2 can lead to the disassociation of complex I. TRADD and RIP then associate with FADD and caspase-8 to form complex II, the so-called death complex. The intrinsic death receptor pathway is initiated by BH3-only protein under intracellular stress; BH3-only protein can inactivate Bcl-2 and prevent Bcl-2 from effectively neutralizing Bax and Bak, leading to activation of Bax and Bak. The activated Bax and Bak on the mitochondrial membrane result in the release of cytochrome c and Smac from mitochondria. Cytoplasmic cytochrome c associates with Apaf-1 and caspase-9 to form the apoptosome, which activates caspase-9 and downstream executing caspases. Smac can regulate apoptosis by inhibiting the inhibitor of apoptosis proteins (IAPs).

The extrinsic death receptor pathway

The extrinsic pathway is initiated by the binding of ligands to the transmembrane death receptors. Several death ligands and corresponding death receptors have been described, including Fas ligand and Fas receptor (FasL/FasR), tumor necrosis factor (TNF) and its

receptor 1 (TNF-α/TNFR1), Apo3L/DR3, Apo2L/DR4 and Apol2L/DR5 (8). As death receptors, members of TNF receptor superfamily share similar cysteine-rich extracellular domains and a cytoplasmic death domain (32). This death domain plays a critical role in transmitting the extracellular death signaling to the intracellular apoptotic machinery to elicit cell death through recruiting adaptor proteins, such as Fas-associated death domain (FADD) and TNF receptor associated death domain (TRADD). To date, the extrinsic pathways of apoptosis mediated by FasL/FasR and TNF-α/TNFR1 are the best-characterized. The binding of FasL to its FasR results in the recruitment of the adaptor protein FADD (33). FADD then associates with procaspase-8 to form the death-inducing signaling complex (DISC), which results in the activation of procaspase-8 (33, 34). Activated caspase-8 then triggers the execution phase of apoptosis including the activation of caspase 3 and downstream pathways. In contrast, the binding of TNF to TNFR results in the transient recruitment of TRADD, TNF receptor-associated factor 2 (TRAF2), TRAF5, cellular inhibitor of apoptosis 1 and 2 (cIAP1/2) and receptor interacting protein 1 (RIP1) to form pro-survival complex I (35, 36). Complex I can activate nuclear factor κB (NF-κB) and JNK pathways to regulate the expression of pro-survival genes, including the cellular FLICE-like inhibitory protein (cFLIP) (36, 37). However, TRADD and RIP1 can be disassociated from complex I once RIP1 is deubiquitinated under certain circumstances. RIP1 then associates with FADD and caspase-8 to form complex II, the so-called death complex, to trigger cell death (36). Death receptor mediated apoptosis can be inhibited by affecting the recruitment of caspase-8 to the DISC. cFLIP competitively displaces caspase-8 from the DISC due to the structure similarities with caspase-8 (38, 39). The cIAP1/2 also inhibits caspase-8 activation via suppressing TNF-α apoptotic signaling and inducing pro-survival signaling, such as the NF-κB pathway (40).

The intrinsic mitochondrial pathway

The intrinsic pathway is initiated within the cell and involves the intracellular organelles mitochondria (41). The intrinsic pathway is regulated by the members of Bcl-2 family, which has been divided into three groups with different functions, including 1) antiapoptotic proteins (Bcl-2, Bcl-xL, etc.), 2) proapoptotic proteins (BAX, BAK, etc.) and 3) regulatory BH3-only proteins (BAD, BIK, BIM, etc.) (42, 43). The proapoptotic proteins are inhibited by the antiapoptotic proteins, while the BH3-only proteins can counteract the antiapoptotic proteins and thereby release the proapoptotic proteins to trigger the intrinsic apoptotic pathway upon the cytotoxic stimuli (42, 43). Upon the internal stimuli such as hypoxia, DNA damage and toxins, two proapoptotic BCL2 proteins, BAX and BAK, are activated by BH3-only proteins (44). Activated BAX and BAK oligomerize on the mitochondria and introduce pores into the mitochondrial surface, which results in the release of cytochrome c (45). Cytoplasmic cytochrome c associates with the caspase adaptor

molecule Apaf-1 and procaspase-9 to form the apoptosome, in which procaspase-9 is activated (45). The activation of caspase-9 then triggers the execution phase of apoptosis. The IAPs are also the negative regulators of the intrinsic apoptosis pathway by several mechanisms (46). In addition to cytochrome c, the BAX/BAK proteins can also induce the release of the second mitochondrial-derived activator of caspase (Smac)/direct IAP binding protein with low pl (DIABLO) from mitochondria, which disrupts the interaction of IAPs with caspase-3/9, and then promotes caspase activation (47, 48).

