Biochimica et Biophysica Acta 1812 (2011) 1272–1280

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Review

Apoptosis in polycystic kidney disease ☆

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ARTICLE INFO

Article history:
Received 26 August 2010
Received in revised form 5 January 2011
Accepted 7 January 2011
Available online 15 January 2011

Keywords:
Polycystic kidney disease
Apoptosis
Cell Cycle
Caspases

ABSTRACT

Apoptosis is the process of programmed cell death. It is a ubiquitous, controlled process consuming cellular energy and designed to avoid cytokine release despite activation of local immune cells, which clear the cell fragments. The process occurs during organ development and in maintenance of homeostasis. Abnormalities in any step of the apoptotic process are associated with autoimmune diseases and malignancies. Polycystic kidney disease (PKD) is the most common inherited kidney disease leading to end-stage renal disease (ESRD). Cyst formation requires multiple mechanisms and apoptosis is considered one of them. Abnormalities in apoptotic processes have been described in various murine and rodent models of PKD as well as in human PKD kidneys. The purpose of this review is to outline the role of apoptosis in progression of PKD as well as to describe the mechanisms involved. This article is part of a Special Issue entitled: Polycystic Kidney Disease.

1. Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common life threatening hereditary disease in the USA [1]. It affects about 1:500 people. Approximately 50% of people with ADPKD develop renal failure. ADPKD accounts for about 5–10% of end-stage renal disease requiring dialysis and renal transplantation [1]. *PKD1* encodes polycystin-1 and mutations in this gene account for about 85% of cases of ADPKD, while mutations in *PKD2* encoding polycystin-2 are responsible for 10–15%. Autosomal recessive polycystic kidney disease (ARPKD) affects 1 in 20,000 live births and is an important cause of renal and liver-related morbidity and mortality in neonates and infants [2,3]. This disease is caused by mutations in the polycystic kidney and hepatic disease gene 1 (*PKHD1*) which encodes fibrocystin/polyductin (FC1), a 500-kDa type I membrane protein expressed in the primary cilium and plasma membrane of renal and bile duct epithelial cells, as well as in the extrahepatic biliary duct [4,5]. Abnormalities of both cyst-lining epithelium and the noncystic tubular epithelium contribute to cyst formation in the kidney and are key to the disease progression.

The following molecular abnormalities of the tubular epithelium lining the cysts have been extensively described: (1) abnormal fluid secretion, (2) abnormalities of cell–matrix interactions, (3) alterations in polarity of membrane proteins, (4) abnormal ciliary function, and (5) disturbance in the balance between tubular cell proliferation and apoptosis [6].

Apoptosis is central to normal kidney development and has been implicated in kidney diseases of various origins, including PKD [7,8]. The cystic epithelia in both ADPKD and ARPKD display dedifferentiated cells with polarization defects, high rates of division and apoptosis [8–12].

2. Mechanisms of apoptosis

Apoptosis is the Greek word for "falling off", such as petals falling off a plant. The term was coined by Kerr, Currie, and Wyllie in 1972 [13], although the principle of apoptosis was first described as early as 1842 by the German scientist Carl Vogt. The term expresses the understanding that programmed cell death is a physiologic and active process. Apoptosis is tightly regulated to ensure safe elimination of defective cells while sparing adjacent normal cells. The decision for a cell to die is based on the balance between intracellular pro- and antiapoptotic factors, i.e., the caspases and intracellular inhibitors of apoptosis, respectively. The latter can be roughly divided into two separate families: 1) the inhibitors of apoptosis proteins (IAPs) and 2) several members of the Bcl-2 family, which are further discussed below. The process of apoptosis involves cell volume reduction, blebbing and loss of cell membrane asymmetry and cell–cell contacts. It leads to nuclear fragmentation, chromatin condensation, and internucleosomal DNA fragmentation [14]. Cells undergoing apoptosis have been described as performing the "Dance of Death", or *Danse Macabre*, which is not to be confused with the late-medieval allegory

☆ This article is part of a Special Issue entitled: Polycystic Kidney Disease.

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doi:10.1016/j.bbadis.2011.01.006

on the universality of death [15], but is rather an excellent description of the level of cellular activity apoptosis requires. Apoptosis takes only about one to six hours and occurs about 50 to 70 billion times per day in an adult person. The loss of membrane asymmetry leads to exposure of phosphatidylserine at the surface of the cell, which in turn is a strong signal for phagocytosis [16].

