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Autophagy in Kidney Disease

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Abstract

Autophagy is a cellular homeostatic program for the turnover of cellular organelles and proteins, in which double-membraned vesicles (autophagosomes) sequester cytoplasmic cargos, which are subsequently delivered to the lysosome for degradation. Emerging evidence implicates autophagy as an important modulator of human disease. Macroautophagy and selective autophagy (e.g., mitophagy, aggrephagy) can influence cellular processes, including cell death, inflammation, and immune responses, and thereby exert both adaptive and maladaptive roles in disease pathogenesis. Autophagy has been implicated in acute kidney injury, which can arise in response to nephrotoxins, sepsis, and ischemia/reperfusion, and in chronic kidney diseases. The latter includes comorbidities of diabetes and recent evidence for chronic obstructive pulmonary disease–associated kidney injury. Roles of autophagy in polycystic kidney disease and kidney cancer have also been described. Targeting the autophagy pathway may have therapeutic benefit in the treatment of kidney disorders.

INTRODUCTION

Autophagy, a genetically regulated and lysosome-dependent cellular program, has emerged as a fundamental process in cell biology and medicine (1–2). The ubiquitin proteasome system degrades most cellular proteins, whereas autophagy provides a pathway for the degradation of long-lived or aggregated proteins, as well as of dysfunctional organelles. Autophagy is a highly conserved process that maintains cellular homeostasis during normal physiology and in pathophysiological states. During autophagy, various substrates such as aggregated proteins and damaged mitochondria are isolated in double-membraned vacuoles termed autophagosomes, which subsequently deliver the cargo to the lysosomes for protease-dependent degradation (3). Autophagy is regulated by a complex signaling network consisting of autophagy-related genes (*ATG*), which are highly evolutionarily conserved, and for which mammalian homologues have been identified (1, 4).

Macroautophagy, commonly referred to as autophagy, designates the bulk processing of cytoplasmic cargos by autophagosome-dependent lysosomal delivery (1). In addition to macroautophagy, other forms of autophagy designated as chaperone-mediated autophagy (CMA) and microautophagy have been described (5). In CMA, proteins targeted for degradation form complexes with the chaperone protein heat shock cognate (hsc70) via intrinsic KFERQ-motifs, which are subsequently imported into the lysosomes by interaction with lysosome-associated membrane protein (LAMP) 2A (5). In microautophagy, cytoplasmic components are directly assimilated into either the lysosome or endosome by membrane invagination (6).

Autophagy facilitates the lysosomal degradation of target substrates, including protein aggregates, damaged organelles (i.e., mitochondria, peroxisomes), carbohydrates, lipids, nucleic acids, and pathogens. Lysosomal degradation of complex molecules releases amino acids, fatty acids, and nucleotides, thus identifying autophagy as a mechanism for the recycling of metabolic precursors (3). The assimilation of cytoplasmic material by autophagosomes is subject to highly selective and genetically regulated responses to the accumulation of specific classes of substrates that are collectively referred to as selective autophagy (1, 7). Selective autophagy programs have been identified for many types of cellular constituents and named after their specific cargo, as exemplified by mitophagy, the selective autophagic processing of mitochondria (1, 7–8).

Experimental findings linking autophagy to the pathogenesis of disease have been demonstrated in animal models and implicate autophagy as a pathogenic mediator of human diseases (1, 9–11). The Nobel Prize in Physiology or Medicine was awarded to Dr. Yoshinori Ohsumi in 2016 for his contributions toward understanding the regulation and functional role of autophagy, setting the stage for the exploration of autophagy in diseases (2, 12).

Autophagy is induced as a cellular stress response to a multiplicity of adverse environmental cues, such as hypoxia, oxidative stress, and nutrient depletion, and is commonly associated with cellular protection or survival (3). Prosurvival roles of autophagy were initially described under conditions of starvation or nutrient deprivation and associated energy decline (3, 13). In contrast, recent studies have implicated dysregulated or maladaptive autophagy with propathogenic responses in select models of disease (1, 14). Autophagy-dependent cell death represents a form of regulated cell death that mechanistically relies on the autophagic machinery and may occur in a context-specific fashion that contributes to the pathogenesis of disorders (14–15). In addition to its involvement in regulated cell death, autophagy can impact other fundamental processes relating to aging or disease, including the regulation of inflammation, innate immunity, and host defense (16–17).