Apoptosis in polycystic kidney disease

Apoptosis was first described as a pathological feature of PKD by Woo in 1995 (49), in that apoptotic DNA fragmentation and nuclei were detected in cystic renal cells and tissues from human ADPKD and ARPKD, the congenital polycystic kidney (cpk) mouse model of ARPKD, and the pcy mouse model that is orthologous to adolescent nephronophthisis (49). Abnormal apoptosis observed in cyst lining epithelial cells and noncystic tubular epithelial cells, as well as in cells within glomeruli, has been suggested to contribute to cyst extension in human PKD (49, 50). This hypothesis has been supported by different studies, including those in ARPKD animal models and in 3D cultures with Pkd1 overexpression or knockdown (51, 52). Whether apoptosis is changed in Pkd1 and Pkd2 knockout animal models is uncertain. Recent studies have indicated that induction of cyst lining epithelial cell apoptosis may delay renal cyst growth in Pkd1 knockout mouse models (53-58). These studies suggest that the role of apoptosis in promotion or retardation of cyst growth in PKD could be disparate, depending on confounding factors such as animals compared to humans, early compared to late disease, tubules and interstitial cells compared to cyst-lining epithelial cells and different primary end-points to measure the levels of apoptosis (53). The nature of the mutation responsible for renal cystic disease may be relevant as well.

Apoptosis and apoptotic pathways in animal models resembling ARPKD

The cpk mouse develops renal cysts via the disruption of a cilia-associated protein, cystin (59). In kidneys from cpk mice, widespread apoptosis is detected in the interstitium, while few apoptotic cells are found in cystic epithelia or noncystic tubules (59). Since the activity of caspase-3 was increased in cpk mice (59) and knockout of caspase-3 in these mice delayed cyst growth and prolonged survival compared to the control mice (mean survival of 117 days versus 32 days, $P < 0.01$), it seemed that caspase-3 mediated apoptotic signaling might somehow promote cyst growth in cpk mice. However, the findings that apoptotic index, the expression level of Bcl2, BAX and caspase-7 showed no difference in cpk mice

with or without knockout of caspase-3 (51), contradicting this conclusion. In addition, another study found that inhibition of the Pax2 gene, which is essential for the differentiation and proliferation of the renal epithelium, inhibited renal cyst growth in Pax2 heterozygous cpk mice due to increased apoptosis but not reduced proliferation of cystic epithelium (60, 61). These results suggested that apoptosis might have more complicated roles in cpk mice than its proposed role in promoting cyst growth.

Juvenile cystic kidneys (jck) mice, which have a missense mutation in the Nek8 gene encoding serine/threonine kinase, are fertile and generally survive to four or more months of age. In kidneys from jck mice, apoptotic nuclei were very common in cystic epithelia, but few were seen in normal tubular epithelial cells (62). Therefore, jck cyst enlargement was accompanied by a high rate of cystic renal epithelial cell proliferation and increased apoptosis. Apoptosis in jck mice may be mediated by the pro-apoptotic protein Apaf1 and caspase-2 as well as Bcl-2 and Bcl-xL (63).

The PCK rat, an orthologous model for human ARPKD, has many features that resemble human ADPKD, such as focal development of cysts, although the pattern of inheritance is autosomal recessive. In the PCK rat, apoptotic cells are commonly found in the normal tubules and dilated tubules, but are less observed in cysts lined by flat epithelium (64). Apoptosis in the PCK rat may be mediated by p38 mitogen activated protein kinase (MAPK) signaling and caspase-7 but not caspase-3 (65).

Another example of a recessive model in which apoptosis was shown to contribute to cyst formation is the knockout mice for AP-2β (a transcription factor), which develop numerous, small cysts in the distal tubule and collecting duct. In the AP-2β knockout mice, embryonic development was completed but the mice died at postnatal day 1 or day 2 due to the PKD. In this case, there was increased apoptosis in collecting duct and distal tubular epithelia (66). The expression of antiapoptotic proteins Bcl-X(L), Bcl-w and Bcl-2 in AP-2β knockout mice was downregulated at the end of embryonic development, which suggest the apoptosis induced by loss of AP-2β may be dependent on the antiapoptotic Bcl-2 protein (66).

Bcl-2 as an antiapoptotic protein inhibits the activity of proapoptotic proteins BAX and BAK. Bcl-2-deficient mice also develop polycystic kidneys accompanied by abnormal apoptosis of the kidney tubular epithelium and interstitium (67-69). Bcl-2 knockout mice reassemble oligomeganephronic hypoplasia (70), which presents hypoplastic kidneys in the embryonic stage due to a reduced number of nephrons mediated by excessive apoptosis. In Bcl-2 knockout mice, apoptosis appears to be mediated primarily by the unopposed proapoptotic activity of the BH3-only protein Bim as deletion of a single allele

of Bim is sufficient to abolish PKD in this model (71). This suggests that apoptosis is the essential driver of cystogenesis in Bcl-2 knock out mice (71).

In summary, abnormal apoptosis was detected in experimental ARPKD animal models, including cpk mice (59), jck mice (62), PCK rats (64), AP-2β $^{-/-}$ mice (66), and Bcl-2 knockout mice (67). The mechanisms of apoptosis in these animal models are regulated through intrinsic Bcl-2 family member-mediated pathways. The involvement of extrinsic pathways in these animal models has not been reported thus far.