Caspases are cysteine proteases that can be divided into two groups, (*i*) mediators of apoptosis and, (*ii*) mediators of inflammation (Table 1). The apoptotic caspases are subdivided into initiating and executing caspases [17]. Caspases are present in the cytosol in an inactive pro-form [18]. They are activated to fully functional proteases by two cleavage steps. The first divides the chain into large and small caspase subunits, and the second removes the N-terminal prodomain [18]. Since proteolytic cleavage generates the mature caspases, one way in which these enzymes are activated is via the action of proteases, including other caspases. Thus, caspases can function in an activation cascade. Caspases-8 and -9 trigger apoptosis by activating the executing caspases [19]. Caspase-3 cuts off contacts with surrounding cells, reorganizes the cytoskeleton, shuts down DNA replication, interrupts splicing, disrupts the nuclear structure, marks the cell for phagocytosis and disintegrates the cell into apoptotic bodies [20–23].

Cells contain natural inhibitors of caspases. These inhibitors of apoptosis proteins (IAPs) were first identified in baculovirus and subsequently found in human cells (XIAP, c-IAP1, and c-IAP2) [24,25]. IAPs are recognized by the presence of a ~70 amino acid BIR (baculovirus IAP repeat), a zinc-finger-fold present at least once in each family member [26]. IAPs can act as direct inhibitors of caspases-3 and -7, and are able to suppress the activation of caspases-8 and -9 [25,27,28]. The eight IAPs in humans contain one to three BIRs, typically arranged at the N-terminus of the protein. Compared with other IAPs, survivin is structurally unique. It is the smallest mammalian IAP, containing a single BIR and a long C-terminal α-helix, but no other identifiable protein domain. Structural data suggest that survivin forms a stable homodimer in solution [29]. Survivin is implicated in both control of apoptosis and regulation of cell division and it was shown that phosphorylated survivin can interact with and inhibit caspase-9 [30].

Two major intracellular apoptotic pathways have been described (Fig. 1); In the mitochondrial or "intrinsic" pathway, stress-induced signals act via B cell CLL/lymphoma-2 (Bcl-2) proteins to cause cytochrome c release from mitochondria due to mitochondrial outer membrane permeabilization. Cytochrome c binds to the cytosolic protein, apoptosis protease-activating factor-1 (APAF-1) in the presence of ATP, forming the apoptosome, which recruits and activates caspase-9. Active caspase-9 in turn recruits and activates procaspase-3 and -7. The post-mitochondrial events are tightly regulated by heat shock proteins and IAPs [31,32]. Bcl-2 and its relatives are functionally classified as either antiapoptotic or proapoptotic. Most cells express a variety of antiapoptotic and proapoptotic Bcl-2 proteins, and the regulation of their interactions dictates survival or commitment to apoptosis. The main effector proapoptotic Bcl-2 members are Bcl-2 antagonist killer 1 (Bak) and Bcl-2-associated x protein (Bax) [33]. Upon activation, Bak and Bax homo-oligomerize into proteolipid pores within the outer mitochondrial membrane (OMM) to promote mitochondrial outer membrane permeabilization. Antiapoptotic Bcl-2 proteins are generally integrated within the OMM. Bcl-2-related gene A1, Bcl-2, Bcl-2-related gene, long isoform (Bcl-xL), Bcl-w, and myeloid cell leukemia 1 are the major members of the antiapoptotic Bcl-2 repertoire and preserve OMM integrity by directly inhibiting the proapoptotic Bcl-2 proteins [34,35].

In the "extrinsic" pathway, the binding of a ligand to its death receptor at the surface of the cell recruits an adaptor protein that in turn recruits and cleaves procaspase-8, which then leads to activation of executing caspases [36].

Apoptosis can be detected by various molecular methods. Morphological cellular changes remain a gold standard for apoptosis identification. In cell culture, nuclear chromatin staining with a cell-permeable, DNA-binding fluorochrome is a simple, yet accurate method [37]. DNA fragmentation can be demonstrated by terminal transferase-mediated dUTP nick end-labeling (TUNEL) [38] (Fig. 2). Apoptosis is also associated with an outward movement of phosphatidylserine (PS) in the plasma membrane as a consequence of loss of ATP [39]. Annexin V is a Ca^{2+}-dependent phospholipids-binding protein with high affinity for PS and can be coupled to a fluorochrome, thus allowing detection of apoptotic cells [40]. Alteration of mitochondrial integrity such as occurs during activation of the intrinsic apoptotic pathway, can be measured in various ways, including production of reactive oxygen species, translocation of soluble inter-membrane proteins, and loss of mitochondrial membrane potential [41,42].

Table 1
Caspases are divided into two sub-groups: caspases involved in the inflammatory process and those involved in the apoptotic process. The apoptotic caspases are further subdivided into initiating and executing caspases. Initiating caspases are activated by either external stimuli via activation of extracellular death receptors or by loss of the mitochondrial outer membrane potential leading to release of cytochrome c into the cytosol.