The kidneys are vital organs involved in blood filtration and osmotic balance. Diseases of the kidney represent a major public health burden and can occur as comorbidities with diabetes, cardiovascular disease, and chronic lung disease. This review focuses specifically on the functional

roles of autophagy in the normal kidney, acute kidney injury (AKI), chronic kidney diseases (CKD), and other renal pathologies such as inherited kidney diseases and kidney cancer. A thorough understanding of the role of autophagy in kidney diseases, whether ultimately adaptive or maladaptive, may lead to elucidation of the molecular mechanisms underlying kidney pathologies as well as provide strategies and targets for therapeutic intervention.

MOLECULAR REGULATION OF THE AUTOPHAGY PATHWAY

Sequence of the Autophagy Pathway

The autophagy pathway proceeds through a series of sequential phases that are highly regulated by ATG proteins and other accessory factors (1, 4). Autophagy initiation results in formation of the phagophore, also called autophagosome nucleation. The next phase involves elongation of the nascent membrane to form an autophagosome. The maturation phase results in a double-membraned autophagosome that completely encloses the autophagic cargo. Finally, the autophagosome-lysosome fusion step results in the formation of an autophagolysosome, in which proteolytic degradation of the cargo takes place (**Figure 1**). The activity of the autophagy pathway, with respect to the relative rate of autophagic turnover of substrates, is termed autophagic flux (3).

Regulation of the Autophagy Pathway by Metabolic Cues

The autophagy pathway is regulated by complex signaling mechanisms that favor activation during starvation or low energy states and general suppression during normal or nutrient replete states (10, 18). Furthermore, autophagy is a highly inducible system that responds to activation or suppression by cellular stresses or application of various pharmacological compounds. Chemicals such as 3-methyl-adenine (3-MA) and chloroquine suppress autophagy initiation and fusion, respectively. Drugs such as metformin, which targets 5′-adenosine monophosphate-activating kinase (AMPK), and rapamycin (sirolimus), which targets mechanistic target of rapamycin (mTOR), are typically used to activate autophagy in experimental systems (19–20).

Metabolic regulation of autophagy requires the mTOR pathway (10, 18, 21) (**Figure 2**). As the major regulatory protein in this pathway, mTOR presides in a macromolecular complex [mTOR complex 1 (mTORC1)] in association with other factors: the regulatory-associated protein of mTOR (Raptor), G protein β-subunit-like protein/mammalian lethal with SEC13 protein 8 (GβL/mLST8), and proline-rich Akt/PKB substrate 40 kDa (PRAS40) (10). The activation of mTORC1 activity in response to nutrient-associated signals (i.e., amino acids and growth factors) results in negative regulation of autophagy (18).

Activated mTORC1 negatively regulates a downstream substrate complex, the mammalian uncoordinated-51-like protein kinase (ULK1) complex, which consists of ULK1, ATG13, ATG101, and RB_1CC_1/FIP200 (18, 22–23). Inhibition of mTORC1 by starvation or energy exhaustion causes mTORC1 to dissociate from the ULK1 substrate complex, leading to dephosphorylation of ULK1 and ATG13 and the phosphorylation of RB_1CC_1/FIP200 that results in the initiation of autophagy (22, 24).

Autophagosome formation is also regulated by the major autophagy protein Beclin-1 (the mammalian homolog of yeast Atg6) (25). Beclin-1 associates with a macromolecular complex that includes the vacuolar protein sorting 34 (VPS34) class III phosphatidylinositol-3 kinase (PI3KC3), VPS15, and ATG14L, which regulates autophagosomal nucleation (1, 26). A second alternate Beclin-1 complex, consisting of Beclin-1, PI3KC3, VPS15, and UVRAG, regulates endosomal and autophagosomal maturation (1, 27).