Apoptosis and apoptotic pathways in animal models resembling ADPKD

Increased apoptotic cells are also shown in Han:SPRD rats and c-Myc transgenic SBM mice that resemble ADPKD. The heterozygotes of Han:SPRD male rats (Cy/+) develop renal cysts and renal failure over several months, while homozygous (Cy/Cy) animals die in a few weeks post-birth due to the rapidly progressive renal enlargement (72). The TUNEL-positive apoptotic cells were increased in two week old heterozygous (Cy/+) and homozygous (Cy/Cy) rat kidneys compared to normal littermate controls and more than half of the apoptotic cells were from cystic tubules in kidneys of Han:SPRD rats (73). The activity of caspase-3, caspase-7 and caspase-8 is increased in the kidneys of homozygous Han:SPRD rats (Cy/Cy) compared with wild type rats (+/+), while it shows no difference in the kidneys from heterozygous Han-SPRD rats (Cy/+) and wild type rats (+/+) (73, 74). However, administration of the pan-caspase inhibitor IDN-8050 could slow disease progression by reducing tubular proliferation and apoptosis in the heterozygous Han-SPRD rats (Cy/+) (75).

c-Myc, an oncogene involved in cell proliferation, apoptosis, differentiation and neoplasia, has been found to be upregulated in human ADPKD and PKD animal models, including the Han:SPRD rat (Cy/Cy) (72, 76-79). The c-Myc transgenic SBM mouse developed polycystic kidney disease as displayed by elevation of proliferation index (10-fold) and apoptotic index (10 to 100-fold) in kidneys compared with nontransgenic controls (80-82). In c-Myc transgenic mice, apoptosis was induced by c-Myc-mediated activation of the pro-apoptotic protein Bax, leading to the release of cytochrome c from the mitochondria to the cytosol (83). However, c-Myc induced-apoptosis in polycystic kidney disease is Bcl-2 independent as overexpression of both Bcl-2 and c-Myc in vivo produce a similar PKD phenotype with a high apoptotic index compared to overexpression of c-Myc only (81). Although the expression of FasL was elevated in the c-Myc transgenic mouse kidneys, mutation of FasL in these mice was incapable of affecting apoptosis, which suggests that c-Myc-induced apoptosis in PKD is independent of FasL/Fas signaling (84). Because c-Myc has been reported to be upregulated in cystic epithelia from Han:SPRD rats (72), the

involvement of c-Myc signaling in regulating apoptosis in these rats needs to be investigated further.

Apoptosis in Pkd1 or Pkd2 mutant animal models

It is controversial as to whether abnormal apoptosis occurs in Pkd1 or Pkd2 mutant mouse models. In $Pkd1^{-/-}/LZ^{+}$ chimeric mice generated by aggregation of $Pkd1^{-/-}$ ES cells and $Pkd1^{+/+}$ morulae from ROSA26 mice, the cyst epithelia of the kidney were composed of both $Pkd1^{-/-}$ and $Pkd1^{+/+}$ ($Pkd1^{-/-}/LZ^{+}$) renal tubular epithelial cells in the early stages of cystogenesis. During cyst development, $Pkd1^{-/-}$ cyst epithelial cells became dominant due to increased proliferation, which gradually replaced $Pkd1^{+/+}$ ($Pkd1^{-/-}/LZ^{+}$) cyst epithelial cells due to JNK-mediated apoptosis. It is important to note that, in this animal model, apoptosis was observed mostly in $Pkd1^{+/+}$ ($Pkd1^{-/-}/LZ^{+}$) cyst epithelial cells but less in $Pkd1^{-/-}$ cyst epithelial cells (85) and was decreased when cyst enlarged, which suggested that if apoptosis contributed to cystogenesis it should be effect at early stage of cyst development.

However, in $Pkd1^{flox/flox}$:Tamoxifen-Cre mice, induction the deletion of Pkd1 before postnatal day 13 results in severely cystic kidneys within 3 weeks, while deletion of Pkd1 after postnatal day 13 results in cysts only after 5 months. However, only a small number of apoptotic cells in the medulla and cortex were observed in cystic kidneys from $Pkd1^{flox/flox}$:Tamoxifen-Cre mice but showed no difference in the kidneys from developing stage to adult stage, which suggests that apoptosis may not be the primary factor of cystogenesis (86).

Furthermore, recent studies indicated that, although deletion of *Pkd1* results in an incremental increase in cell proliferation in the *Pkd1* conditional knockout $Pkd1^{flox/-}$:Ksp-Cre mice, $Pkd1^{flox/-}$Pkhd1-Cre mice and Pkd1 hypomorphic $Pkd1^{nl/nl}$ mice, increased apoptosis was not a feature in kidneys from these mice as the TUNEL-positive nuclei are negligible and had no difference between cystic and non-cystic kidneys (53, 54, 57, 87). This provided further evidence that apoptosis is not the primary factor of cystogenesis at least in Pkd1 knockout animals.