	Inflammatory caspase	Apoptotic caspase	Initiating apoptotic caspase	Executing apoptotic caspase
Caspase-1	√			
Caspase-2		√	√	
Caspase-3		√		√
Caspase-4	√			
Caspase-5	√			
Caspase-6		√		√
Caspase-7		√		√
Caspase-8		√	√	
Caspase-9		√	√	
Caspase-10		√	√	
Caspase-11	√			
Caspase-12	√			

3. Role and mechanisms of apoptosis in PKD

In PKD, abnormal proliferation in tubular epithelial cells plays a crucial role in cyst development and/or growth [43]. Kidneys from patients with ADPKD have high levels of apoptosis as well as cellular proliferation [44]. The SBM mouse is a transgenic murine model that bears similarities to human adult ADPKD. It carries a fusion gene including the *c-myc* coding region, the β-globin promoter and the simian virus 40 (SV40) enhancer, which is expressed at high levels in the renal tubular epithelium [45]. These animals reproducibly develop renal morphological and functional alterations characteristic of PKD, and die of renal failure. In this mouse model, there is a 10–100 fold increase in both apoptosis and proliferation [46,47]. Increased apoptosis and proliferation occur early in the course of the disease and precede cystogenesis in SBM mice [48]. Another frequently used model of ADPKD is the Han:SPRD rat [49]. It is derived from a spontaneous mutation in the Sprague–Dawley strain and inherits the disease in an autosomal dominant fashion [50]. Heterozygous animals of this strain develop slowly progressive renal cystic disease, whereas homozygous rats develop massive renal enlargement leading to early death [51]. This rodent model is considered highly suitable to study initial processes in tubular cystic transformation [52]. In Han:SPRD rats fed soy protein, the observed improved renal function and decreased cyst formation are accompanied by decreases in both tubular cell proliferation and apoptosis [53].

Apoptosis in PKD seems to act like a double-edged sword. The literature can be split into two groups: one accusing apoptosis of

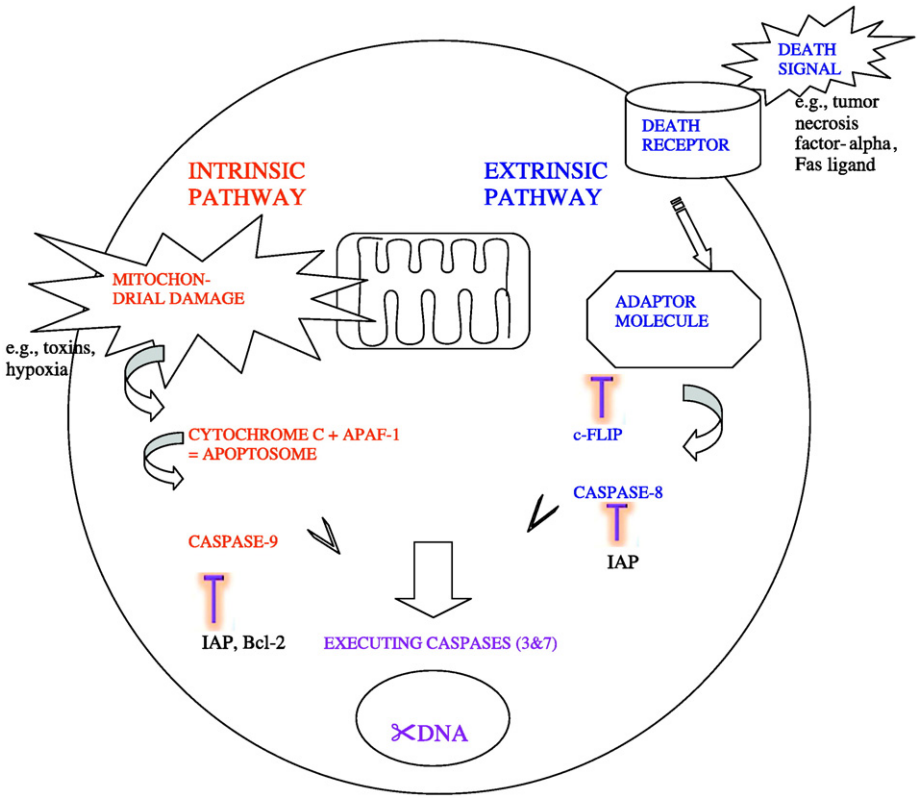

Fig. 1. Intracellular pathways of apoptosis. External apoptotic stimuli (extrinsic) lead to activation of caspase-8, while mitochondrial damage (intrinsic) leads to activation of caspase-9. Both pathways converge to activation of executing caspases. Activation of executing caspases is considered the point of no return. Both apoptotic pathways can be inhibited by IAPs. The intrinsic pathway can also be inhibited by antiapoptotic members of the Bcl-2 family of proteins and activation of caspase-8 in the extrinsic pathway can be inhibited by c-FLIP.

