How Many Ways Can a Podocyte Die?

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Summary: Podocytes are highly specialized epithelial cells that line the urinary surface of the glomerular capillary tuft. To maintain kidney filtration, podocytes oppose the high intraglomerular hydrostatic pressure, form a molecular sieve, secrete soluble factors to regulate other glomerular cell types, and provide synthesis and maintenance of the glomerular basement membrane. Impairment of any of these functions after podocyte injury results in proteinuria and possibly renal failure. Loss of glomerular podocytes is a key feature for the progression of renal diseases, and detached podocytes can be retrieved in the urine of patients with progressive glomerular diseases. Thus, the concept of podocyte loss as a hallmark of progressive glomerular disease has been widely accepted. However, the nature of events that promote podocyte detachment and whether detachment is preceded by any kind of podocyte cell death, such as apoptosis, necroptosis, or necrosis, still remains unclear and is discussed in this review.

Semin Nephrol 32:394-404

Keywords: Podocyte, apoptosis, necrosis, necroptosis, autophagy

The development of glomerular sclerosis in several human and experimental diseases is associated with a decrease in the number of podocytes in the glomerular capillary tuft.[1-5] In particular, glomerular injury and proteinuria in diabetes (types 1 and 2)[4-8] and IgA nephropathy[3,9] are related to the degree of podocyte depletion in human beings. In agreement with these findings, a number of seminal studies in Japan have described podocalyxin-positive cells in urinary sediments of patients suffering from a variety of kidney diseases.[9-15] In addition, changes in semiquantitative measures of podocyturia seem to correlate directly with disease activity as assessed by biopsy and to decline with treatment,[16,17] suggesting that urinary shedding of podocytes may represent a real-time measure of podocyte loss from the glomerulus. Experimentally, the causal relationship between podocyte depletion and glomerulosclerosis was examined in toxic models of podocyte depletion. Sequential podocyte depletion by 15%, 31%, and 53% was achieved by the administration of one, two, or three, respectively, injections of puromycin aminonucleoside (PAN) at 30-day intervals in rats. Regions of the glomerulus devoid of podocytes developed glomerulosclerosis, and the sclerosis progressed with the ongoing depletion of podocytes.[1] Similar results were obtained by using a transgenic rat strain in which the human diphtheria toxin receptor was expressed specifically in podocytes.[18] In this model, glomerulosclerosis also was correlated to the extent of podocyte diphtheria toxin (DT)-induced depletion. By using a mouse model with podocyte expression of the human alpha chain of the IL-2 receptor (hCD25), which is the receptor for the immunotoxin LMB2 (a fusion of a single-chain Fv fragment of the CD25-specific, anti-Tac mAb to a truncated form of the bacterial *Pseudomonas* exotoxin A), Matsusaka et al[19,20] observed that, again, injection of the anti-Tac (Fv)-PE38 (LMB2) immunotoxin dose-dependently promoted podocyte foot process effacement, vacuolar degeneration, detachment, and down-regulation of synaptopodin, WT-1, nephrin, and podocalyxin, with subsequent focal segmental glomerular sclerosis. Furthermore, this group observed spreading of podocyte damage and detachment to podocytes that had not been targeted initially, suggesting that podocyte damage may propagate injury by triggering secondary damage of remnant podocytes.[20] During glomerular injury, podocytes retract and broaden their foot processes and may detach from the glomerular basement membrane. As a consequence of significant podocyte loss, the remaining podocytes are likely to fail to completely cover the outer surface of the glomerular basement membrane (GBM).

However, despite strong evidence that podocyte loss is a prerequisite for glomerulosclerosis, there is very limited insight into the nature of events that promote podocyte detachment. In particular, it is not clear whether

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Financial support: Supported by INSERM and by a grant from the Agence Nationale de la Recherche (grant ANR-08-EBIO-003 to P.L.T.), and by grants from the Deutsche Forschungsgemeinschaft (DFG), Bundesministerium für Bildung und Forschung (BMBF) Gerontosys II-NephAge (031 5896A to T.B.H.), as well as by the Excellence Initiative of the German Federal and State Governments (EXC 294).
Conflict of interest statement: none.
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0270-9295/

http://dx.doi.org/10.1016/j.semnephrol.2012.06.011

podocytes detach because they die or because of the primary loss of adhesion.

In other conditions, such as collapsing glomerulopathy (CGP) and crescentic glomerulonephritis (GN), podocyte loss occurs after a transient phase of proliferation. This could induce severe detrimental effects in such terminally differentiated cells, which are not equipped to face a sustained mitotic process.

We suggest that improved knowledge of the pathways that mediate podocyte loss should help identify potential upstream and downstream signals as novel therapeutic targets to protect against glomerular disease progression.