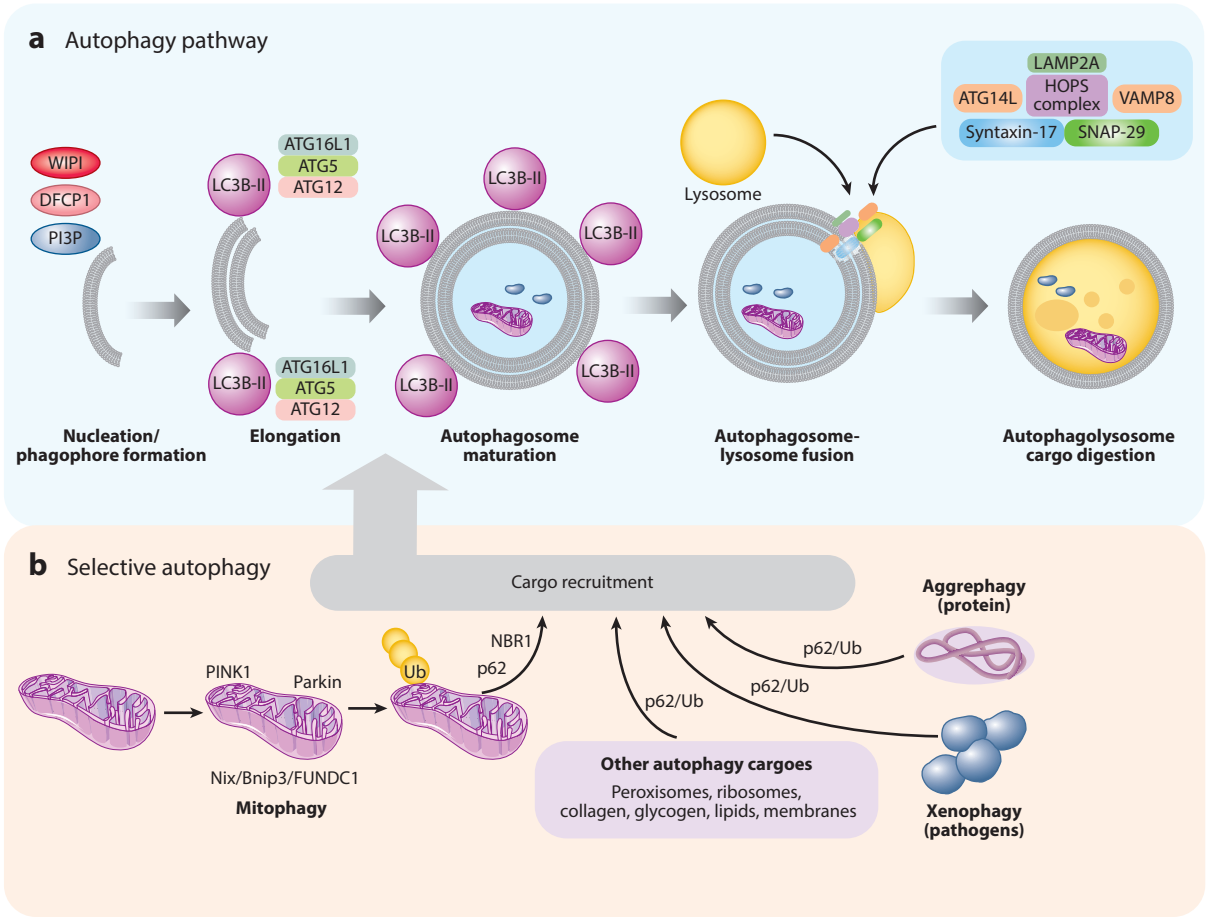

Figure 1

Sequence of the autophagy pathway and selective autophagy. (*a*) The autophagy pathway involves sequential steps beginning with phagophore formation and membrane elongation, culminating in autophagosome maturation with cargo assimilation. Subsequently, the mature autophagosome fuses to the lysosome to form an autophagolysosome, in which the contents are degraded. During initiation of autophagy, the second messenger phosphatidylinositol-3-phosphate (PI3P) recruits double FYVE-containing protein-1 (DFCP1) and WD-repeat protein interacting with phosphoinositides (WIPI) to the phagophore. The activated phosphatidylethanolamine-conjugated form of LC3B (LC3B-II) and trimeric protein complexes consisting of ATG12–ATG5-ATG16L1 assist in autophagosome membrane elongation. The GTPase Rab7 regulates the autophagic vacuole maturation. Autophagosome-lysosome fusion is facilitated by the homotypic fusion and protein sorting (HOPS) tethering complex through molecular interactions with the autophagosomal Qa-SNARE, syntaxin-17, ATG14L, SNAP-29, VAMP8, and LAMP2A. In the final stages of autophagy, autophagic cargoes are degraded by lysosomal acid hydrolases, and the recycling of nutrients is facilitated by lysosomal permeases. (*b*) Selective autophagy refers to specific targeting of cellular components for autophagic degradation. The p62/sequestosome-1 