The role of apoptosis in cyst formation in Pkd2 mutant mice remains unclear because of conflicting reports. Increased apoptosis was observed in Pkd2 transgenic mice and in Pkd2 knockout renal cells isolated from Pkd2 conditional knockout mice (88, 89). Calcium influx mediated by transient receptor potential (TRP) channels in the plasma membrane triggers the cell death (90). PC2, also known as TRPP2, is enriched in the endoplasmic reticulum (ER) membrane. ER-resident PC2 reduces the Ca2+ release from ER and then decreases

cytosolic and mitochondrial Ca^{2+} signals, which results in the protection from apoptotic stimuli (91). However, an opposite apoptotic phenomenon was reported in $Pkd2^{WS25}$ mice, in that Stroope et al. found that the apoptotic indices were increased in kidneys of $Pkd2^{WS25}$ mice compared with those in wild type mice (92), while Wei et al. found that apoptosis was not increased in these mice compared to the controls (54). In summary, increased apoptotic cells are shown in Han:SPRD rats and c-Myc transgenic SBM mice that resemble ADPKD. However, recent studies suggest that apoptosis may not be the predominant factor of cyst expansion in ADPKD.

Apoptotic pathways in ADPKD

As we described above, the extrinsic pathway-mediated apoptosis involves the death ligand, death receptors, initiator caspase-8 and the common downstream executioner caspase-3 and caspase-7. Caspase-3 and caspase-8 are activated in the small cysts and normal-appearing tubules but are not seen in the larger cysts in human ADPKD (93), which implies the involvement of these caspases in regulating apoptosis in the small cysts and normal-appearing tubules in human ADPKD. The death ligand TNF-α and death receptor TNFR1 are upregulated in the Pkd1 mutant cystic renal epithelial cells, however, the role of TNF-α/TNFR1 signaling is to inhibit apoptosis in these cells via association with the upregulated cIAP1, RIP1 and TRADD to form the pro-survival complex I (53, 94), leading to activation of NF-κB and the upregulation of c-FLIP to inhibit caspase-8. Thus, the involvement of the extrinsic pathway in mediating apoptosis associated with human ADPKD needs be investigated further.

The intrinsic pathway-mediated apoptosis involves caspase-9, cytochrome c and the Bcl-2 family proteins. Abnormal apoptosis in the kidney from homozygous Han-SPRD rats (Cy/Cy) may be mediated by decreased expression of anti-apoptotic Bcl-XL and Bcl-2 compared with wild type rats, leading to the release of procaspase-9 and cytochrome c into the cytosol (73). Increased apoptosis in c-Myc transgenic SBM mice (81) may also be mediated via Bcl-2 family proteins. However, studies by Hughes et al. demonstrated that loss of Pkd1 and loss of Bcl-2 elicit cyst formation through distinct mechanisms (95). They found that ablation of one or both alleles of the pro-apoptotic gene Bim prevented cyst formation in mice deficient for Bcl-2 while loss of Bim had no effect on cyst development in Pkd1 homozygous mutant mice. Nor did loss of Bcl-2 alleles significantly influence the Pkd1 mutant phenotype (95). These studies suggest that Bcl-2 mediated apoptosis is not involved in cystogenesis in mice with Pkd1 deficiency.

In addition to Bcl-2, the changes of the other members of the IAP family protein, cFLIP, cIAPs and survivin, have been observed in ADPKD. cFLIP inhibits the activation of

caspase-8 due to its structure similarity. cFLIP can only be detected in large cysts, whereas caspase-8 can be detected in small cysts and normal appearing tubules in end stage human ADPKD kidneys, which may explain why apoptosis can be observed in small cysts and normal-appearing tubules but not in large cysts (5). The expression of cFLIP is upregulated in Pkd1 mutant cystic renal epithelial cells and mouse kidney tissue, and can be induced by TNF-α, which constantly exists in cyst fluid (53, 94). The upregulated cFLIP in turn inhibits the TNF-α/TNFR1 mediated extrinsic apoptotic pathway in Pkd1 knockout mouse kidneys (53). In addition to cFLIP, cIAP1 is also upregulated in Pkd1 mutant cystic renal epithelial cells and kidney tissues, and is induced by TNF-α (53). The upregulated cIAP1 associates with TNF-α/TNFR1, TRADD, TRAF2 to form the pro-survival complex I to activate NF-κB signaling, resulting in the survival of cystic renal epithelial cell (53). The TNF-α/TNFR1-mediated extrinsic apoptotic pathway can only be triggered until cIAP1 is degraded by the pro-apoptotic protein Smac or Smac-mimetics (53). Survivin, as another IAP, is undetectable in normal adult tissue but is expressed in cancer, such as renal cancers (96). Survivin inhibits the intrinsic apoptotic pathway via inhibition of Smac (97), and interaction with caspase-9 (96). Survivin is increased in cystic kidneys from Han-SPRD rats with increased caspase-9 and apoptosis (98). Thus, it is unlikely that survivin could inhibit the intrinsic apoptotic pathway in Han-SPRD rats. In contrast, AbouAlaiwi et al. reported that the expression of survivin was decreased in human ADPKD kidneys and kidneys from PKD mice (99, 100). In mouse and zebrafish models, deletion of survivin resulted in the cystic phenotype, which may be due to the abnormal oriented cell division since survivin plays an important role in regulating ploidy, in addition to regulating apoptosis (100).