MECHANISMS OF PODOCYTE CELL DEATH

Three major morphologies of cell death have been described: apoptosis (type I), cell death associated with autophagy (type II), and necrosis (type III), with a recently described subcategory of regulated necrosis (necroptosis).[21-24] Apoptosis and cell death associated with autophagy can be distinguished by certain biochemical events. Morphologic changes associated with apoptosis are evident in the nucleus with chromatin condensation followed by nuclear fragmentation, zeiosis (membrane blebbing), and formation of intranuclear and extranuclear apoptotic bodies. The membrane-bound apoptotic bodies contain organelles that appear intact, unlike during autophagy, and some of these bodies contain nuclear fragments. As apoptosis evolves in cultured cells, massive cell swelling is observed, suggesting plasma membrane fragmentation. Necrosis is characterized mostly in negative terms by the absence of features associated with apoptosis, such as caspase activation, cytochrome c release, and DNA oligonucleosomal fragmentation. A particular difficulty in defining necrosis is that in the absence of phagocytosis, a situation that applies to podocytes, apoptotic cells can become secondary necrotic cells that have many morphologic features of primary necrosis.

APOPTOSIS

In the absence of a molecular marker for apoptosis, electron microscopy remains the gold standard for the identification of this elusive form of cell death.[25,26] Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) is a sensitive method, but it can generate false positives.[27,28] A critical point for the quantitation of apoptosis is that, irrespective of the initiating insult, the time-course of apoptosis is very fast.[29,30] Usually, the clearance of the resultant debris (either by professional phagocytes or bystander phagocytes) is rapid and completed within hours. In the putative case of podocyte apoptosis, phagocytosis is not likely to occur, but detachment may occur rapidly, resulting in a low probability that apoptotic cells are observed.

Nevertheless, the first signs of apoptotic cell death are a condensation of the nuclear material, with a marked accumulation of densely stained chromatin, typically at the edge of the nucleus. This is accompanied by cell shrinkage. Cytoplasmic condensation is accompanied by break up of the cell into a number of membrane-bound, ultrastructurally well-preserved fragments. Cytoplasmic blebs appear on the cell surface,[31] and the cell detaches from its neighbors. In the second stage, these apoptotic bodies are shed from epithelial-lined surfaces or are taken up by other cells. Eventually, the cells themselves fragment, with the formation of a number of membrane-bound apoptotic bodies. The morphologic features of apoptosis have been reviewed extensively.[21,32-34]

Evidence for Apoptosis in Experimental Diabetic Nephropathy

Evidence for TUNEL-positive podocyte death is scarce. In one study, positive TUNEL staining indicated that apoptosis was present in approximately half of the urinary podocytes examined.[35] Apoptosis of glomerular podocytes in situ has been described in animal models of progressive glomerular sclerosis,[36-38] at the onset of hyperglycemia in Ins2 (Akita) mice with type 1 diabetes and Lepr (db/db) mice with obesity and type 2 diabetes, rats after chronic infusion of angiotensin II (AngII),[39] and in the very specific transforming growth factor-β1 (TGF-β1) transgenic mice model. TGF-β1 and Smad7 each induce apoptosis in cultured podocytes, and their coexpression has an additive effect. Activation of p38 mitogen-activated protein kinase and caspase-3 is required for TGF-β–mediated apoptosis, but not for Smad7-mediated apoptosis, which inhibits nuclear translocation and transcriptional activity of the cell survival factor nuclear factor-κB (NF-κB).[38] Interestingly, in diabetic models, podocyte apoptosis coincided with the onset of urinary albumin excretion and preceded significant losses of podocytes in Akita (37% reduction) and db/db (27% reduction) mice.[40] Such events may be transient or specific to pathophysiological conditions, with few other reports of in vivo apoptosis of podocytes, such as in models with forced expression of PDGF-D[41] or deletion of the estrogen receptor.[42] In addition, it should be noted that the detected rates of TUNEL-positive podocytes in vivo have been very small.

Extrinsic Apoptosis Downstream the TNF-alpha Pathway

Some mechanistic insight is provided by the autosomal-recessive Alport nephropathy in collagen 4a3–deficient mice. This model is associated with increased intrarenal expression of the pro-apoptotic cytokine tumor necrosis factor-α (TNF-α) in glomerular cells, including podocytes as well as infiltrating leukocytes. Podocyte rarification develops over time with glomerulosclerosis. TNF-α dose-dependently promotes podocyte apoptosis in culture.[43] A beneficial effect of the TNF-α antagonist etanercept shown on a 5-week survival period was associated with significant improvement of the glomerulosclerosis score, proteinuria, and the glomerular filtration