being the primary cause of the decline in renal function and the other, claiming that inducing apoptosis slows down disease progression [54]. Clearly, both epithelial cell apoptosis and proliferation are dysregulated in ADPKD and may represent a general mechanism for cyst growth and tissue remodeling [1,44]. By applying simple logic, it is evident that apoptosis per se cannot be the main culprit for PKD, because polycystic kidneys are massively enlarged and if apoptosis were to be the predominant feature, it would be expected that the kidneys would eventually involute, such as is the case in the majority of congenital dysplastic kidneys [55]. It seems that it is an imbalance between proapoptotic and pro-proliferative factors, rather than the absolute expression levels that play a critical role in the development of cystic kidney disease [56]. This imbalance leads to aberrant cell cycle progression in a similar fashion to cancer, such that PKD has been labeled “neoplasia in disguise” [57]. Cancers are generally characterized by suppression of apoptosis and the goal of cancer therapy is to induce apoptosis in malignant cells [58]. In the Bcl-2 transgenic mouse model, Bcl-2 was retrovirally introduced into the bone marrow of wild-type mice or transgenic mice overexpressing the c-myc oncogene (Em-myc) [59]. Bcl-2 cooperated with c-myc to promote transformation of B cell precursors [60]. Although developmentally normal, the adult Bcl-$2^{-/-}$ mouse displays thymus and spleen involution, polycystic kidney disease, and hypopigmented hair due to increased apoptosis. Mice deficient in the proapoptotic Bcl-2 gene have hyperproliferation as well as apoptosis with renal cysts [46,61]. Transgenic expression of human Bcl-2 in all organs except the kidneys resulted in early death due to renal failure due to PKD in this mouse model [62]. However, in an *in vitro* model of PKD, apoptosis was shown to be causally linked to cyst formation [63]. Apoptosis was essential for Madin–Darby canine kidney (MDCK) cell cyst cavitation in collagen type 1 matrix. Cystogenesis in this system was inhibited by overexpression of the antiapoptotic gene, Bcl-2. The timing between apoptosis and proliferation is also a chicken-or-egg type of controversy. While the latter seems to have been solved as of recent [64], whether apoptosis is secondary to proliferation or vice versa, is still a subject of debate. It seems that the controversy in the PKD literature is generated by several confounding factors in the experimental designs of the studies: (*i*) animal models versus human PKD, (*ii*) early versus late disease, (*iii*) cyst-lining epithelium versus normal-appearing tubules, and (*iv*) primary end-points being decreased cyst formation or regression in kidney size. In a seminal paper by David Woo, apoptosis was detected in kidneys of humans with ADPKD regardless of degree of renal dysfunction, but not in normal kidneys. Particularly, apoptosis was detected in normal-appearing, noncystic tubules suggesting that it may be associated with the progressive loss of normal nephrons in PKD [8]. Overall, however, the scientific community agrees that dysregulation of apoptosis contributes to the progression of ARPKD, ADPKD, juvenile nephronophthisis, and dysplastic renal disease associated with cyst formation in both humans and animal models [8,44,65,66]. In summary, there is much evidence that apoptosis is abnormally persistent in PKD and may result in cyst formation. The following are the main points suggesting a pathophysiological role for apoptosis in PKD: 1) Tubular cell apoptosis occurs in most animal models of PKD and in kidneys from humans with PKD. 2) Mice overexpressing the proto-oncogene c-myc (SBM mice), mice lacking the transcription factor AP-2β, and Bcl-2 deficient mice have increased apoptosis and develop cysts in the kidney. 3) Apoptosis is essential for MDCK cell cyst cavitation in collagen type 1 matrix; cystogenesis in this system is inhibited by overexpression of the antiapoptotic gene, Bcl-2, and tubule formation is induced with expression of *PKD1*, and 4) a direct cause and effect relationship

