The regulation of apoptosis in kidney development: implications for nephron number and pattern?

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Apoptosis is essential to remodel developing structures and eliminate superfluous cells in a controlled manner during normal development, and continues to be an important component of tissue remodeling and regeneration during an organism's lifespan, or as a response to injury. This mini review will discuss recent studies that have provided insights into the roles of apoptosis in the determination of nephron number and pattern, during normal and abnormal kidney development. The regulation of congenital nephron endowment has implications for risk of chronic kidney disease in later life, whereas abnormalities in nephron pattern are associated with congenital anomalies of the kidney and urinary tract (the leading cause of renal disease in children). Tight regulation of apoptosis is required in normal renal morphogenesis, although many questions remain regarding the regulation of apoptosis by genetic, epigenetic, and environmental factors, in addition to the functional requirement of different components of the apoptotic pathway.

Keywords: kidney development, apoptosis, nephron number, nephron pattern, cystic kidney disease

Apoptosis is a process used by multicellular organisms to dispose of unwanted cells in a controlled manner that minimizes damage and disruption to neighboring cells [reviewed in Ref. (1)]. In the context of development, apoptosis is an essential component in the establishment of tissue architecture, and serves to both remodel developing structures to their mature form and ablate superfluous cells [reviewed in Ref. (2)]. Moreover, apoptosis plays a crucial role in tissue remodeling and regeneration that occurs constitutively, or in response to insult or injury, throughout the lifespan of vertebrates. Thus, dysfunctions in apoptosis can manifest as developmental abnormalities, and in adult pathologies such as cancer or degenerative disease.

In the genitourinary tract, the most obvious examples of a requirement for apoptosis during development include the regression of the pronephros and the selective loss of portions of the mesonephros in females during kidney development (3, 4). In addition to these examples, studies have implicated apoptosis more generally in determining nephron number and pattern. Since the mammalian kidney is unable to compensate for nephron loss due to renal injury by the *de novo* generation of nephrons, the number of nephrons generated at birth in any one individual is thought to be an important determinant of adult kidney health (5, 6). In keeping with this concept, reduced nephron number has been associated with hypertension and chronic kidney disease in human beings (7, 8). Moreover, the function of the mammalian kidney is critically dependent on the complex structural arrangement of multiple cell types in the nephron (or nephron pattern) within the kidney itself. This review will discuss recent studies that have provided insights into the roles of apoptosis in the determination of nephron number and pattern during normal and abnormal kidney development.

THE MECHANICS OF APOPTOSIS

Cells undergoing apoptosis are characterized by a series of distinct morphological events (cell shrinkage and retraction from neighboring cells, blebbing of the plasma membrane, and cellular fragmentation into apoptotic bodies) that ultimately result in these cells being rapidly engulfed by phagocytes (9). These events are accompanied by subcellular changes, such as nuclear condensation and DNA fragmentation in apoptotic cells. Together, these morphological changes are thought to distinguish specific cells for removal in a controlled fashion, without the activation of inflammatory cells (in contrast to cell death attributed to necrosis). A wide variety of normal and pathological stimuli have been identified that trigger apoptosis, and are thought to do so through two pathways: the intrinsic and the extrinsic pathways.