rate at 9 weeks of age. Etanercept treatment specifically reduced the numbers of apoptotic podocytes (TUNEL+), increased total podocyte counts, and increased the renal messenger RNA expression of nephrin and podocin without affecting markers of renal inflammation.[44] Thus, this study suggests that some degree of "extrinsic apoptosis" may occur in podocytes of collagen 4a3–deficient mice. The term extrinsic apoptosis has been used extensively to indicate instances of apoptotic cell death induced by extracellular stress signals that are sensed and propagated by specific transmembrane receptors.[45,46] Extrinsic apoptosis can be initiated by the binding of lethal ligands, such as the apoptosis stimulating fragment FAS/CD95 ligand, TNF-α, and TNF (ligand) superfamily member 10 (best known as TNF-related apoptosis-inducing ligand), to various death receptors (ie, FAS/CD95, TNF-a receptor 1).[24] There is a current consensus that extrinsic apoptosis is a caspase-dependent cell death subroutine and hence can be suppressed by pancaspase chemical inhibitors. Notably, TNF-α stimulation of a human podocyte cell line did not lead to any detectable levels of phosphatidylserine exposure to the extracellular environment,[47] indicating that specific in vivo conditions such as specific glomerular basement membrane composition may be required to promote extrinsic apoptosis. Extrinsic apoptosis would feature one of three major lethal signaling cascades: (1) death receptor signaling and activation of the caspase-8 (or −10)–caspase-3 cascade; (2) death receptor signaling and activation of the caspase-8-truncated BH3 interacting-domain death agonist (tBID)- mitochondrial outer membrane permeabilization (MOMP)-caspase-9– caspase-3 pathway; or (3) ligand deprivation-induced dependence receptor signaling followed by (direct or MOMP-dependent) activation of the caspase-9–caspase-3 cascade.[24] Other stimuli such as increased oxidative stress also were shown to promote apoptosis of cultured podocytes.[40] Eight days after the onset of experimental crescentic rapidly progressive glomerulonephritis,[48] or after 28 days of chronic AngII infusion (unpublished data), we failed to find TUNEL-positive cells and electron microscopy features of apoptosis in mouse glomeruli, suggesting that apoptosis is scarce or an early event in these conditions. During aging, the amount of apoptotic cells assessed by TUNEL staining in 9-month-old rats was increased compared with 1-month-old rats. Apoptotic cells were rare overall but increased continuously with age. Interestingly, they almost always were found in tubules and interstitial cells, rather than in glomeruli.[49]

Taken together, experimental data indicate that apoptosis is observed much more often in cultured podocytes than in podocytes in vivo, where apoptosis has been reported only under a few conditions and at very low rates. This might suggest that podocyte apoptosis is not a general pathway of podocyte loss in vivo, and it remains unclear whether podocytes are shed because they undergo necrosis or apoptosis in situ, or if they still are viable when they detach from the glomerular basement membrane.

Loss of Mitotic Arrest, Abnormal Balance of Cell-Cycle Proteins

As reviewed elsewhere, during development, podocytes lose their mitotic activity concurrent with the formation of foot processes and the expression of specific makers.[50] At the capillary loop stage, a switch of expression in cyclins and cyclin-dependent kinase inhibitors is observed with the disappearance of cyclin A, B1, and D1 along with up-regulation of p27 and p57 and specific podocyte markers.[51] Thus, podocytes are locked in the G1 arrest of the cell cycle. In the context of crescentic glomerulonephritis, epithelial cells forming the crescent were shown to be negative for p27 and p57 and positive for proliferating cell nuclear antigen (PCNA).[52] Expression of cyclin-dependent kinases such as cyclin-dependent kinase (CDK)2 is enhanced in experimental crescentic glomerulonephritis in mice, and diminution of CDK2 activity with the purine analogue roscovitine ameliorates histopathologic damage.[53,54]

CGP was recognized early as an entity distinct from focal segmental glomerulosclerosis (FSGS).[55] Podocyte injury in CGP is characterized by a dedifferentiated phenotype as indicated by the loss of expression of maturity markers, re-expression of proliferative markers (Fig. 1)[52,56,57] and the acquisition of a macrophage-like phenotype.[58,59] These features are partially reminiscent of crescentic GN with abnormal distribution of WT1 and PAX2 and extensive loss of podocyte markers, as reported in idiopathic collapsing glomerulopathy and human immunodeficiency virus (HIV)-associated nephropathy. This

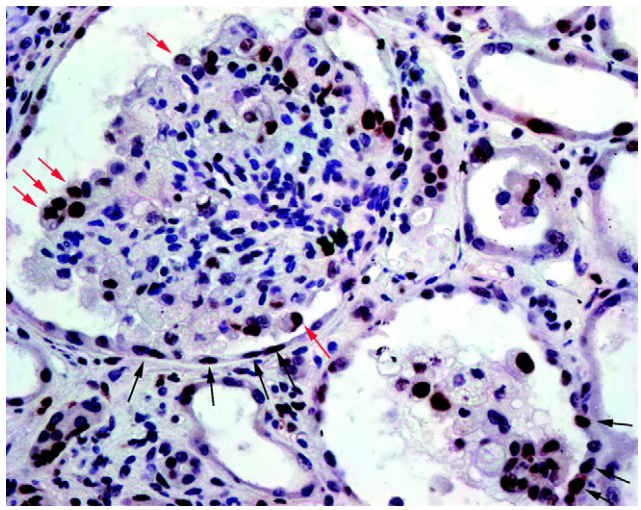

Figure 1. PCNA (Proliferating Cell Nuclear Antigen) staining in FSGS. In the upper glomerulus, black nuclear positivity is seen in numerous podocytes at the periphery of the glomerular tuft (red arrows), as well as in parietal epithelial cells (black arrows). In the lower glomerulus, several parietal epithelial cells are marked as well as a cell of macrophagic appearance adjacent to the tuft, likely of podocyte origin. Original magnification x350. Image courtesy of Gary S. Hill and Dominique Nochy.