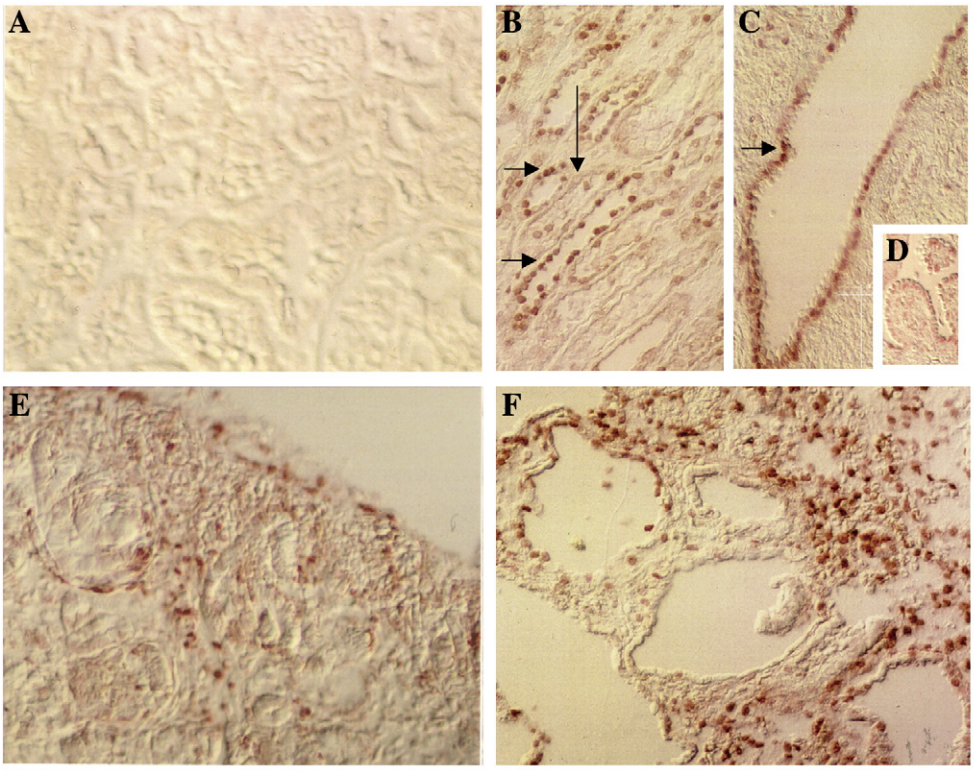

Fig. 2. In situ hybridization using terminal transferase-mediated dUTP nick end-labeling (TUNEL) method in a kidney from a patient with early-stage ADPKD (B), normal age-matched kidney (A), a fetal ARPKD kidney (F), and an age-matched fetal kidney (E). Note the lack of apoptosis in the normal adult kidney and the relative abundance in the nephrogenic zone of the normal fetal kidney. Long arrows in (B) point on sporadic apoptotic cells in cyst-lining epithelia of small cysts and short arrows point on apoptosis in epithelium of normal-appearing tubules (D) depict an area within a section from an end-stage ADPKD kidney (patient is on renal replacement therapy), demonstrating hyperplastic cyst-lining epithelium, accompanied by extensive apoptosis.
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between apoptosis and cyst formation is demonstrated by a study in Han:SPRD rats in which caspase and apoptosis inhibition with a pan-caspase inhibitor decreases apoptosis and proliferation in cystic and noncystic tubules, inhibits renal enlargement and cystogenesis, and attenuates the loss of kidney function. [1,8,46,48,63,67–70] On the other hand, there is also evidence that increased apoptosis in tubular cells may be associated with decreased cyst formation. For example, Pax2 deficient mice that have increased apoptosis, were backcrossed into congenital polycystic kidney (cpk) mice have increased renal apoptosis yet less cystic disease [44,46–48,53,71,72].

4. Implications of *PKD1*, *PKD2*, and *PKHD1* in control of apoptosis

A study implicated *PKD1* in the regulation of apoptosis, proliferation and cyst formation. Expression of human *PKD1* in MDCK cells slows the rate of proliferation and is protective from apoptosis [70]. Also, there is a tendency towards tubule formation rather than cystogenesis [70]. The gene may therefore be directly involved in the regulation of both apoptosis and proliferation pathways, allowing cells to enter a differentiation pathway that results in tubule formation. This study provided evidence that (*i*) polycystin-1 inhibits apoptosis and (*ii*) that increased apoptosis is associated with cyst formation in MDCK cells. Another evidence for a direct role of polycystin-1 in apoptosis regulation was provided by a study demonstrating an interaction between polycystin-1 and nephrocystin-1 that is required for polycystin-1-induced resistance to apoptosis [73]. Recent studies also demonstrated that polycystin-1 expression levels controlled G-protein-stimulated apoptosis in that polycystin-1 was able to inhibit apoptosis [74] and a specific binding site between polycystin-1 and the G protein was detected, which could act as a potential new pharmacological target [75].

In a mouse model of ADPKD due to *PKD2* mutations, both mitotic and apoptotic indices were increased compared with wild-type controls. As a matter of fact, apoptotic indices increased gradually with disease progression [76].

Knockdown of *PKHD1* by short hairpin RNA interference in IMCD cells results in disrupted normal tubulo-morphogenesis and increased apoptosis as well as proliferation [77]. Nuclear factor kappa B (NFκB) is a transcription factor, which plays a pivotal role in promotion of cell proliferation and inflammation, as well as suppression of apoptosis [78,79]. siRNA-induced knockdown of *PKHD1* in human kidney cells results in an increase in apoptosis [80]. NFκB inactivation is required for the suppression of caspase-3 activity and reduction of cell death in FC1-depleted cells, thus showing that NFκB may play a proapoptotic role in polycystic cells. Hence, FC1 is involved in the control of apoptosis through the modulation of NFκB [80]. Cell features observed in FC1-depleted cells are consistent with previous findings showing increased apoptosis and reduced proliferation in mouse renal tubular IMCD cells after *PKHD1*-silencing [77].