Activation of the intrinsic pathway is regulated via complex interactions between the pro- and anti-apoptotic members of the B-cell lymphoma 2 (Bcl2) family of proteins, and it is thought that it is the balance between these factors that determines whether a cell will undergo apoptosis (**Figure 1A**) [reviewed in Ref. (10)]. The proapoptotic Bcl2 family proteins are divided into effector proteins (Bax, Bak), which are required for mitochondrial outer membrane permeabilization, and the BH3-only proteins, which either interact with the anti-apoptotic Bcl2 members, or the effector proteins. The anti-apoptotic Bcl2 family members inhibit apoptosis by binding proapoptotic Bcl2 family members and activated Bax or Bak. Mitochondrial outer membrane permeabilization leads to the release of proapoptotic proteins from the mitochondrial intermembrane space, and is the crucial event that drives subsequent activation of caspases via a protein complex termed the apoptosome. The eventual outcome is activation of the executioner caspases, 3 and 7, and subsequent apoptosis.
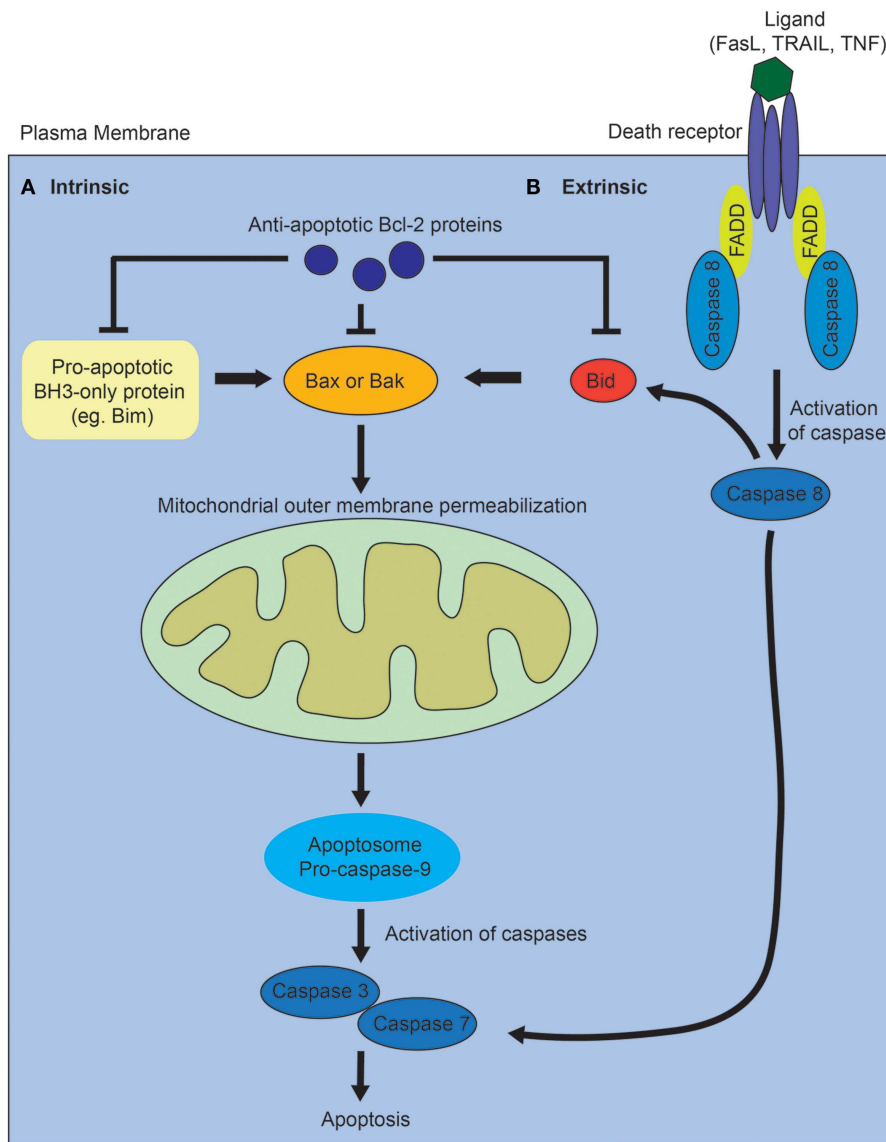
FIGURE 1 | Diagram of intrinsic and extrinsic pathways of apoptosis.
(A) In the intrinsic pathway, the proapoptotic BH3-only family members
activate Bax or Bak, leading to mitochrondrial outer membrane
permeabilization, which drives formation of the apoptosome, activation of the
executioner caspases, 3 and 7, and subsequent apoptosis. The proapoptotic
BH3-only proteins are inhibited via interactions with the anti-apoptotic Bcl-2
family of proteins. **(B)** In the extrinsic pathway, ligands such as Fas, tumor
necrosis factor (TNF), or tumor necrosis factor-related apoptosis-inducing
(TRAIL) ligand bind to death receptors. This results in the recruitment of
Fas-associated death domain protein (FADD) and activation of caspase 8.
Caspase 8 directly activates caspase 3 and 7. The two pathways interact via
caspase 8-mediated cleavage of Bid.

In contrast, the extrinsic apoptotic pathway is initiated when a
death receptor (Fas, tumor necrosis factor receptors) is bound
by its ligand (**Figure 1B**) [reviewed in Ref. (10)]. This results
in the subsequent recruitment of adaptor proteins such as Fas-
associated death domain protein and procaspase 8. Activated
caspase 8 directly cleaves and activates the executioner caspases,
3 and 7. The intrinsic and extrinsic pathways also interact via cas-
pase 8-mediated cleavage of Bid, which leads to mitochondrial
outer membrane permeabilization. In vertebrates, most apoptotic
stimuli are thought to require mitochondrial outer membrane
permeabilization for caspase activation and apoptosis. The relative
contribution of the extrinsic versus intrinsic pathways of apoptosis
during normal kidney development remains unclear.

