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# Programmed Cell Death in Diabetic Nephropathy: A Review of Apoptosis, Autophagy, and Necroptosis

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Diabetic nephropathy is a common complication of type I and type II diabetes, in which renal glomeruli are destroyed, resulting in renal damage, proteinuria, and hypertension. Apoptosis, autophagy, and necroptosis are 3 forms of programmed cell death that have been implicated in the pathogenesis of diabetic nephropathy. Apoptosis of podocytes leads to glomerular injury and podocyte depletion, which are associated with proteinuria and glomerular structural damage in diabetic nephropathy. Additionally, epithelial cells in the proximal convoluted tubules also undergo apoptosis in diabetic nephropathy, leading to tubular atrophy, which causes tubular cell depletion and the subsequent formation of atubular glomeruli in association with the loss of renal function. On the other hand, insufficiency of autophagy has been correlated with the pathogenesis of diabetic nephropathy. For instance, decreased autophagic activity has been shown in podocytes of the diabetic kidney, causing variations in podocyte function and subsequent disruption to the glomerular filtration barrier. Furthermore, attenuated autophagic activity has also been demonstrated in proximal tubular cells of the diabetic kidney, resulting in the buildup of impaired molecules and organelles, which are normally broken down by autophagy, leading to proteinuria. Moreover, necroptosis might have a key role in podocyte damage and subsequent decline in diabetic nephropathy. Thus, this article aims to review the mechanisms and effects of programmed cell death in diabetic nephropathy, including the roles of apoptosis, autophagy, and necroptosis.

**Keywords:** **Apoptosis • Diabetic Nephropathies • Review • Necroptosis • Hyperglycemia • Autophagy**

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## Background

Diabetes is a metabolic disorder that is characterized by hyperglycemia, which is high blood glucose level, as a consequence of deficiencies in insulin action, insulin secretion, or both [1]. Chronic hyperglycemia results in the dysfunction and failure of different organs, particularly the heart, blood vessels, eyes, and kidneys [2]. Diabetes can be classified into 3 types: type I, type II, and gestational diabetes [3]. Type I diabetes is insulin-dependent diabetes [4], whereas type II diabetes is non-insulin-dependent diabetes [5]. On the other hand, gestational diabetes is characterized by glucose intolerance that starts or that is recognized during pregnancy [3,6], and it increases the risk of development of type II diabetes [7,8]. Diabetes can have both microvascular and macrovascular complications, including nephropathy, cardiomyopathy, retinopathy, and peripheral neuropathy [1].

Diabetic nephropathy is a clinical syndrome, which is regarded as the leading cause of end-stage renal disease (ESRD) [9-11]. Diabetic nephropathy is characterized by albuminuria, which is increased albumin excretion in the urine [12]. Albuminuria is indicative of significantly increased cardiovascular mortality and morbidity in either type I or type II diabetes [13]. Albuminuria can be classified into 2 stages – macroalbuminuria and microalbuminuria – according to the amounts of albumin excreted in the urine [13]. Albumin excreted in the urine exceeds 200g/min in macroalbuminuria, and it is less than 199 g/min in microalbuminuria [13].

Diabetic nephropathy develops through several distinct phases [13]. Initially, functional alterations appear in the nephron at the level of the glomerulus, starting as glomerular hyperfiltration and hyperperfusion [14]. Then, the glomerular basement membrane thickens, and glomerular hypertrophy and sclerosis occur due to intraglomerular hypertension, and mesangial expansion that is caused by extracellular matrix accumulation [15].

The main risk factors for the progression of diabetic nephropathy comprise hyperglycemia, hypertension associated with obesity, and metabolic syndrome [13]. Diabetic nephropathy can be screened at the time of diagnosis in patients with type II diabetes by several methods, including measuring albumin-to-creatinine ratio in a random spot urine sample [16].

Apoptosis, autophagy, and necroptosis are 3 forms of programmed cell death that are implicated in the pathogenesis of diabetic nephropathy [17,18]. Apoptosis occurs via 2 well-characterized pathways, which are the extrinsic and intrinsic pathways [19]. Both apoptotic pathways are mediated by initiator and executioner caspases [20-22]. Apoptosis takes place in podocytes and epithelial cells of proximal convoluted

tubules in association with the development of diabetic nephropathy [23,24].

Autophagy means self-eating and it is necessary for maintaining cell homeostasis under many stress circumstances [25]. Autophagy has been classified into macroautophagy, microautophagy, and chaperone-mediated autophagy [26]. Impaired autophagy in podocytes and proximal tubular cells has been correlated with the pathogenesis of diabetic nephropathy [27-29].