deregulation was associated with podocyte proliferation (PCNA+) without detectable apoptosis (TUNEL).[60] In contrast, no podocyte changes were detected in minimal change nephrotic syndrome (MCNS) or membranous glomerulonephritis (MGN). Interestingly, in children with minimal change disease and classic FSGS, Srivastava et al[61] showed that podocytes are physiologically unable to overcome the G1/S transition phase, in contrast to the deregulated proliferative phenotype of idiopathic CGP in adults. This interpretation was supported by observation of de novo cyclin A staining in children with CGP only, by comparison with children with FSGS and minimal change disease. The staining of both cyclins D and A was increased and p27 and p21 was decreased in CGP, suggesting that during CGP, podocytes are able to overcome the G1/S transition phase.[62] A pathophysiological role of activation of cyclins has been shown in a mouse model.[63] The upstream promoters of podocyte proliferation in CGP still are unknown, although a src-Sta3–mitogen-activated protein kinase–vascular endothelial growth factor–vascular endothelial growth factor receptor 2/neuropilin-1 was shown to be involved in nef-transfected podocytes and transgenic mice.[64-66] Notably, in mouse embryonic kidneys, transgenic HIV mice, and rats with experimental membranous nephropathy (passive Heymann nephritis), there was an increase in podocyte immunochemical expression of mitotic cell-cycle proteins Cdc2, cyclin B1, and B2, and phosphorylated histone 3 in passive Heymann nephritis rats and in HIV transgenic mice.[67] Thus, mitotic cell-cycle proteins also may increase in podocytes in conditions with mild or lack of proliferation.

It still is unknown whether these features of HIV-associated nephropathy are generalizable to other CGP and to crescentic GN. The role of the epidermal growth factor receptor (EGFR) pathway, a potent promoter of crescent formation,[48] should be evaluated in this specific context as well. It is uncertain how this pathway would lead to podocyte death. For example, heparin binding-EGF (HB-EGF)/EGFR activation promotes proliferation[48] and anchorage-independent growth in podocytes, as may stat3 in nef-expressing podocytes.[68] Nevertheless, histopathologic evidence suggests that neither in the case of crescentic glomerulonephritis, nor in collapsing glomerulopathy, do podocytes proliferate indefinitely. Decellularization of glomeruli occurs over time, indicating that some mode of cell death should take place.

MITOTIC CATASTROPHE

Interestingly, restrictions in a cell's ability to halt the cell cycle can cause mitotic catastrophe. Indeed, during mitosis, cells segregate duplicated chromosomes with high fidelity to maintain genome stability. Proper attachment of sister kinetochores to spindle microtubules is critical for accurate chromosome segregation and is driven by complex mechanisms that promote the capture of unattached kinetochores and the resolution of erroneously attached kinetochores.[69,70] Defects in these surveillance systems promote failing mitoses that often are associated with chromosomal breaks and deficient karyokinesis, which lead to gross nuclear alterations (micronucleation and multinucleation) that constitute the most prominent morphologic traits of the mitotic catastrophe. However, features of apoptosis and necrosis also have been observed in cells dying from mitotic failure, raising the possibility that mitotic catastrophe might constitute a prelude to apoptotic or necrotic cell death rather than a cell death mechanism.[71,72] Vakifahmetoglu et al[72,73] observed that activation of p53 by cellular stress caused by cisplatin in cultured ovarian carcinoma cells may lead to either cell-cycle arrest or apoptotic cell death. Functional p53 and caspase-2 were required for the apoptotic response. In the absence of functional p53, cisplatin treatment resulted in caspase-2–independent mitotic catastrophe, followed by necrosis. Nevertheless, scarce but noticeable evidence suggests that a mitotic catastrophe occurs in cultured human glomerular progenitor cells upon activation of Notch.[74] Similarly, Kriz et al[75] encountered features of "abortive mitosis" in a rat model of FSGS, which is highly suggestive of mitotic catastrophe in podocytes.