5. Caspase activation

Increased rates of apoptosis and increased caspase-3 activity have been demonstrated in kidneys from ARPKD rodent models [81,82]. Apoptosis is localized primarily to the interstitium with little evidence of cell death in cyst epithelium or noncystic tubules. A marked

increase in caspase-3 and -7 activity has also been reported in the Han:SPRD rat [56,83]. In this model, pan-caspase inhibition reduces tubular apoptosis and proliferation and slows cystic kidney disease progression and in a mouse model of ARPKD, targeted caspase-3 gene deletion prolongs survival [68,84]. In human PKD kidneys, the initiator caspase-8 and the executing caspase-3 are activated early in ADPKD, before the patient has progressed to ESRD and is mainly found in normal-appearing tubules [12]. Hence, apoptosis in normal tubules further contributes to the progression of loss of renal function in ADPKD. The extrinsic pathway does not seem to play a role in human ARPKD, although there is increased expression of caspase-3 [12]. In homozygous cystic kidneys of Han:SPRD rats, activation of both the intrinsic and extrinsic pathway can be detected. However, no differences in FasL mRNA are seen, suggesting that the extrinsic pathway is independent of that particular ligand [83].

6. Downregulation of inhibitors of apoptosis

Bcl-2 deficient mice have increased apoptosis in the kidneys, and renal failure results from severe PKD characterized by dilated proximal and distal tubular segments and hyperproliferation of epithelium and interstitium [46,71,72]. Alternatively, increased Bcl-2 expression has been demonstrated in animal models of both ARPKD and ADPKD [44,56,82]. High levels of Bcl-2 prevent apoptosis as well as cyst formation in MDCK cells [63]. In Han:SPRD rats, apoptotic cells are significantly increased in two-week mutant animals, compared to normal littermate controls. Decreased expression of Bcl-xL coincides with increased caspase-3 activity at two weeks of age while expression of Bcl-2 increases at six weeks of age. Proapoptotic Bax and Bad expression was unchanged at two weeks of age in both heterozygous and homozygous rat kidneys. This study demonstrates that dysregulation of the balance between pro- and antiapoptotic Bcl-2 family members, specifically a downregulation of antiapoptotic Bcl-xL, correlates with increased apoptosis in the kidneys [56].

The IAP survivin is increased in renal cancers and also in two-week old homozygous Han:SPRD rat kidneys in association with activation of caspase-9 and increased apoptosis [67].

Cellular (FADD)-like interleukin-1β-converting enzyme inhibitory protein (c-FLIP) is structurally related to caspase-8, but its caspase domain is altered, rendering it inactive. It is therefore a natural inhibitor of activated caspase-8. It is present in normal human kidney tissue, but undetectable in small cysts and normal-appearing tubules in human ADPKD kidney tissues from pre-dialysis patients [85]. c-FLIP was detectable in large cysts from end-stage ADPKD kidneys. Essentially, the expression pattern of c-FLIP was a mirror image of the expression of caspase-8. Hence, c-FLIP may be a candidate involved in the modulation of proliferation and apoptosis in ADPKD.

7. Proto-oncogenes

Abnormal expression of proto-oncogenes, in particular, c-myc may also contribute to abnormalities in proliferation and apoptosis, leading to cyst development. In both murine ARPKD and human ADPKD kidneys, c-myc is overexpressed in cystic tissue [44,86,87] and is associated with a marked increase in both tubular cellular proliferation and apoptosis. Overexpression of c-myc is thought to play a role in the dysregulation of both proliferation and apoptosis in ADPKD [48,88,89]. In addition, c-myc antisense oligonucleotides ameliorate cystic kidney disease in a murine ARPKD model [89]. The pathway of c-myc induced apoptosis is thought to be mediated by caspase-9 and caspase-3 [90].

8. Cell cycle abnormalities

Two key classes of regulatory molecules, cyclins and cyclin-dependent kinases (CDKs), determine progression through the cell cycle [91]. Most genes encoding cyclins and CDKs are conserved among all eukaryotes [92]. Cyclins form the regulatory subunits and CDKs the catalytic subunits of an activated heterodimer. CDKs are constitutively expressed in cells whereas cyclins are synthesized at specific stages of the cell cycle, in response to various molecular signals [93].