On the other hand, necroptosis is a programmed form of necrosis [30]. It might have a key role in podocyte damage and decline in diabetic nephropathy, particularly because it has been shown to be triggered subsequent to the inhibition of apoptosis that is induced by hyperglycemia [31].

Therefore, this article aims to review the mechanisms and effects of programmed cell death in diabetic nephropathy, including the roles of apoptosis, autophagy, and necroptosis.

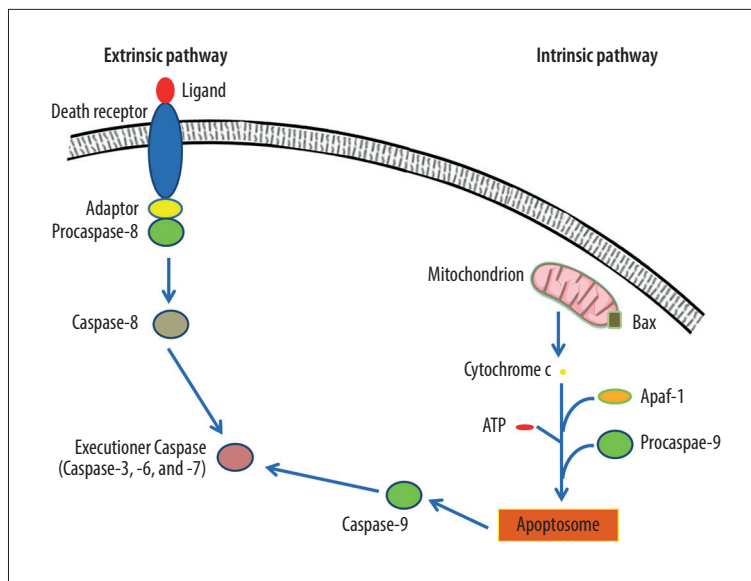
## Apoptosis

Apoptosis is also called type I programmed cell death [32]. It is morphologically characterized by cell shrinkage, chromatin condensation, nuclear deoxyribonucleic acid (DNA) fragmentation, and the formation of apoptotic bodies, which are eventually removed via phagocytosis, preventing the elicitation of any inflammation [33-35].

The initiation and execution of apoptosis are carried out by caspases, which are cysteine proteases that are specific for aspartate [36]. Caspases normally exist in the cytoplasm as inactive zymogens, which can be stimulated via cleavage [32,37]. Apoptotic caspases are functionally classified into initiator caspases (caspases-8, -9, and -10) and executioner caspases (caspases-3, -6, and -7) [38-40]. The initiator caspases are triggered by upstream signals, such as external cell death molecules and internal stress [20]. Initiator caspases are activated by autocleavage, and they subsequently stimulate the downstream executioner caspases [21,22].

There are 2 well-characterized apoptotic pathways (**Figure 1**), which are the extrinsic pathway (death receptor-mediated pathway) and the intrinsic pathway (mitochondria-mediated pathway) [19].

In the extrinsic pathway, the apoptotic signaling is initiated by the binding of extracellular ligands to the transmembrane domains of the extracellular death receptors [32]. Extracellular ligands include tumor necrosis factor (TNF) and TNF-related apoptosis-inducing ligand (TRAIL) that bind to type 1 TNF receptor (TNFR1), and TRAIL receptor, respectively [41]. Consequently,



**Figure 1.** Extrinsic and intrinsic pathways of apoptosis. The extrinsic apoptotic pathway is triggered by the binding of a particular death ligand to death receptor, leading to its trimerization and the consequent recruitment of procaspase-8, which is subsequently activated. In contrast, the intrinsic apoptotic pathway starts in response to apoptotic stimuli that induce pro-apoptotic proteins, such as B-cell lymphoma 2-associated X protein (Bax), triggering the permeabilization of the outer mitochondrial membrane, and the consequent release of cytochrome c into the cytosol, where it contributes to the formation of a multimeric apoptotic peptidase activating factor 1 (Apaf-1)/cytochrome c complex that recruits procaspase-9, forming the apoptosome. Hence, procaspase-9 is activated and consequently detached from the complex. Ultimately, active caspase-8 and active caspase-9 activate executioner caspases-3, -6, and/or -7, which mediate apoptosis. *This figure was adapted with permission from John Wiley and Sons, from "Apoptosis and its therapeutic implications in neurodegenerative diseases" by Nour S. Erekat, 2022, Clinical Anatomy [34].*

death receptors trimerize and engage adaptor molecules to their death domains, leading to the stimulation of caspase-8, which then activates the executioner caspases that mediate the proteolytic events of the apoptotic cell death, leading to destruction of the nucleus and other intracellular structures [41,42].