Thus, the final mode of cell death may be determined by the profile of proteins involved in the regulation of the cell cycle. For example, complex spatiotemporary regulation of cyclin-dependent kinase 1 (Cdk1) contributes to the mitotic prophase and metaphase but also could lead to apoptosis or to mitotic catastrophe if prematurely activated.[76] We refer to excellent recent reviews on this phenomenon.[77] Whether such a definition applies to terminally differentiated cells such as podocytes requires further investigation. Interestingly, the Stuart Shankland's laboratory found that high levels of cyclin I in podocytes activated Cdk5 in isolated mouse podocytes and neurons, and they proposed that cyclin I-Cdk5 activates the mitogenactivated protein kinase (MAPK)/extracellular signal regulated kinase (ERK) kinase pathway and results in increased Bcl-2 and Bcl-X(L) messenger RNA and protein levels. This pathway was found to be defective in mice with increased caspase-3 activation in cyclin I–deficient podocytes after experimental glomerulonephritis induced by the administration of antiglomerular serum.[78] In fact, lack of cyclin I renders podocytes more susceptible to apoptosis in vitro and in vivo, a feature not observed in mesangial or in tubular cells.[54] In a nonproliferative model of glomerulopathy, the fa/fa Zucker rat, increased expression of cyclin-dependent kinase inhibitors co-exists with vacuolation of podocytes, suggesting promotion of cell death.[50]

We thus suspect that proliferation and quiescence are the result of different podocyte survival strategies. A quiescent phenotype with a G1 arrest of the cell cycle is compatible with high autophagic flux, whereas proliferative pathways restrain autophagy and disorganize the specific podocyte cytoskeleton. Two fates then can be predicted and should be investigated in further depth: (1)

forced progression to G1/S would require loss of polarity protein complexes,[79] formation of a mitotic spindle, and loss of foot processes and detachment; and (2) forced mitosis in such a terminally differentiated postmitotic cell type also may lead to abnormal mitosis and genomic instability. Overall, even in diseases associated with transient proliferation of podocytes, loss of podocytes may occur not only though dedifferentiation but also through mitotic catastrophe and detachment. Molecular pathways still need to be deciphered in more detail in the podocyte.

MORPHOLOGIC AND ULTRASTRUCTURAL CHARACTERISTICS OF PODOCYTE INJURY AND LOSS

Because available biochemical and fluorescent data are not conclusive with respect to the mode of podocyte cell death in vivo, the description of specific morphologic features of damaged podocytes is important for understanding the sequence of events leading to podocyte detachment and loss.

Mitochondrial Damage

Podocytes are characterized by a prominent nucleus, a well-developed Golgi system and endoplasmic reticulum, and abundant mitochondria. The latter was attributed to the high energy demand of podocytes to maintain various cellular functions. Mitochondria play a primary role in maintaining podocyte energy homeostasis[80] and represent one of several possible sources of cellular reactive oxygen species (ROS). The apoptotic demise of cells can be triggered by a plethora of intracellular stress conditions, including DNA damage, oxidative stress, cytosolic Ca^{2+} overload, mild excitotoxicity (related to glutamate-receptor overstimulation in the nervous system), accumulation of unfolded proteins in the endoplasmic reticulum, and many other factors. Although the signaling cascades that trigger intrinsic apoptosis are highly heterogeneous as far as the initiating stimuli are concerned, they are all wired to a mitochondrion-centered control mechanism.[81] Frequently, along with the propagation of the pro-apoptotic signaling cascade, anti-apoptotic mechanisms also are engaged in an attempt to allow cells to cope with stress. In this scenario, both proapoptotic and antiapoptotic signals converge at mitochondrial membranes, which become permeabilized when the former predominate over the latter.[81] Furthermore, the mitochondrial copy number has been shown to be decreased in focal segmental glomerulosclerosis induced by PAN.[82] However, the number of podocyte mitochondria was reported to be increased during the early stage of diabetic nephropathy. Dysmorphic mitochondria frequently are observed in podocytes, and further studies are required to evaluate potential significance.

In fact, the impact of mitochondrial dysfunction is equivocal. Necrotic cell death represents a rapid cellular response involving the production of mitochondrial ROS, decreased adenosine triphosphate concentration, and other cellular insults, whereas autophagic cell death first starts as a survival attempt by the clean up of ROS-damaged mitochondria.[83] Major MOMP constitutes a point-of no-return of intrinsic apoptosis.[81]

Genetic alteration of the mitochondrial genome proved to influence podocyte fate rather than other glomerular cells. An A to G transition at position 3243 in mitochondrial DNA has been described mainly in association with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes or progressive external ophthalmoplegia. However, two recent studies of patients carrying the A3243G mitochondrial DNA mutation with FSGS reported severely damaged podocyte changes containing extraordinary dysmorphic mitochondria, which may participate in the development of FSGS.[84,85] It should be noted that in these specimens, abnormal mitochondria were accumulated exclusively in podocytes, whereas both mesangial cells and capillary endothelial cells in glomeruli appeared intact. Because podocytes are highly differentiated terminal cells that do not undergo cell division in the postnatal period, these cells may be susceptible to the accumulation of mutated mitochondria, similar to neural and muscle cells. Because of the accumulation of these abnormal mitochondria, mitochondrial protein synthesis and energy supply may be impaired,[85] leading to the induction of podocyte dysfunction followed by the development of FSGS. However, mitochondrial dysfunction can lead to collapsing GN and crescentic GN. This is the case in primary coenzyme Q_{10} (CoQ_{10}) deficiency owing to mutation in the coenzyme Q2 homolog (COQ2) gene that encodes the parahydroxybenzoate-polyprenyl-transferase enzyme of the CoQ_{10} synthesis pathway.[86] COQ2 nephropathy should be suspected when electron microscopy shows an increased number of abnormal mitochondria in podocytes and other glomerular cells. Interestingly, in the mouse kd/kd model of collapsing glomerulopathy, electron micrographs showed collapsed capillaries, extensive foot process effacement, and dysmorphic mitochondria in podocytes.[86] In this model, mitochondrial dysfunction occurs as a result of a mutation in the gene encoding for a prenyltransferase-like mitochondrial protein that has extensive homologies with the human transprenyltransferase (*PDSS2*) gene, which is involved in the CoQ_{10} synthesis pathway.