The role of apoptosis in ADPKD

Apoptosis may act as a double-edged sword in human ADPKD. Apoptotic loss of renal tissue may be responsible for the progressive deterioration of renal function (49). Apoptosis detected in the normal-appearing, non-cystic tubules may result in the progressive loss of normal nephrons in PKD, which may be due to the direct compression from adjacent expanding cysts, apoptotic stimuli secreted by cystic epithelia and inflammatory cells. On the other hand, increased apoptosis in ADPKD may counteract hyperproliferation, which may prevent kidneys from progression of PKD into the renal cell carcinoma despite the high rate of epithelial cell proliferation. Recent retrospective study showed that cancer incidence was lower in PKD renal transplant recipients than in non-PKD renal transplant recipients (101). The exact mechanisms of the low cancer risk in PKD recipients are unclear, but may be associated with the increased apoptosis.

Cell model systems for the investigation of apoptosis and cyst formation

Early studies to delineate the role of apoptosis on cystogenesis involved Madin Darby canine kidney (MDCK) cell cultures. Apoptosis was proposed to be important for lumenization and other morphogenetic processes in kidney development and this prediction was supported by the following studies. The cyst cavitation of MDCK cells in a collagen-type I matrix resulted from apoptosis, and mimiced the proapoptotic state during renal development (102). It has been reported that MDCK cells overexpressing PC1 are resistant to apoptosis induced by serum starvation and are decreased in cell growth, which results in the formation of the branching tubule but not the simple cysts as in control cells (52). Studies also suggest that PC1 was able to inhibit apoptosis, in that: 1) MDCK cells overexpressing PC1 were resistant to Gα12 stimulated apoptosis, which is through the JNK activation and Bcl-2 degradation (103, 104); 2) knockdown of nephrocystin-1 increased apoptosis in PC1-overexpressed MDCK cells stimulated with TNF-α, which suggested that the interaction of PC1 with nephrocystin-1 was required for overexpression of PC1-mediated resistance to apoptosis in these cells (105); and 3) in 3D cell culture, apoptosis measured by staining for cleaved caspase-3 was detected in cyst lining cells in the large and spherical cysts formed by $Pkd1^{-/-}$ cells, apoptosis was not detected in the extended, tubule-like structures formed by control $Pkd1^{flox/-}$ cells, and reintroducing PC1 c-terminal tail (CTT) can suppress apoptosis in this system (106). In addition, Lin et al. reported that overexpression of the anti-apoptotic protein Bcl-2, which is essential for survival of renal stem cells during nephrogenesis, inhibited the cystogenesis in the MDCK system by inhibiting apoptosis (107). These studies, together with the findings that abnormally increased apoptosis was observed in animal models resembling ADPKD and ARPKD, suggest that apoptosis may be causally linked to the development of renal cystic disease. However, recent studies that apoptosis is negligible in kidneys from Pkd1 mutant mice challenges the role of apoptosis in Pkd1 knockout-mediated cyst development.

The relationship of apoptosis and cystic renal epithelial cell proliferation in PKD

PKD has been labeled "neoplasia in disguise" since the aberrant cell cycle progression and hyperproliferation are similar to cancer development (108,109). Hence, if hyperproliferation is a predominant factor that contributes to cystogenesis, it is important to understand the relationship of proliferation and apoptosis in the pathogenesis of ADPKD. Positive and negative correlations between apoptosis and proliferation have been reported in other diseases. For example, increased apoptosis and proliferation have been identified in dextran sulfate sodium-induced colitis (110) and neoplastic transformation of the colon (111) as well as in the progression of prostatic intraepithelial neoplasia (PIN) (112). In comparison, decreased apoptosis has been reported during progression from

adenoma to carcinoma in the colon (111) as well as from intraepithelial neoplasia to carcinoma in the human prostate (113), suggesting apoptosis may oppose proliferation and may be an important determinant of net tissue growth. The upregulation of c-Myc signaling in human ADPKD and PKD animal models (72, 76-79) may act as a model to explain the positive relationship between apoptosis and proliferation in PKD. In addition to its growth promoting activity, c-Myc is also an apoptosis inducer under stress conditions (114). c-Myc promotes apoptosis by triggering the release of cytochrome c from mitochondria and by activating caspase-9, and c-Myc-induced apoptosis can be inhibited by the antiapoptotic protein Bcl-2 (114, 115). In cancer cells, overexpressed Bcl-2 potentiates the oncogenic action of c-Myc by allowing c-Myc-induced proliferation to proceed without apoptosis (116), which suggests that apoptosis may act as part of a cellular fail-safe mechanism to forestall continued proliferation (114, 115). Thus, apoptosis in cystic epithelium may also act as a protective mechanism to limit the consequences of aberrant proliferation. In contrast, recent evidence suggests that caspases and other proapoptotic proteins can induce proliferation of neighboring surviving cells to replace dying cells through apoptotic cells secreting mitogens, such as Wg and Dpp mediated by JNK and dp53, in Drosophila (117). This process is called"apoptosis-induced proliferation", and it may be critical for tissue regeneration and tumor repopulation during cancer irradiation (117). Whether tubular cell apoptosis contributes to increased cell proliferation of neighboring cysts and tubules is not determined.