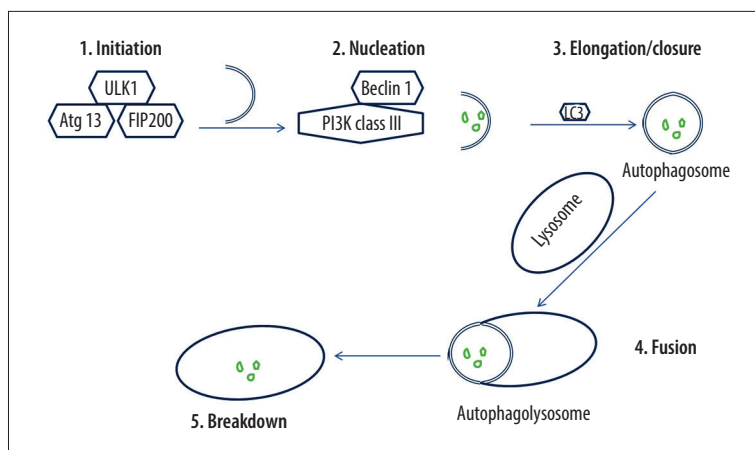
The intrinsic pathway is triggered by cellular stresses, such as DNA impairment and metabolic stress [43]. These stimuli cause an alteration in the equilibrium between pro-apoptotic B-cell lymphoma 2 (Bcl-2) family members, such as Bcl-2-associated X protein (Bax) and Brassinosteroid-insensitive 1 (BRI1)-associated kinase (Bak), and anti-apoptotic members, such as Bcl-2 and B-cell lymphoma-extra-large (Bcl-xL) [44]. Consequently, pro-apoptotic Bcl-2 family members accumulate on the outer membrane of mitochondria, resulting in mitochondrial outer membrane permeabilization (MOMP) [45]. Thus, cytochrome c is discharged from the mitochondrial intermembranous space to the cytoplasm [45]. Once in the cytoplasm, cytochrome c participates in generation of a multimeric apoptotic peptidase activating factor 1 (Apaf-1)/cytochrome c complex by binding to Apaf-1 in the presence of deoxyadenosine triphosphate (dATP) or adenosine triphosphate (ATP) [46]. Subsequently, the resultant complex recruits and activates caspase-9, leading to production of the apoptosome and consequent dissociation of active caspase-9, which cleaves and consequently stimulates the executioner caspases-3 and -7 [47,48].

Tumor suppressor protein (p53) is a transcription factor that is physiologically maintained at low levels [49-51]. P53 can stimulate apoptosis, principally depending on its transcriptional activity [52]. P53 can trigger the intrinsic apoptotic pathway by activating the transcription of many pro-apoptotic genes, such as the genes encoding Bax, phorbol-12-myristate-13-acetate-induced protein 1 (Noxa), and p53-upregulated modulator of

apoptosis (Puma), which are pro-apoptotic homology (BH-3)-only Bcl-2 proteins [52]. Additionally, p53 stimulates the expression of Apaf-1, causing formation of the apoptosome [52]. On the other hand, p53 can repress anti-apoptotic genes, thus inducing apoptosis [52]. Moreover, p53 can trigger the extrinsic apoptotic pathway by activating the transcription of F57-associated cell surface antigen (Fas) [52,53].

## Autophagy

Autophagy is referred to as type II programmed cell death, and it means "self-eating" [54,55]. Normally, it happens at low basal levels, being essential for the clearance of impaired proteins and long-lived organelles [56]. Autophagy is essential for maintaining cell homeostasis under many stress circumstances [25]. Autophagy has been classified into 3 types, which vary in their mechanisms and functions [26]. Those 3 types are macroautophagy, microautophagy, and chaperone-mediated autophagy [26]. Macroautophagy is the predominant type, and it is denoted as "autophagy" [26].