Two families of genes, the cip/kip family and the INK4a/ARF (Inhibitor of Kinase 4/Alternative Reading Frame) encode CDK inhibitors. These genes are considered to be tumor suppressors [94]. The cip/kip family includes the genes *p21*, *p27*, and *p57*. They halt cell cycle in G1 phase and inactivate, cyclin-CDK complexes. *p21* is activated by *p53*. The INK4a/ARF family includes p16INK4a, which binds to CDK4 and arrests the cell cycle in G1 phase, and p14arf, which prevents *p53* degradation.

Abnormal cell cycle regulation has been suggested to contribute to PKD. Polycystin-1 increases expression of *p21*, and *p21* is decreased in human and a rat model of PKD [95,96]. These findings are consistent with the observed augmentation of both cell proliferation and apoptosis in this disease.

9. Therapeutic interventions implicating apoptotic pathways

9.1. Mammalian target of rapamycin (mTOR)

The mammalian target of rapamycin (mTOR) pathway is an evolutionarily conserved pathway that transmits cell growth and survival signals in cells. It senses the availability of nutrients and growth factors in the environment and responds by signaling for cell growth and division [97]. The mTOR pathway has become of interest in the pathophysiology of PKD because it also integrates signals from growth factors (including EGFR) and G-protein coupled receptors (which generate cAMP) [98,99]. Mutated *TSC1* and *TSC2* genes cause tuberous sclerosis, a disease in which renal cystic lesions may accompany the more classical renal angiomyolipomas [100] and *TSC1/2* mutations are associated with upregulate mTOR signaling. The *TSC2* and *PKD1* genes lie adjacent to each other on human chromosome 16p13.3 and the cytoplasmic tail of polycystin-1 interacts with mTOR. This sparked the interest for a possible mTOR activity in PKD. In a variety of animal models, as well as in human ADPKD and ARPKD cyst-lining epithelia, expression of phospho-mTOR and p70S6K is increased [101]. Indeed, in the absence of polycystin-1, activation of mTOR results in increased cell growth. Inhibition of the mTOR pathway reverses renal cystogenesis in PKD and has been associated with an increased rate in apoptosis *in vitro* [102]. Rapamycin is an immunosuppressive drug that inhibits mTOR and is used to prevent transplant rejection. Rapamycin and a related drug everolimus were evaluated in the Han:SPRD rat [103,104]. Inhibition of mTOR significantly slows cyst growth in this model. Other studies have tested the efficacy of rapamycin in three non-orthologous mouse models of PKD, Tg737orpk/orpk mice (mutation in the ciliary protein Polaris), bpk mice, and kidney-specific BHD (gene mutated in patients with Birt–Hogg–Dubé syndrome) mutant mice [101,105]. These studies also revealed significant improvement in cyst size and azotemia compared to control animals. In a retrospective study, Shillingford examined a small group of patients who had undergone kidney transplantation for ESRD due to ADPKD and retained their native cystic kidneys [101]. In four patients who received rapamycin as part of their post-transplant immunosuppression, the volume of the native cystic kidneys was reduced by 24% compared to 8.6% in three patients treated with other immunosuppressants. The change in kidney size was significantly different between the rapamycin and non-rapamycin groups. Another retrospective study by Qian demonstrated that rapamycin decreased the size and number of liver cysts but failed to show a benefit on kidney cysts [106]. Nonetheless, these results were encouraging enough to lead to prospective

clinical trials in adult patients with ADPKD, evaluating the role of everolimus [107] and sirolimus [108] in patients with early ADPKD. However, neither of the studies demonstrated slowing of the disease progression, despite a slowing of the increase in kidney volume. The lack of demonstrable effects may have been due to a relatively short follow-up period (2 years and 18 months, respectively). Another caveat to the studies was that both sirolimus and everolimus had significant side effect profiles and the patients did not tolerate what was considered a therapeutic dose.

9.2. Cyclin-dependent kinase inhibitors

The CDK inhibitors, both endogenous (*p21*) and synthetic (roscovitine), have shown promise in the treatment of cancer [10], and more recently, of ADPKD [109]. Germino was the first to suggest a relationship between *p21* and PKD by showing that *p21* is induced polycystin-1 [95]. Increased apoptotic loss of renal tissue was found in subsequent studies in the presence of the reduced levels of *p21* [110–112]. Roscovitine, an inhibitor of cyclin-dependent kinases was administered to two non-orthologous mouse models of recessive PKD, jck mice and cpk mice and resulted in arrest of cyst growth [10]. Intermittent administration of roscovitine has a long-lasting anti-cystic effect [113]. At high concentrations, roscovitine results in caspase-3 activity being significantly increased from control cells. Low doses of roscovitine likely induce minor repairable damage to cells, which in turn cause activation of ATM, *p53*, and *p21*, and activate the Akt pathway, which prevents cells from entering the apoptosis pathway rather than the survival pathway. As a result, cells enter the permanent cell cycle arrest stage, or replicative senescence, which is an ideal result for a potential PKD therapeutic. That low-dose roscovitine induces senescent markers in association with *p21* augmentation is consistent with the known role of *p21* in causing replicative senescence and its absence in avoiding this fate, and suggests that these events are mechanistically related [114–117]. This state of irreversible cell cycle arrest has the potential to beneficially modulate the aberrant cell proliferation seen in PKD [118]. Recent studies revealed that the p53 pathway monitors the mTOR pathways; in response to stress, the p53 pathway negatively regulates the IGF-1/AKT and mTOR pathways to shut down cell growth and division [119].