APOPTOSIS IN THE ESTABLISHMENT OF NEPHRON NUMBER
How is nephron number determined? The mature mammalian
kidney arises from reciprocal interactions between two tissues
derived from the intermediate mesoderm: the ureteric bud and
the metanephric mesenchyme (11). A series of inductive signals
between these two tissues result in the iterative branching of the
ureteric bud (which will subsequently become the collecting sys-
tem of the kidney), and the formation of a "cap" of nephron

progenitors (which are fated to become nephrons) around each ureteric bud tip (**Figure 2**). These nephron progenitors possess the ability to proliferate and self-renew throughout development, to generate an appropriate number of nephrons, and to differentiate into the multiple cell types required to form a functioning nephron (12, 13). The process of new nephron formation continues until around 36 weeks of gestation in human beings, and then terminates, via mechanisms that are largely unknown. Interestingly, the number of nephrons that are created appears to be limited by the number of nephron progenitors generated during kidney development. Thus, ablation of a subset of nephron progenitors results in decreased ureteric bud branching and adult nephron endowment (14). The converse is also true, in which mutations that affect ureteric bud branching result in impaired adult nephron endowment (15–21).

Taken together, these findings suggest that mechanisms that regulate apoptosis of the metanephric mesenchyme (from which nephron progenitors are derived), and of the progenitors themselves, would have a significant impact on congenital nephron endowment. Conceptually, those mechanisms could be considered broadly into two categories: (1) apoptosis resulting from paracrine or autocrine signals to eliminate unwanted cells; or (2) apoptosis

FIGURE 2 | Diagram of kidney development. Kidney development begins with the outgrowth of the ureteric bud (red) from the Wolffian duct into the metanephric mesenchyme (light blue). In response to signals from the ureteric bud, the metanephric mesenchyme condenses around the ureteric bud tip, becoming specified as nephron progenitors (dark blue). Nephron progenitors give rise to multiple cell types of the nephron, including podocytes, proximal tubules, loops of Henle, and distal tubules. The ureteric bud branches in response to signals from the metanephric mesenchyme to form the collecting system of the kidney.

as the result of a default intrinsic pathway from which some cells are rescued by survival signals. Classic studies using embryonic kidney explants demonstrated that isolated metanephric mesenchyme undergoes apoptosis in the absence of inducing signals from the ureteric bud (22). Subsequent work to characterize the growth factors that promote survival of the metanephric mesenchyme or nephron progenitors *in vitro* have identified several candidates including transforming growth factor-β2 (TGF-β2), TGFα, leukemia inhibitory factor (LIF), epidermal growth factor (EGF), fibroblast growth factor 2 (FGF2), FGF9, FGF20, and bone morphogenetic protein 7 (BMP7) (23–27). More recently, *in vivo* transgenic mouse studies showed that *Fgf9* and *Fgf20* are critical for maintaining nephron progenitor survival; furthermore, FGF20 mutations in human beings were shown to be associated with severe renal dysplasia (28). The extent to which some or all of these factors act endogenously to promote nephron progenitor survival remains to be determined.

In keeping with the idea that the metanephric mesenchyme is programed to undergo apoptosis is the observation that an inability to respond to inducing and/or survival signals also results in apoptosis. Thus, conditional deletion of both *Fgfr1/Fgfr2* in the metanephric mesenchyme causes marked apoptosis in the metanephric mesenchyme early in kidney development (19). Another example is that of the transcription factor, Wilms tumor-1 (Wt1), which is one of several transcription factors thought to be critical in specification of nephron progenitors from the metanephric mesenchyme. Wt1 null mice have an intrinsic defect in the metanephric mesenchyme that results in abnormal formation of this tissue, which subsequently undergoes rapid apoptosis (29). Finally, impairment of the cell–cell interactions between the metanephric mesenchyme and the ureteric bud can result in subsequent apoptosis of the mesenchyme, as seen in alpha8-integrin null mice (30).

Recent studies have also implicated epigenetic mechanisms (defined as heritable changes in gene activity that are not caused by changes in DNA sequence) in regulating apoptosis in nephron progenitors. microRNAs (miRNAs) are endogenous, small non-coding RNAs that bind to specific mRNA targets to block translation and/or promote mRNA degradation. Conditional targeting of *dicer*, an enzyme required for processing of mature miRNAs, in mouse nephron progenitors, led to premature depletion of nephron progenitors due to excessive apoptosis, likely from upregulation of the proapoptotic protein, Bim (31). In addition to genetic and epigenetic factors, there is also emerging data suggesting that there are environmental influences that impact apoptosis of the metanephric mesenchyme. Thus, disruption of the G protein-coupled bradykinin B2 receptor gene (*Bdkrb2*) in combination with gestational high salt results in increased apoptosis of the metanephric mesenchyme (32). Together, these data suggest that multiple factors (epigenetic, genetic, and environmental) converge to regulate apoptosis in nephron progenitors, with consequent effects on congenital nephron endowment, and thus, the risk of chronic kidney disease in human beings.