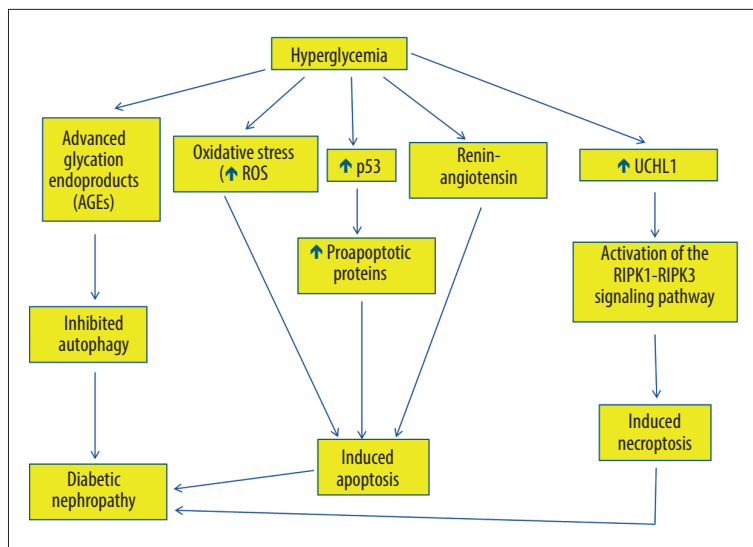
**Figure 2.** The process of autophagy. The autophagic process occurs via 5 major stages, which are initiation, nucleation, elongation/closure, fusion, and breakdown. Initiation stage: The unc51-like kinase 1 (Ulk1) complex consists of Ulk1 serine/threonine protein kinase, autophagy-related gene 13 (Atg13), and focal adhesion kinase family interacting protein of 200 kD (FIP200). Ulk1 is required for the initiation stage. 2. Nucleation stage: Beclin 1, which exists with class III phosphoinositide 3-kinase (PI3K), is essential for the nucleation stage. 3. Elongation/closure stage: A membrane sac isolation membrane wraps around the degradation substrates and ultimately closes to become an autophagosome. This stage requires the microtubule-associated protein light chain 3 (LC3). 4. Fusion stage: the outer membrane of the autophagosome completely fuses with the outer lysosomal membrane. 5. Breakdown stage: contents of the autophagosome are released and subsequently degraded into the lysosomal cavity. This figure was generated using Microsoft PowerPoint Software, version 10, Microsoft Corp., USA.

Isolation membranes used in autophagy are assumed to derive from the endoplasmic reticulum (ER) membrane, and they lengthen and fuse, forming autophagosomes [57]. Autophagosomes engulf a portion of the cytoplasm [58]. Hence, the autophagic process occurs via 5 major stages (Figure 2), which are initiation, nucleation, elongation/closure, fusion, and breakdown [59]. Throughout these stages, autophagy-related genes (Atg) and proteins are implicated [60]. For instance, the mammalian ortholog of yeast Atg1 is called the unc51-like kinase 1 (Ulk1) complex, and it consists of Ulk1 serine/threonine protein kinase, Atg13, and focal adhesion kinase family interacting protein of 200 kD (FIP200), which is the mammalian homolog of yeast Atg17 [61,62]. Ulk1 can initiate autophagy by phosphorylating Atg13 and FIP200 [63]. Additionally, the mammalian ortholog of yeast Atg6 is called Beclin 1, and it is present with class III phosphoinositide 3-kinase (PI3K) in a complex product that is called phosphatidylinositol 3-phosphate (PtdIns(3)P) [64,65]. Beclin 1 is essential for phagophore nucleation [66]. Thus, autophagy is triggered by management with PtdIns(3)P, and it is prevented by blocking class III PI3K [67,68].

Once autophagy is initiated, a membrane sac isolation membrane extends around the substrate to be eliminated and eventually forms an autophagosome or autophagic vacuole [69]. Microtubule-associated protein light chain 3 (LC3) is the mammalian ortholog of yeast Atg8, and it is essential for autophagosome elongation/closure [70]. LC3 engaged on the surface of autophagosomes persists on the autophagosomal membrane [71]. It has been demonstrated that autophagosome formation needs 2 ubiquitin-like conjugation systems [72]. Subsequent to its formation, the autophagosome fuses with the lysosome [72]. Consequently, substrates enclosed by the autophagosome are broken down by hydrolases within the lysosomal cavity [72]. Protein p62, which is also called sequestosome 1, is continually hydrolyzed by autophagy-lysosome system after being recruited to the autophagosomes by LC3 interaction [73,74]. Thus, p62 buildup is detected in autophagy-deficient cells [74].

Autophagy is augmented by nutrient deprivation and extracellular or intracellular stress [75,76]. Autophagosome formation is intensely stimulated in response to starvation [77]. Nutrient status is sensed by 2 well-described signaling pathways, which are the pathways of adenosine monophosphate-activated protein kinase (AMPK) and mammalian target of rapamycin complex 1 (mTORC1) [78].

Autophagy is activated by AMPK, which is a critical energy sensor of adenosine monophosphate (AMP), and it is increased by a rise in intracellular levels of AMP [78]. AMPK senses the energy state of a cell by detecting the AMP/ATP ratio [79]. For example, AMPK is stimulated in low glucose conditions, which lead to decreased levels of ATP and activation of autophagy [80,81]. AMPK can be activated by several kinases, such as liver kinase B1, calcium/calmodulin kinase, and transforming growth factor  $\beta$ -activated kinase 1 (TAK1) [82]. AMPK can trigger autophagy by reducing mTORC1 activity and/or regulating Ulk1 phosphorylation directly [83,84]. mTOR is a protein kinase that makes up 2 functional complexes, which are known as mTORC1 and mTORC2 [85]. mTORC1 is a rapamycin-sensitive protein kinase complex that controls various cellular processes, such as autophagy, based on the cellular nutritional status [85]. mTORC1 activity is definitely controlled by