Autophagy

Autophagy is an evolutionarily conserved lysosomal process wherein a cell degrades its own cytoplasmic contents.[87,88] The term *autophagy* was coined by Christian de Duve soon after his discovery of lysosomes.[89] Autophagy is extremely important in housekeeping, particularly in terminally differentiated cells such as neurons[88-90] and podocytes.[91] Notably, podocytes were identified as the cell type with the highest autophagic activity in the kidney.[91,92] Accumulating evidence indicates that the exact role of autophagy in cell survival versus death is both

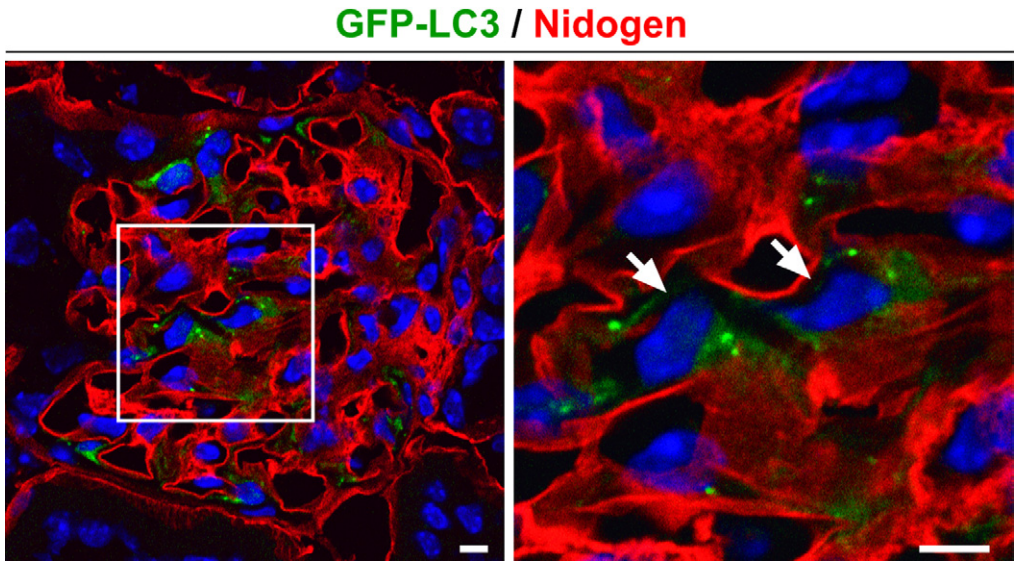

Figure 2. Transgenic green fluorescent protein (GFP)-LC3 mouse model showing the high number of GFP-positive dots, indicating autophagosomes, in podocytes. Right panel: higher magnification view of a field within the white square box in the left panel. Clusters of GFP-LC3 fluorescent molecules are visualized in autophagosomes in podocytes (white arrows). Scale bars: 5 μm. Image courtesy of Björn Hartleben.