NEPHRON PATTERN AND CELL DEATH

Apart from its role in the determination of nephron number, apoptosis is tightly regulated in kidney development to eliminate
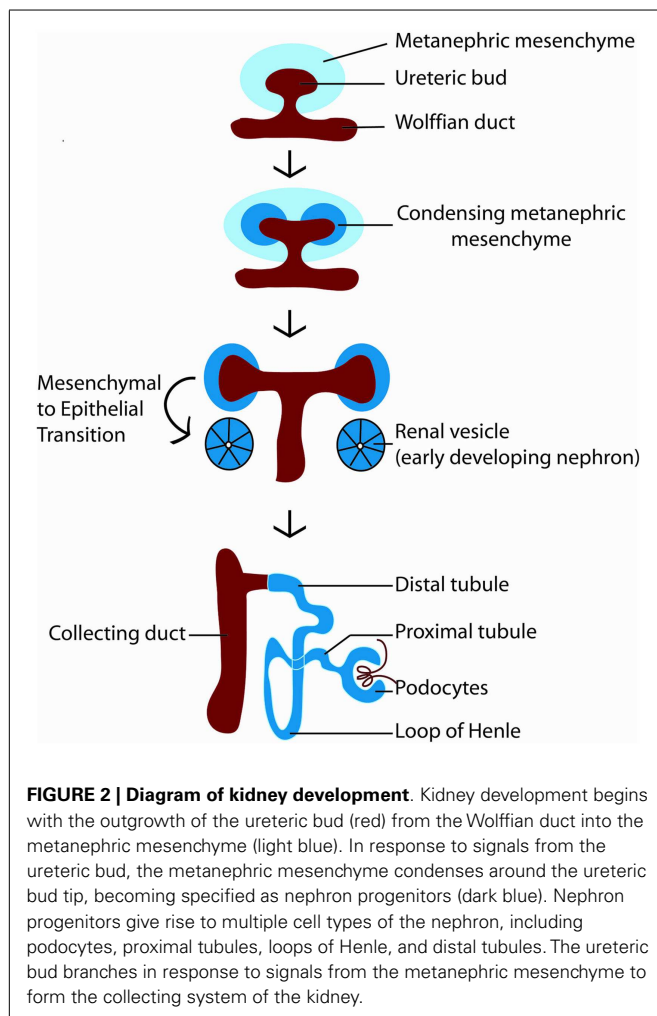
unwanted cells as nephrons and the surrounding tissues are patterned to form the complex three-dimensional architecture of the kidney. Experimental surveys of apoptosis in the normal developing kidney have identified a distinct developmental time course for apoptosis in two main areas of the developing kidney: the nephrogenic zone (where new developing nephrons are produced) and the medullary papilla (which gives rise to the calyces, renal pelvis, and renal papilla) (33–35). Interestingly, the cell death rate was highest in the nephrogenic zone of embryonic rat kidneys, concurrent with high rates of cell proliferation, and declined postnatally, suggesting tight regulation of both proliferation and cell survival in this compartment during the process of nephron formation (33). In contrast, the cell death rate in the rat medullary papilla peaked at around postnatal day 6–7, with a subsequent decline (33). In terms of location, most of the apoptotic cells detected in the nephrogenic zone were in the stromal mesenchyme surrounding nephron progenitors and developing nephrons, with fewer apoptotic nephron progenitors (33). In the developing collecting system, apoptosis is infrequently detected in the ureteric bud and is prominent in the medullary papillary region (33). This suggests a potential role for apoptosis in remodeling the first 3–5 generations of the branched ureteric bud/developing collecting duct system. Other suggested roles for medullary apoptosis include elimination of interstitial cells as a mechanism for making room for new blood vessel ingrowth (36).

During nephron formation, nephron progenitors undergo a mesenchymal to epithelial transition to form the renal vesicle, which subsequently differentiates into the comma-shaped body, followed by the S-shaped body, and then the mature nephron (11). Survival signals are equally important in this process, which establishes proximal–distal pattern of the developing nephron. One example is that loss of FGF8 signaling in the metanephric mesenchyme results in progressive loss of the progenitor population, along with a failure to form S-shaped bodies (37). Interestingly, mice that are hypomorphic for FGF8 do develop S-shaped bodies; however, the nephrons that form are truncated due to apoptosis (37).