**Figure 3.** The role of hyperglycemia in the suppression of autophagy and induction of apoptosis and necroptosis in diabetic nephropathy. Hyperglycemia causes overproduction of advanced glycation end-products (AGEs), which inhibit autophagy, leading to diabetic nephropathy. Hyperglycemia induces reactive oxygen species generation, which initiates apoptosis. Hyperglycemia also increases tumor suppressor p53 messenger ribonucleic acid (mRNA) and protein expression, thus activating the transcription of many pro-apoptotic proteins that stimulate the extrinsic and the intrinsic apoptotic pathways. Additionally, hyperglycemia stimulates renin-angiotensin, which is involved in apoptosis and is involved in diabetic nephropathy. Hyperglycemia can also trigger necroptosis in diabetic nephropathy via activating the interacting serine/threonine kinase 1 (RIPK1)-RIPK3 signaling pathway. This figure was generated using Microsoft PowerPoint Software, version 10, Microsoft Corp., USA.

Ras homology enriched in brain (Rheb), which is a GTP-binding protein [85-87]. Protein kinase B (Akt) is phosphorylated by insulin signal via PI3K and phosphoinositide-dependent kinase-1, and it consequently attenuates tuberous sclerosis 2, which is a potent Rheb suppressor [88,89]. Thus, an insulin signal reduces autophagy through activating mTORC1 in cells [90]. Activation of mTORC1 also needs amino acids, and it is associated with the movement of mTORC1 from the cytoplasm to lysosomal membranes [91]. Once activated, mTORC1 phosphorylates Ulk1 inhibiting it and subsequently preventing autophagosome formation [85].

Autophagy can also be triggered by reactive oxygen species (ROS) [92]. For instance, exogenous hydrogen peroxide can eventually activate Atg4 proteases, resulting in upregulation of proteolytic mature LC3 and the suppression of mTORC1 activity [93]. Consequent to increased ROS, mitogen-activated protein kinases, such as c-Jun N-terminal kinase 1 (JNK1), are often activated, leading to activation of autophagy [94]. Thus, autophagy of damaged mitochondria, which is called mitophagy, is essential to inhibit ROS buildup [95]. Additionally, autophagy is induced by hypoxia via activating hypoxia-inducible factor 1 (HIF1) transcription factor, which ultimately leads to release of Beclin-1, which triggers autophagy [96].

## Necroptosis

Necroptosis is a type of programmed cell death that has morphological features similar to those of necrosis, including swollen organelles and fragmented nuclear membrane [30]. Additionally, the integrity of the plasma membrane is impaired, leading to its rupture and the subsequent leakage of cellular substances, eventually resulting in inflammation [97].

Necroptosis is triggered by interleukin-1beta (IL-1 $\beta$ ), TNF, certain viral infections, and other factors [98]. Furthermore, necroptosis depends on receptor-interacting serine/threonine kinase 1 (RIPK1) to RIPK3, which form an RIPK1/RIPK3 complex, which is known as the necrosome, which activates the necroptotic pathway in the absence of caspase-8 [98]. Thus, RIPK1 and RIPK3 are phosphorylated, leading to the recruitment and activation of pseudokinase [98]. Consequently, plasma membrane integrity is impaired and subsequently ruptured, leading to discharge of damage-associated molecular patterns (DAMPs) [97]. DAMPs, which comprise IL-1 family members, stimulate the inflammasome, activate inflammation, and provoke immune reactions [97]. RIPK3-dependent necroptosis is regulated by the ubiquitin-proteasome system [97]. Thus, RIPK1 and RIPK3 are activated by deubiquitinating enzymes, stimulating necroptosis [97,99].

## Apoptosis in Diabetic Nephropathy

Hyperglycemia and insulin resistance are implicated in the pathogenesis of diabetic nephropathy [9,100-102]. Hyperglycemia is believed to cause microvascular injury, particularly in the renal glomerulus, leading to development of diabetic nephropathy [103]. Hyperglycemia induces reactive oxygen species generation, which initiates podocyte

apoptosis (Figure 3) and subsequent podocyte decline, both in vitro and in vivo [104]. Apoptosis leads to glomerular injury in diabetic kidneys, decreasing podocyte numbers [23]. The decreased number of podocytes is associated with proteinuria and glomerular structural damage in diabetic nephropathy [105,106]. Podocyte apoptosis and the subsequent podocyte depletion may indeed represent early events affecting the diabetic kidney and underlying diabetic glomerulopathy, which leads to diabetic nephropathy in type I and type II diabetes [107,108]. Podocyte apoptosis coincides with the onset of diabetes and hyperglycemia in type I and type II diabetes, indicating that glucotoxicity may be underlying the stimulation of pro-apoptotic signaling and subsequent podocyte injury in diabetic kidneys. Decreased glomerular podocyte density precedes microalbuminuria [109].