and NBR1 (next to BRCA1 gene 1 protein) are selective autophagy substrates that are degraded by the autophagosome. NBR1 and p62 can interact with ATG8 proteins through a specific sequence motif, the ATG8/LC3-interacting region (LIR). These proteins associate with polyubiquitinated proteins and/or mitochondria and assist in their delivery to autophagosomes. Mitophagy, the selective degradation of depolarized mitochondria by autophagy, is orchestrated by several proteins, including PINK1 and Parkin. PINK1 facilitates the mitochondrial recruitment of Parkin, which catalyzes the polyubitquitination (Ub) of damaged mitochondria. PINK1-/Parkin-independent mitophagy involves interaction of LC3B with outer membrane proteins: FUN14 domain-containing 1 (FUNDC1), NIP3-like protein X (Nix), and BCL2 adenovirus E1B 19 KDa interacting protein 3 (Bnip3) via the LIR. This results in removal of mitochondria through mitophagy. In a process called aggrephagy, p62 facilitates the autophagosomal delivery of ubiquitinated protein and histone deacetylase-6 complexes (aggresomes). The term xenophagy refers to the selective autophagic degradation of invading pathogens. Other forms of selective autophagy targeting specific substrates such as peroxisomes, ribosomes, collagen, glycogen, lipids, endoplasmic reticulum membranes, and other biomolecules (e.g., mRNA and inflammasome complexes) have been described.