9.3. Caspase inhibition

Heterozygous Han:SPRD rats treated with the pan-caspase inhibitor IDN-8050 or vehicle for five weeks [68] develop the following phenotypic changes: (1) decreased apoptosis and proliferation in cystic and noncystic tubules, (2) inhibited renal enlargement and cystogenesis and (3) attenuated loss of kidney function [68]. Caspase inhibition may impede a common pathway for both apoptosis and proliferation and the decrease in proliferation may be beneficial in reducing cyst formation. Another model of PKD, the cpk mouse, dies from renal failure and PKD at a mean age of 32 days. Complete deficiency of caspase-3 prolongs the life of the cpk mouse [67].

10. Conclusion

The heightened cellular proliferation and apoptosis observed in SBM mice and human ADPKD resemble the process occurring during renal organogenesis [120]. Cystic epithelia in PKD are characterized by mispolarization of channels and cell surface receptor reminiscent of a fetal phenotype [121]. During renal development, apoptosis and proliferation are the main mechanisms by which extensive remodeling of the kidney structures occurs. Experiments interfering with these processes result in dysplastic, non-functioning kidneys [122]. It is unclear what the underlying cause of the acquisition of a fetal phenotype in cystic tubular epithelial cells is. However, a study comparing pathways of apoptosis in adult and fetal kidneys as well as human PKD kidneys failed to show similarities between nephrogenesis and PKD [12]. Therefore, the dedifferentiation occurring in cystic epithelial cells is not simply a “step backwards”, but likely a reaction to the environment encountered in PKD kidneys. Analysis of cyst fluid has revealed the presence of multiple cytokines, among them are TGF-β, TGF-α, and EGF [123,124]. These cytokines are closely involved in apoptosis, proliferation, and differentiation. They are also cytokines encountered in inflammatory states, such as ischemia-reperfusion injury. The consequence to ischemia of renal tubular epithelial cells is clinically referred to as acute tubular necrosis (ATN), which is a partial misnomer, since tubular epithelial cells do not just die due to necrosis, but also undergo apoptosis [125]. The cells slough off the tubular basement membrane and can be found in the urinary sediment of the patient [125]. Once a favorable environment has been restored in the kidney, dedifferentiated tubular cells migrate back onto the tubular basement membrane and proliferate in order to restore tubular transport functions. The phenotype of the dedifferentiated cells is similar to cyst-lining epithelial cells in that they are not terminally differentiated and that they have a large proliferative capacity. Nonetheless, despite the similarities between cyst-lining epithelial cells and tubular epithelial cells involved in regeneration, in ATN, once the tubular basement membrane is covered with cells and the cell–cell junctions as well as the cell–matrix adherence have been restored, terminal differentiation occurs, and proliferation stops. This is not true for PKD. Whether this phenomenon is due to ongoing cytokine release and ischemia, is beyond the scope of this review, but has been discussed elsewhere in the past [126]. Evidently, mechanisms governing apoptosis and proliferation in PKD seem to be of a different nature than the ones involved in nephrogenesis or ischemia-reperfusion injury.

The precise pathways that link apoptosis and proliferation in PKD remain to be determined. There are two lines of evidence for a common pathway of apoptosis and proliferation involving 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 [127]. 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 seen in the same cyst and caspase inhibition would attenuate both processes. Advances in understanding of the apoptotic pathways have led to the development of drugs, which suppress apoptosis. However, apoptosis in PKD, beyond its role in cyst cavitation, contributes to the loss of renal tissue and may be responsible for the progressive deterioration of renal function that occurs in patients with ADPKD [8]. On the other hand, however, apoptosis can be viewed as the last line of defense against oxidative or other form of DNA damage, thus reducing the risk for neoplastic transformation and explaining the lack of a convincingly increased risk for renal cell carcinoma in this condition despite the high rate of epithelial cell proliferation. Therefore, elucidation of the mechanisms responsible for the coupled stimulation of cell proliferation and apoptosis in ADPKD may provide opportunities for interventions that avoid the potential risk of selectively inhibiting apoptosis alone. Exciting times are awaiting us in the near future.

Acknowledgments

The author would like to thank Massimo Cohen for stimulating discussions.

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