P53 mediates podocyte apoptosis in cells exposed to high glucose [110,111]. Indeed, high glucose increases p53 messenger ribonucleic acid (mRNA) and protein expression (Figure 3), which activates Bax and the intrinsic apoptotic pathway, with the eventual activation of caspase-3 in podocytes, leading to their apoptosis [110,111]. P53 downregulation blocks podocyte apoptosis that is induced by high glucose [112].

Epithelial cells in the proximal convoluted tubules also undergo apoptosis in diabetic nephropathy, leading to tubular atrophy [24]. Thus, diabetic nephropathy is characterized by the absence of a connection between glomeruli and proximal convoluted tubules due to tubular atrophy, which is associated with loss of renal function [113]. Tubular atrophy leads to tubular cell depletion and the subsequent formation of atubular glomeruli [114].

Hyperglycemia modulates the expression of apoptosis regulatory genes in renal proximal tubular cells, leading to their cell death [115]. Hyperglycemia induces angiotensinogen gene expression through reactive oxygen species formation in the proximal convoluted tubules [115]. Renin-angiotensin has been suggested to be involved in tubular epithelial cell apoptosis in diabetic kidneys (Figure 3), since blockade of renin-angiotensin system has been demonstrated to attenuate the apoptosis of tubular epithelial cells [115]. Additionally, hyperglycemia is associated with increased reactive oxygen species generation, which induces apoptosis in proximal convoluted tubules, leading to tubular atrophy and subsequent formation of atubular glomeruli [116-118].

Tubular apoptosis has been reported to be associated with glycogen accumulation, as well as other factors of the diabetic condition [119]. Glycogen-accumulating cells undergo apoptosis, leading to renal tubular atrophy, which contributes to depletion of renal tubular epithelial cells, representing a hallmark feature of end-stage diabetic nephropathy [119].

## Autophagy in Diabetic Nephropathy

Hyperglycemia inhibits autophagy, because it causes overproduction of irreversibly glycosylated proteins, which are called advanced glycation end-products (AGEs) [120]. Consistently, AGEs have been demonstrated to inhibit autophagy in cultured proximal tubular epithelial cells (PTECs) [121]. Thus, AGE degradation, which takes place in PTECs, is impaired, causing their accumulation and subsequent inflammation, which is a risk factor for development of diabetic nephropathy [121]. Consequently, AGE surplus disturbs the permeabilization of lysosomal membrane and consequently impairs autophagy in diabetic nephropathy, causing tubular injury, inflammation, and interstitial fibrosis [121].

Diabetic nephropathy is characterized by proteinuria, which results from cell death of podocytes and dysfunction of their foot processes, leading to destruction of the glomerular filtration barrier [122]. Podocytes are highly specialized glomerular epithelial cells that enable the glomerular filtration barrier (GFB) to perform its function by wrapping around the outer aspect of the glomerular basement membrane (GBM) with their interdigitating foot processes [122]. However, podocyte loss frequently occurs in diabetic nephropathy, leading to proteinuria [122]. Podocytes have constitutively high basal autophagic activity under normal conditions, suggesting the necessity of autophagy for maintaining podocyte homeostasis [123]. Accordingly, conserving podocyte cell homeostasis is considered as a therapeutic approach to inhibit the progression of diabetic nephropathy to nephrotic syndrome [124]. Indeed, impaired autophagy causes podocyte loss [125]. For instance, deleting Atg5 gene in podocytes leads to glomerular lesions that are concomitant with podocyte loss and albuminuria [126].

Autophagy is speculated to be involved in the pathogenesis of diabetic nephropathy [127]. Decreased autophagic activity has been demonstrated in podocytes after induction of diabetes using streptozotocin [27]. Consistently, hyperglycemia has been suggested to decrease autophagic activity (Figure 3), causing variations in podocyte function and subsequent disruption of the glomerular filtration barrier [27]. For example, cultured podocytes exhibited decreased autophagic activity and associated proteins, including Beclin-1 and the Atg5-Atg12 complex, consequent to their exposure to high concentrations of glucose [27,28]. Likewise, an in vitro study showed that underexpression of Beclin-1 in podocytes eventually caused albumin leakage [128]. Additionally, morphologically abnormal lysosomes were substantially increased subsequent to autophagy deficiency in the podocytes of obese type II diabetic rodents [129,130]. Thus, impaired lysosomes are proposed to be a significant degradation target of autophagy in podocytes under diabetic circumstances [130].