Although proliferation of cyst lining epithelia was increased, no obvious apoptosis was detected in several Pkd1 conditional knockout mice and Pkd1 hypomorphic Pkd1$^{nl/nl}$ mice. Growing evidence supports a mechanism that slowing cyst growth is through cell cycle arrest and restoration of pro-apoptotic activities to induce cell death, which is similar to some effective anti-cancer therapies (114). For example, inhibition of SIRT1 with nicotinamide or EX-527 decreased cystic renal epithelial cell proliferation by inactivation of p-Rb, and induced its apoptosis by activating p53 in a Pkd1 conditional knockout mouse model (58). In addition, inhibition of macrophage migration inhibitory factor (MIF) with ISO-1 decreased cystic renal epithelial cell proliferation but increased apoptosis (57). However, the direct role that inducing apoptosis within proliferating cystic renal epithelium has in delaying cyst growth in ADPKD is not provided by these studies, since inhibiting cell proliferation may be the predominant effect. A recent report demonstrated, for the first time, that induction of cystic epithelial cell death with Smac-mimetics delayed renal cyst growth with no effect on cell proliferation (53). Smac-mimetics are cell-permeable compounds designed to mimic the N-terminal 4 amino acid of Smac, a mitochondrial protein that binds to and antagonizes IAPs, including cIAP1, cIAP2 and xIAP (97, 118). TNF-α together with a Smac-mimetic induced cancer cell death (97, 118). Smac-mimetics induced the degradation of cIAP1, leading to its disassociation from

complex I, which then promoted the formation of complex II to induce apoptosis of cystic renal epithelial cells (53). Treatment of $Pkd1^{flox/flox}$:Ksp-Cre mice and $Pkd1^{nl/nl}$ mice with Smac-mimetics strikingly delayed cyst growth and preserved renal function, which resulted from the increased apoptosis of cyst lining epithelia (53) (Figure 2). Thus, this study suggests that apoptosis does not contribute to cystogenesis in PKD1 animal models; rather, it reduces the expansion of cysts and may be a new therapeutic target for the treatment of ADPKD.