stimulus- and context-dependent.[93,94] Indeed, autophagy is well recognized as a survival mechanism during nutrient limitation: through the bulk degradation of cytoplasmic material, autophagy generates both nutrients and energy in starving cells.[87,88] Accordingly, during nutrient starvation, inhibition of autophagy promotes apoptosis,[95] and anti-apoptotic Bcl2 inhibits beclin1-dependent autophagy.[94,96] In contrast, excessive autophagy has been proposed to mediate autophagic or type 2 programmed cell death. For example, L929 fibrosarcoma cells die in the absence of caspase activity with involvement of autophagy; autophagy related 7 homolog (*ATG7*) and *beclin-1* genes are required for this death process.[97] In this model, caspase inhibition induces the selective autophagic degradation of catalase, a major ROS scavenger, and the resulting ROS accumulation promotes type 2 programmed cell death.[97,98] Thus, caspase inhibitors may arrest apoptosis but also have the unanticipated effect of promoting autophagic cell death in certain conditions. Interestingly, accumulation of autophagic vacuoles can precede apoptotic cell death in several cell types or necroptosis in acute lymphoblastic leukemia cells.[99] However, mutual exclusion between the two processes, apoptosis and autophagy, as described earlier, is common. For example, RAGE is a positive regulator of autophagy and a negative regulator of apoptosis during oxidative stress in pancreatic cancer cells.[100] This deserves to be investigated in podocyte diseases.[101,102] Interestingly, glomerular podocytes display very high levels of basal autophagic activity that appear to be crucial for intracellular protein quality control. Importantly, a negative association between PAN-induced nephrosis and the amount of microtubule-associated protein 1 light chain 3 alpha (LC3) in podocytes was recognized.[91] Levels of LC3-II, a marker of autophagosomes, (Fig. 2) rapidly decrease in podocytes damaged by PAN but increase during their recovery from the damage,[91] suggesting an association of autophagy with healthy and differentiated cell status. Beyond this seminal observation, recent mechanistic evidence has suggested that podocyte autophagy is a protective pathway in glomerular disease and aging.[92] Podocyte-specific deletion of the *Atg5* gene therefore leads to the accumulation of damaged mitochondria oxidized proteins and protein aggregates. This loss of cellular protein homeostasis in autophagy-deficient podocytes results in a dramatic acceleration of glomerular diseases, identifying autophagy as a key homeostatic mechanism to maintain podocyte integrity under physiological and stress conditions.[92] These recent findings underscore the need to decipher the network of local regulators of autophagy in podocytes.

In podocytes, co-activation of the TGF-β1 pathway through engagement of the Notch ligand Jagged 1 was reported to induce apoptosis, resulting in cell depletion and FSGS.[102] AngII, a known inducer of TGF-β activity,[103] also was found to increase staurosporine-induced apoptosis in vitro,[104] likely via the calcineurin-NFAT (nuclear factor of activated T-cells) cascade.[105] Increase in the calcium influx in response to AngII, caused by mutation of the transient receptor potential canonical (TRPC6) channel, promotes excessive podocyte apoptosis acutely[106,107] and FSGS over time.[108] Interestingly, some features evoking AngII-dependent podocyte apoptosis have been found in vivo,[39] particularly after a first initial diphtheria toxin–induced podocyte injury,[109] suggesting again that AngII may be more of a co-factor than a trigger of apoptosis. Paradoxically, AngII may acutely promote autophagy in cultured podocytes,[103] and in vivo investigations thus are required. More in-depth examina-

Table 1. Summary of the Currently Suspected Death Modes of Podocytes

Main Suspected Death Modes for Podocytes	Main Biochemical Description
Anoikis	Lack of β1 integrin activity
	Overexpression of BIM (Bcl-2 interacting mediator of cell death)
	Caspase 3 activation
	Inhibited by B-cell leukemia-2 (Bcl-2) expression or caspase Inhibition by N-benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone (Z-VAD-fmk)
	Down-regulation of the EGFR pathway?
Caspase-dependent intrinsic apoptosis	Activation of Bcl-2 homolog region (BH)3-only proteins, which activate or de-repress the BH1–3 proteins, such as Bax and Bak, producing MOMP
	Loss of mitochondrial transmembrane potential
	Unligated integrin β3 (ITGB3) induced apoptosis by recruitment of caspase-8
	Inhibited by Bcl-2 expression or caspase inhibition (Z-VAD-fmk)
Caspase-independent intrinsic apoptosis	Accumulation of apoptosis-inducing factor (AIF) and endonuclease G proteins in the intermembrane space of mitochondria
	Inhibited by Bcl-2-family proteins
Extrinsic apoptosis caused by death receptors	Death-receptor activation
	BH3 interacting-domain death agonist (BID) cleavage
	MOMP
	Caspase 3 and caspase 8 activation
	Inhibited by caspase 3 and 8 inhibition or Z-VAD-fmk
Extrinsic apoptosis caused by death receptors	Ras hyperactivation
	Lamp-1–positive vacuoles
	No morphologic features of apoptosis
Methuausis	Ras hyperactivation
	Lamp-1–positive vacuoles
	No morphologic features of apoptosis
Necroptosis	Death-receptor signaling with receptor-interacting protein 1 or 3 (RIP1 or RIP3) activation
	No caspase 8 activation
	Receptor tyrosine kinase, growth factors, overstimulation?
	Inhibited by necrostatins or genetic alteration of RIP1 and RIP3
Autophagic cell death	High LC3-phosphatidylethanolamine content
	P62/sequestosome 1 accumulation
	Inhibited by genetic alteration of Ambra1 or Beclin1 or Atg5, Atg7, Atg12 genes
Mitotic catastrophe	Leads to necrosis or apoptosis
	Mitotic arrest
	Aneuploidy
	Caspase 2 activation
	Tumor protein p53 or transactivating isoform of p73 (TP73) activation (in some cases)
	Inhibited by p53 or TP73 deficiency (in some cases) or by caspase 2 inhibition