Unlike podocytes, proximal tubular cells exhibit very low basal levels of autophagy under normal conditions [131]. However, autophagy in proximal tubular cells is increased in stress conditions, such as those caused by acute kidney injury consequent to ischemia and nephrotoxic agents, such as cisplatin [132-134]. Additionally, activation of autophagy in proximal tubular cells was suggested to be renoprotective to manage with both acute and chronic nephrotoxic stresses [134]. Deficiency of autophagy in proximal tubular cells after deletion of Atg5 gene led to proteinuria and subsequent tubulointerstitial lesions [133]. Similar to other proteinuric kidney diseases, proteinuria causes strong nephrotoxic stress and activates autophagy in diabetic nephropathy [124].

On the other hand, induction of diabetes has been demonstrated to attenuate autophagic activity in proximal tubular cells [29]. Indeed, autophagy has been attenuated in kidneys after induction of diabetes using streptozotocin in mice, resulting in buildup of impaired molecules and organelles that are normally broken down by autophagy [29,124]. Additionally, accumulation of damaged molecules indicative of reduced autophagy has been found in biopsies from type II diabetic patients [124]. Furthermore, mTORC1 has been implicated in prevention of autophagy associated with diabetes in proximal tubular cells in type II diabetic humans and mice, indicating an intimate correlation between insufficient autophagy and the pathogenesis of diabetic nephropathy [135]. Thus, enhancing autophagy may be regarded as a potential therapeutic approach for diabetic nephropathy [124].

## Necroptosis in Diabetic Nephropathy

Necroptosis is involved in the pathogenesis of diabetic nephropathy [136]. Podocyte necroptosis has been shown to be stimulated by ubiquitin C-terminal hydrolase L1 (UCHL1), which regulates the ubiquitination state of the RIPK1/RIPK3 pathway [31,137]. Indeed, abnormal overexpression of UCHL1 in podocytes stimulates podocyte injury, leading to polyubiquitin buildup [31]. Therefore, upregulation of UCHL1 in diabetic nephropathy has been associated with the dysregulation of ubiquitination of the RIPK1/RIPK3 pathway, resulting in necroptosis of podocytes [31]. It has been shown that inhibition of apoptosis induced by hyperglycemia could trigger necroptosis [31]. Thus, necroptosis might have a key role in podocyte damage and subsequent decline in diabetic nephropathy via activating the RIPK1-RIPK3 signaling pathway (Figure 3) [31]. Finally, necroptosis was reported to be obstructed by necrostatin-1 (Nec-1), which blocks the RIPK1/RIPK3 pathway, and genetic deletion of UCHL1 decreased the expression of RIPK1 and RIPK3 [31].

## Therapeutic Implications of Programmed Cell Death in Diabetic Nephropathy

Because apoptosis, autophagy, and/or necroptosis are involved in diabetic nephropathy, therapeutic strategies that impede the molecular and biochemical steps involved in one or more types of programmed cell death that occur in diabetic nephropathy could be renoprotective (Table 1).

Since apoptosis is mediated by p53, p53 downregulation has been proposed as a possible anti-apoptotic therapeutic method in diabetic nephropathy that develops via apoptosis [111,138,139]. Additionally, management with caspase inhibitors has been reported to impede the development of diabetic nephropathy, halting cell death [140]. Drugs, such as paricalcitol and/or enalapril, which improve the antioxidant defense system attenuating oxidative stress, have been reported to exert renoprotective effects in streptozotocin-induced diabetic nephropathy by suppressing apoptosis via reducing the renal expression of pro-apoptotic p53 and caspase-3 and augmenting the expression of the anti-apoptotic Bcl-2 [140]. Additionally, catalase overexpression exerts beneficial effects, attenuating apoptosis and pro-apoptotic gene expression in the proximal convoluted tubules [141-143]. Furthermore, taurine, which is a conditionally essential amino acid, has been shown to act as an endogenous antioxidant in renal tubular cells, attenuating hyperglycemia-induced apoptosis in human tubular cells by preventing oxidative stress [144]. Thus, the inhibition of oxidative stress by taurine has been demonstrated to prevent tubulointerstitial injury in diabetic nephropathy [144].

Moreover, long intergenic noncoding RNAs (lincRNAs) are involved in the pathogenesis of diabetic nephropathy [145]. However, miR-27a-3p inhibition, which is one of the microRNAs (miRNAs) that prevent gene expression post-transcriptionally, has been shown to slow the progression of diabetic nephropathy by suppressing podocyte apoptosis via upregulating tissue inhibitor of matrix metalloproteinases-3 (TIMP3) [145].