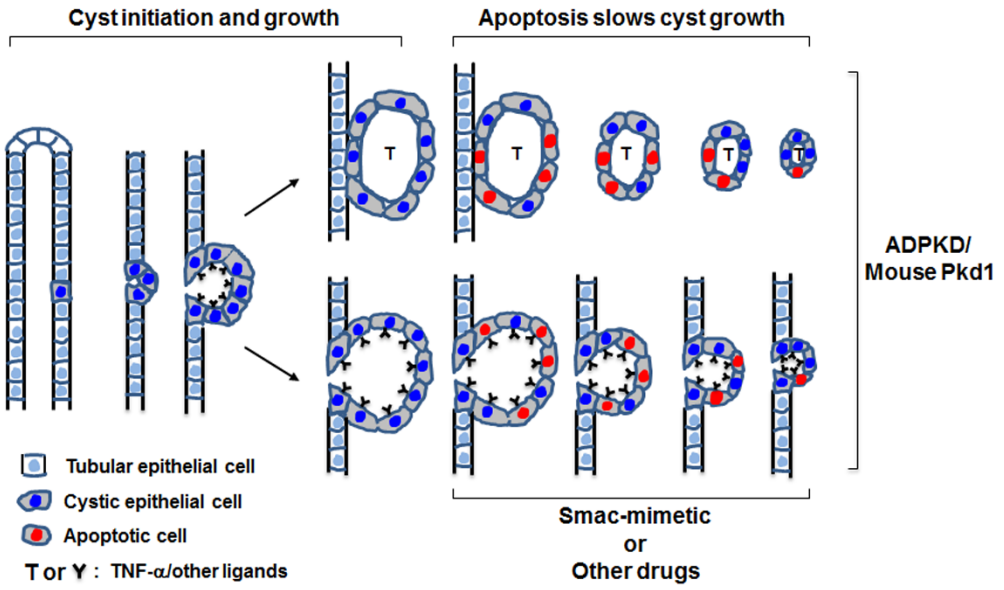

Figure 2. Model depicting the effect of Smac-mimetics or other potential drugs on delaying cyst enlargement in human ADPKD and ADPKD animals. Cysts arise primarily in collecting ducts when cell polycystin-1 levels fall to a critical threshold. A new phenotype arises that proliferates in response to cyclic AMP and other growth factors, and generates cytokines and chemokines, including TNF-α which has been found to accumulate in cyst fluid in an isolated sac formed after the cyst separates from the tubule in ADPKD patients (top panel). While the cyst remains attached to the tubule, some of the TNF-α synthesized in the mutant cells may escape into the urine but some of the TNF-α may retain at cyst developing sites through binding to its receptor, TNFR1 (bottom panel). TNF-α is trapped within the cysts or is trapped with TNFR1 to partner with Smac-mimetics to promote apoptosis of mural cells. Removal of apoptotic mural cells would reduce cyst surface area and decrease the rate of cyst growth. Extensive apoptosis could cause cysts to shrink. Other identified or unidentified drugs to target apoptotic pathways other than TNF-α signaling in ADPKD may shrink cyst or delay cyst growth in a similar manner.

Therapeutic strategies of regulating apoptosis in PKD

The therapeutic interventions involved in regulating apoptosis in PKD animal models are summarized in Table 1. Roscovitine is a cyclin-dependent kinase (CDK) inhibitor, and has shown promise in cancer treatment. Intermittent administration of roscovitine has a long-lasting effect to delay cyst growth in two non-orthologous mouse model of ARPKD, jck mice and cpk mice (63). The apoptotic cells were decreased in kidneys of roscovitine-treated jck mice, which is associated with the decreased expression of proapoptotic protein Apaf1 and caspase-2, and the increase expression of Bcl-2 and Bcl-xL (63). The mechanism of inhibiting the apoptosis by roscovitine may also involve the inhibition of Cdk5, which is responsible for anti-apoptotic effects of roscovitine in neurodegenerative diseases (63, 119). The GlcCer synthase inhibitor, Genz-123346, also slowed cyst growth by decreasing the proliferation and apoptosis in jck mice (120). The angiotensin converting enzyme inhibitor, Lisinopril (121), and water intake (65) have been reported to delay cyst growth associated with the decreased proliferation and apoptosis in the PCK rat. The studies in Han:SPRD rats also suggest that inhibiting proliferation and apoptosis with the caspase inhibitor IDN-8050 (75), soy protein feed (122), catechol-O-methyltransferase (COMT) inhibitor Tolcapone (123) and 2-hydroxyestradiol (124) can slow the cyst expansion. However, it is worth noting that decreased cell apoptosis is consistently accompanied with the decreased epithelial cell proliferation in all these studies. If hyperproliferation is the predominant factor for cyst expansion as described above, the decreased proliferation induced by these therapeutic interventions should be the primary effect on reduction of renal cysts.

The mammalian target of rapamycin (mTOR) pathway is an important pathway to regulate cell growth by sensing and integrating diverse nutritional and environmental signals, including growth factors (such as IGF-1 and IGF-2), energy levels, amino acids, and cellular stress (125, 126). PC-1 associates with tuberin and mTOR to form a complex to downregulate mTOR activity in renal epithelial cells under normal condition (55). N-terminal cytoplasmic PC1 (NTM-PC1) acts a constitutively-active inhibitor of mTOR, since it can inhibit the mTOR activity which results in G1 cell cycle arrest and apoptosis (55). The mTOR pathway is aberrantly activated in cystic epithelial cells in human ADPKD and mouse PKD models (55). Rapamycin treatment reduces renal cysts in the orpk-rescue mutant mouse model, which is a late-onset form of PKD due to defects in cilia protein polaris encoded by Tg737 gene, and Pkd1 conditional knockout Pkd1cond/cond:Nestin-Cre mice, and reduces the size of affected kidneys in ADPKD patients after renal transplantation (55, 56). The selective induction of apoptosis and luminal shedding of cyst-lining epithelial cells by rapamycin may be one of the potential mechanisms to slow cyst growth (55). Overexpression of neutrophil gelatinase-associated lipocali (NGAL) mediated by adenovirus suppressed renal cyst growth in $Pkd1^{flox/-}$:Ksp-Cre mice, partially due to the