Evidence that apoptosis is regulated during renal branching morphogenesis is provided by studies in which dysregulated apoptosis is associated with defective collecting duct development. For example, increased branched ureteric bud cell proliferation and subsequent medullary collecting duct cell apoptosis was observed in *Glypican 3*$^{-/-}$ (*Gpc3*) mice, which exhibit cystic degeneration of the medullary collecting duct system (38, 39). The defect is thought to be caused by an altered cellular response to growth factors, such as FGFs (39–41). Moreover, apoptosis is a prominent feature of dilated collecting ducts in experimental models of fetal and neonatal urinary tract obstruction (42, 43). Together, these data suggest a relationship between collecting duct apoptosis and two frequent features of renal dysplasia – cystogenesis and urinary tract dilatation.

THE APOPTOTIC MACHINERY IN THE KIDNEY

Despite the evidence that apoptosis plays a critical role in multiple facets of kidney development, there have been few studies that implicate specific members of the apoptotic pathway in renal morphogenesis. This is thought to be due to functional redundancy among the molecules in the apoptotic pathway, in addition to other levels of compensation for the loss of any one single component. Thus, overall suppression of apoptosis via pharmaceutical means to inhibit caspase-9 or caspase-3 activity leads to decreased ureteric branching and nephron formation in embryonic kidney explants (44, 45). However, mice that lack caspase-3 display normal cell death and normal kidney development (46).

One notable exception to this is the prosurvival protein, *B-cell lymphoma 2* (*bcl2*), which is expressed in the ureteric bud and metanephric mesenchyme (47). *Bcl2*-null mice demonstrate increased apoptosis in the metanephric mesenchyme, resulting in fewer nephrons, mild renal hypoplasia, and cystogenesis (48–50). As noted above, in broad terms, the determination of whether a cell undergoes apoptosis or survives is dependent on the pairing between Bcl-2 family members that promote cell death and those that promote survival. The proapoptotic protein, Bim, binds to many of the Bcl-2 prosurvival proteins, and is thought to release Bax or Bak proteins from their interaction with Bcl-2 to promote apoptosis (51). Interestingly, the loss of a single *Bim* allele in *Bcl-2* null mice is sufficient to rescue the cystic phenotype of *Bcl-2* null mice, suggesting that gene dosage of *Bim* is critical during nephrogenesis (52). While Bim is expressed in the metanephric mesenchyme, *Bim* null mice do not demonstrate an overt renal structural defect [these mice die at several months of age due to an immune complex glomerulonephritis (53)]. Together, these data suggest that the balance of activity between the prosurvival protein, Bcl-2, and the proapoptotic protein, Bim, regulates subsequent apoptosis of the metanephric mesenchyme via the mitochondrial instrinsic apoptotic pathway. An alternative hypothesis for the requirement for Bcl-2 has also been proposed, in which Bcl-2 interacts with paxillin and focal adhesion kinase (FAK) to bypass the need for integrin-mediated survival signals, allowing cells to migrate as needed for nephrogenesis (54, 55). The relative functional contribution of other Bcl-2 family proapoptotic and prosurvival proteins to nephron progenitor survival or nephron patterning remains unknown.

Apart from components of the "apoptotic machinery," there are also proteins that are known to regulate apoptosis. One example is the transcription factor and tumor suppressor gene, p53, which activates the expression of proapoptotic factors, such as Bax (56). Mice that are null for p53 exhibit defects in terminal nephron differentiation, and on a C57Bl6 background, a spectrum of congenital anomalies of the kidney and urinary tract (57, 58). While these studies have clearly implicated p53 in renal development, the relative role of p53 in the regulation of apoptosis, cell cycle, and/or DNA repair in nephrogenesis remains to be determined.

CONCLUSION

Apoptosis is tightly regulated in space and time during kidney development, and dysregulation of apoptosis is clearly associated with changes in nephron number and pattern. However, many questions remain unanswered, including how do genetic, epigenetic, and environmental factors interact to regulate apoptosis? what is the role of other members of the Bcl-2 family of proteins or other components of the apoptotic cascade in kidney development? what are the relative contributions of the intrinsic versus extrinsic pathways in apoptosis? An improved understanding of

these processes would contribute to our understanding of congenial anomalies of the kidney and urinary tract, the leading cause of pediatric chronic kidney disease. Moreover, the study of nephron progenitor survival has implications for novel therapies, given recent research efforts to develop methods to reliably propagate and differentiate nephron progenitors in culture, and potentially manipulate progenitors *in vivo*.

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