Autophagy in proximal tubular cells is triggered by temporary food deprivation. Additionally, calorie restriction exerts beneficial effects in several kinds of renal injury [146]. Thus, a calorie restriction regimen must be regarded as an effective therapeutic approach to inhibit diabetic nephropathy. Calorie restriction enhances renal impairment in type II diabetic Wistar fatty rats, and it repairs autophagy activity in their proximal tubular cells [147]. Therefore, an agent that can imitate caloric restriction might be a potential therapy for diabetic nephropathy by inducing autophagy in mammalian cells. Moreover, rapamycin, which induces autophagy by inhibiting mTORC1, has been reported to decrease glomerular lesions in experimental diabetic nephropathy [124,148]. Thus, rapamycin might be protective in diabetic nephropathy by activating autophagy.

**Table 1.** Potential therapeutic implications of apoptosis, autophagy, and necroptosis in diabetic nephropathy.

Type of programmed cell death	Therapeutic implications	Reference (s)
Apoptosis	Paricalcitol and/or enalapril attenuated the oxidative stress, and subsequently suppressed apoptosis via reducing the renal expression of pro-apoptotic p53 and caspase-3 and augmenting the expression of the anti-apoptotic Bcl-2	[140]
	Catalase overexpression decreased the upregulated p53 gene	[141-143]
	Taurine acted as an endogenous antioxidant and attenuated hyperglycemia-induced apoptosis by inhibiting oxidative stress	[144]
	miR-27a-3p inhibition slowed the progression of diabetic nephropathy by suppressing podocyte apoptosis via upregulating TIMP3	[145]
Autophagy	Calorie restriction enhanced renal impairment by repairing autophagy activity	[147]
	Rapamycin induced autophagy by inhibiting mTORC1	[124,148]
	Metformin and resveratrol induced autophagy by activating AMPK	[150-153]
Necroptosis	Adiponectin decreased the expression levels of RIPK1 and RIPK3	[156]
	Nec-1 inhibited the expression of RIPK1 and RIPK3 following the inhibition of apoptosis	[31]
	Genetic deletion of UCHL1 decreased the half-life of RIPK1 and RIPK3 proteins and underexpressed RIPK1 and RIPK3	[31]

Bcl-2 – B-cell lymphoma 2; TIMP3 – tissue inhibitor of matrix metalloproteinases-3; mTORC1 – Mammalian target of rapamycin complex 1; AMPK – adenosine monophosphate-activated protein kinase; Nec-1 – necrostatin-1; RIPK – receptor-interacting serine/threonine kinase; UCHL1 – ubiquitin C-terminal hydrolase L1.

However, extreme mTORC1 prevention impairs podocyte function, suggesting that further investigation is required regarding the safety and effectiveness of mTORC1 prevention in patients with diabetic nephropathy [147,148].

Deactivated AMPK may be involved in the development of diabetic nephropathy by suppressing autophagy, suggesting that AMPK stimulation might be targeted for repairing autophagy in diabetic kidneys [149]. Indeed, AMPK deactivation is inverted by agents such as metformin and resveratrol, which also reduce diabetic glomerular and tubular lesions [150-153]. AMPK activation can induce autophagy, which may mediate its renoprotective mechanism, sustaining renal homeostasis in diabetic kidneys [151,153]. Glomeruli and tubules of both type I and type II diabetic animal models display dephosphorylation that deactivates AMPK [154,155].

Finally, inhibition of necroptosis might be a possible therapeutic method in diabetic nephropathy, since it has been demonstrated to improve diabetic nephropathy [156]. For instance, adiponectin could reduce podocyte necroptosis and subsequent damage in diabetic nephropathy by reducing the expression levels of RIPK1 and RIPK3 [156]. In addition, necrostatin-1 (Nec1) inhibited necroptosis and the associated expression of RIPK1 and RIPK3, which were stimulated subsequent to apoptosis

inhibition using caspase inhibitors in podocytes exposed to high glucose [31]. Additionally, genetic deletion of UCHL1 resulted in decreased half-life of RIPK1 and RIPK3 proteins and underexpression of RIPK1 and RIPK3 [31]. Therefore, UCHL1 inhibition might be a potential therapeutic strategy to suppress the necroptotic RIPK1/RIPK3 pathway in an attempt to rescue podocytes in patients with diabetic nephropathy [31].

## Conclusions

There is ample evidence supporting the involvement of programmed cell death, including apoptosis, autophagy, and necroptosis, in the pathogenesis of diabetic nephropathy. A better comprehension of the molecular events regulating apoptosis, autophagy, and necroptosis, which are associated with the pathogenesis of diabetic nephropathy, would be tremendously helpful in discovering factors that can serve as potential therapeutic targets to stop or retard the progression of the diabetic nephropathy. Additionally, further investigation in this field can be predicted to afford promising therapeutic approaches, which might decrease the incidence of diabetic nephropathy development in diabetic patients via manipulating cell death programs.



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