Table 1. Therapeutic intervention involving in the regulation of apoptosis

Therapeutic intervention	Animal models	Mutated gene	Protein	Human disease	Proliferation	Apoptosis	Reference
Roscovitine, CDK inhibitor	jck mice	Nek8	Nek8	Nephronophthisis	decrease	decrease	(63)
	cpk mice	Cys1	Cystin	NA	decrease	decrease	
Genz-123346, GlcCer synthase inhibitor	jck mice	Nek8	Nek8	Nephronophthisis	decrease	decrease	(120)
Water intake	pck rat	Pkhd1	Fibrocystin	ARPKD	decrease	decrease	(121)
Lisinopril, ACE inhibitor	pck rat	Pkhd1	Fibrocystin	ARPKD	decrease	decrease	(65)
IDN-8050, Caspase inhibitor	Han:SPRD rat	Anks6	Ankyrin repeat and SAM domain-containing protein 6	Nephronophthisis	decrease	decrease	(75)
Soy protein	Han:SPRD rat	Anks6	Ankyrin repeat and SAM domain-containing protein	Nephronophthisis	decrease	decrease	(122)
Tolcapone, COMT inhibitor	Han:SPRD rat	Anks6	Ankyrin repeat and SAM domain-containing protein	Nephronophthisis	decrease	decrease	(123)
2-Hydroxyestradiol, 17-β Estradiol metabolites	Han:SPRD rat	Anks6	Ankyrin repeat and SAM domain-containing protein	Nephronophthisis	decrease	decrease	(124)
PP242, mTOR kinase inhibitor	Han:SPRD rat	Anks6	Ankyrin repeat and SAM domain-containing protein	Nephronophthisis	decrease	No change	(131)
Rapamycin, mTOR inhibitor	Orpk mouse	Tg737	Polaris	NA	NA	increase	(55)
mTOR anti-sense oligonucleotide (ASO)	Pkd2WS25/-	Pkd2	Polycystin 2	ADPKD	decrease	decrease	(132)
Rapamycin, mTOR inhibitor	$Pkd1^{flox/flox}$:*Nestin-Cre*	Pkd1	Polycystin 1	ADPKD	decrease	increase	(56)
Neutrophil gelatinase-associated lipocalin	$Pkd1^{flox/-}$:Ksp-Cre	Pkd1	Polycystin 1	ADPKD	decrease	increase	(54)
Nicotinamide and EX-527, Sirtuin 1 inhibitor	$Pkd1^{flox/flox}$:Ksp-Cre	Pkd1	Polycystin 1	ADPKD	decrease	increase	(58)
	$Pkd1^{nl/nl}$	Pkd1	Polycystin 1	ADPKD	decrease	increase	
ISO-1, MIF inhibitor	$Pkd1^{flox/flox}$:Ksp-Cre	Pkd1	Polycystin 1	ADPKD	decrease	increase	(57)
	$Pkd1^{flox/flox}$: Pkhd1-Cre	Pkd1	Polycystin 1	ADPKD	decrease	increase	
	$Pkd1^{nl/nl}$	Pkd1	Polycystin 1	ADPKD	decrease	increase	
Smac-mimetic	$Pkd1^{flox/flox}$:Ksp-Cre	Pkd1	Polycystin 1	ADPKD	No change	increase	(53)
	$Pkd1^{nl/nl}$	Pkd1	Polycystin 1	ADPKD	No change	increase	

induction of apoptosis of cystic epithelial cells by sequestration of intracellular iron and subsequent activation of Bim1 (54, 127). In addition, as we described above, both SIRT1 inhibitors and the MIF inhibitor delayed cyst growth in Pkd1 knockout mice partially through induction of cystic epithelial cell death through the activation of p53 (57, 58), whereas Smac-mimetics slowed cyst growth mostly though disruption of the TNF-α mediated pro-survival NF-κB signaling and then induction of cell death in cystic epithelium but not the non-cystic epithelial cells (53). These studies suggest that induction of cyst lining epithelial cell apoptosis, with or without a decrease in proliferation, can help to slow cyst growth in Pkd1 mutant animal models.

Conclusion and Perspectives

Understanding the potential detrimental and beneficial roles and mechanisms of apoptosis in PKD is necessary for developing therapeutic strategies in the future. Although aberrant apoptosis has been suggested as one of the pathological features in human ADPKD, ARPKD and PKD animal models, more recent evidence indicates that apoptosis is not increased in Pkd1 mutant mouse models, and induction of cyst lining epithelial cell apoptosis indeed slows cyst growth in these animals. Thus, it is necessary to thoroughly evaluate the role and mechanism of apoptosis during cyst development, which should include the origin of apoptotic cells (such as the cystic tubule cells, dilated tubule, normal tubule and interstitial cells), apoptosis in different stages of disease (such as the early stage of cyst initiation, intermediate stage of cyst expansion, and late stage of renal failure) and in different animal models (such as ARPKD and ADPKD models), and the relation of proliferation and apoptosis as well as its associated signaling pathways in PKD. It is possible that 1) apoptosis may be a secondary effect of aberrant cystic epithelial cell proliferation and is not directly related to the induction of cell proliferation; and 2) if we induce apoptosis of cystic cells we can overcome the increased cell proliferation and remove the mutant cells from contributing to cyst growth in the future. The pathogenesis of PKD is relevant to the hallmarks of cancer, such as the aberrant activation of signaling pathways of mTOR, Rb-E2F1, JAK/STAT (58, 128, 129). Several anti-cancer drugs have shown promise in ADPKD, such as rapamycin (56), Hsp90 inhibitor (130), and Smac-mimetics (53). Thus, novel therapeutic interventions may be identified for PKD treatment by repurposing the anti-cancer drugs (Figure 2) either to induce cell apoptosis alone or together with inhibiting cell proliferation.

Conflict of interest

The authors declare that they have no conflicts of interest with respect to research, authorship and/or publication of this book chapter.

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