tion of the potential regulation of autophagy and apoptosis by potent inducers of podocyte damage, such as AngII and endothelin-1 (ET-1), is required. Indeed, podocytes show functional AngII and ET-1 receptors,[110-114] as well as EGFR,[48,115] a potent amplifier of their signaling cascades. Transactivation of the EGFR by the AT1-type AngII receptor or the ETA-type ET-1 receptor[115-117] may in fact inhibit autophagy through activation of the ras-type I phosphoinositide 3 (PI3)-kinase–mammalian target of rapamycin (mTOR) pathway.[118-122] These factors may apply to other tyrosine kinase receptors such as the insulin receptor, because podocyte-specific deletion of the insulin receptor was sufficient to promote glomerulosclerosis.[123] Meanwhile, activation of the EGFR also has been suggested to stimulate autophagy in nonrenal cells.[124-127] Thus, whether part of the nephroprotective actions of anti-AngII, anti–ET-1, or anti-EGFR therapeutic strategies is owing to stimulation of podocyte autophagy remains an open question.[91,92] In part, anti-apoptotic action of autophagy could be attributed to enhanced clearance of mitochondria by autophagy, thereby reducing cytosolic cytochrome c release and downstream caspase activation after pro-apoptotic insults.[128] Therefore, stimulation of autophagy could be a promising strategy for the treatment of podocytopathies.

Resistance to Anoikis, Modulating Podocyte Capacity to Survive in Diseases

Evidence from Lemley's[35] laboratory indicates that nearly half of the podocytes collected from urine of normal subjects are viable. Moreover, it is possible to culture and immortalize some of these cells.[129] The difference in growth behavior between healthy controls and subjects with active glomerular disease suggests that in active disease, viable podocytes detach from the glomer-

ular tuft because of local environmental factors, whereas in healthy individuals it is mostly senescent podocytes that are shed. Thus, detached podocytes may be resistant to anoikis. Anoikis, which literally means "the state of being homeless," is a term of ancient Greek derivation that was introduced by Frisch and Francis[130] in 1994 to describe the apoptotic response of adherent cells owing to the absence of cell-to-matrix interactions.[131] The survival of nontransformed adherent cells does indeed depend on signals transduced by integrins and by some growth factor receptors upon interaction with the extracellular matrix. In some cancer cells, the resistance to anoikis of epithelial cancer cells sustains invasiveness and metastasis. Thus, we hypothesize that during rapidly progressive glomerulonephritis and collapsing GN, transient podocyte proliferation with loss of anatomic patterns may be owing to loss of anoikis with abnormal attachment to the GBM, an effect in part mediated by activation of the EGFR.[48,132] In addition, although stimulation of the TNF receptor may lead to apoptosis (see earlier), de novo expression of the TNF receptor 2 and the activated NF-κB pathway was detected in podocytes before hyperplasic injury in crescentic glomerulonephritis of mice after nephrotoxic nephritis and in collapsing glomerulopathy of Tg26 (HIV/nl) mice, kd/kd mice, and human beings.[133] In this context, some anti-apoptotic pathways may synergize with the activated NF-κB pathway to promote survival and proliferation.

SUMMARY AND OUTLOOK

In summary, although apoptosis and necroptosis both have been observed in podocyte cell lines and primary cultures, our understanding of the modes of active podocyte death in situ/in vivo are based on a handful of studies. A salient finding of the recent decade is that podocytes, some of which still are viable, detach from the GBM under both physiological and pathologic conditions. In the context of nonproliferative scarring glomerulopathies such as FSGS, it is not clear whether detachment occurs primarily and independently of a cell death pathway. Evidence for primary or secondary (anoikis) apoptosis is scarce, possibly because apoptosis unavoidably would lead to rapid podocyte detachment. Another important finding is the recognition of high autophagic activity in podocytes. In chronic conditions such as aging and toxic challenge to podocytes, disruption of autophagy accelerated glomerular damage with extensive vacuolar degeneration of podocyte cell bodies and foot process fusion. It will be important to address whether susceptibility to glomerular aging or scarring disease is influenced by genetic or environmental determinants of autophagy.

At the other extremity of the spectrum of podocytopathies, in collapsing glomerulopathy and in inflammatory extracapillary glomerulonephritis complicating vasculitis, loss of polarization of podocytes and extensions of parietal cells leads to the loss of the separation between the tuft and Bowman's capsule by forming cell bridges between the glomerular and the parietal basement membranes. In this context, it is unclear whether autophagy is involved. Some degree of proliferation is observed in the formation of epithelial crescents, along with a switch in the podocyte phenotype. This switch, associated with re-expression of a fetal gene program, may be incomplete, with progression through the G1/S transition phase but an inability to sustain normal mitosis. Understanding the sequence of events leading to podocyte injury and defining the exact point of no return, beyond which cytoprotection can no longer be achieved, will be important for future podocyte research. (Table 1).

ACKNOWLEDGEMENT

We apologize to the colleagues whose work was not cited because of length restrictions. We thank all members of our laboratories for their support and helpful discussions, Björn Hartleben for help with the figure, and Dr. Cécile Fligny and Suja Thomas for critically reading the manuscript.

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