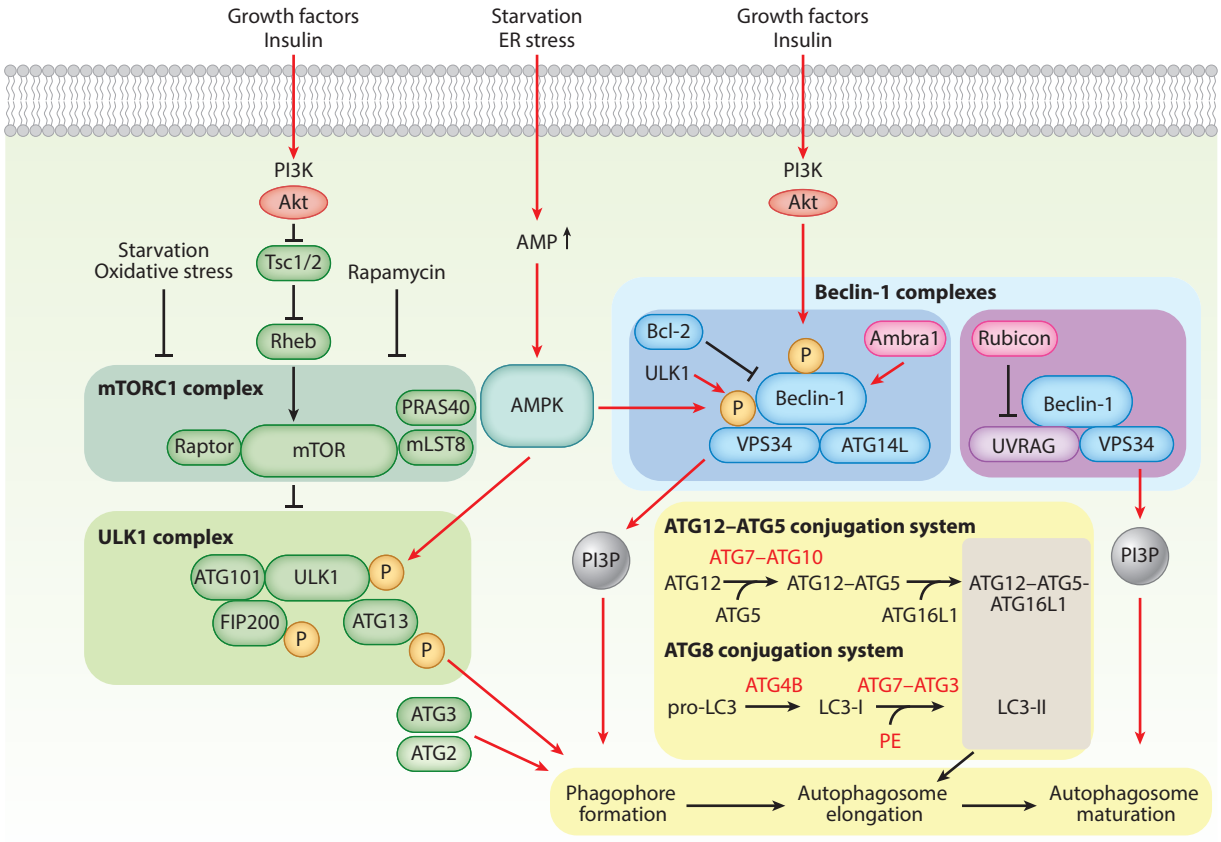

Figure 2

Molecular regulation of autophagy. Growth factors, insulin, or nutrient signals stimulate mTOR complex 1 (mTORC1: mTOR, mLST8, PRAS40, Raptor) via activation of the Class I PI3K/Akt pathway. Akt regulates mTOR upstream of Tsc1/2 and Rheb. Starvation or oxidative stress can inhibit mTORC1. The autophagy pathway is also regulated by energy depletion through activation of the 5′ AMP-activated protein kinase (AMPK), which senses cellular AMP levels. mTORC1 inhibition leads to activation of UNC-51-like autophagy-activating kinase (ULK1). ULK1 resides in a macromolecular complex that contains focal adhesion kinase family interacting protein of 200 kD (FIP200), ATG13, and ATG101. Autophagy initiation also requires the Beclin-1 complex that includes vacuolar protein sorting 34 (VPS34)/phosphatidylinositol 3-kinase class III (PI3KC3), p150/VPS15, and ATG14L. VPS34 generates phosphatidylinositol-3-phosphate (PI3P), which promotes the formation of the isolation membrane. AMPK can activate autophagy via the direct phosphorylation of ULK1 at Ser 317/777 and can phosphorylate Beclin-1 (Thr 388), leading to PI3KC3 activation. ULK1 can induce autophagy by activating Beclin-1 via phosphorylation of Beclin-1 or ATG14L. Ambra1 can activate the Beclin-1 complex while the antiapoptotic protein Bcl-2 can inhibit it. Beclin-1 can also form an alternate complex with the ultraviolet radiation resistance-associated gene protein (UVRAG), which can promote autophagosome or endosome maturation. Rubicon acts as a negative regulator of the Beclin-1-UVRAG complex. ATG9 and ATG2 facilitate the recruitment of membrane and phospholipid components to the nascent autophagosome membrane. The elongation of the phagophore involves two ubiquitin-like conjugation systems. In the ATG12–ATG5 conjugation system, ATG12 is conjugated to ATG5 by ATG7 (E1-like) and ATG10 (E2-like) enzymes. The ATG12–ATG5 complex associates with ATG16L1. In the ATG8 conjugation system, ATG4B cleaves pro-LC3 (and other ATG8 homologues) to generate LC3-I. Conjugation of phosphatidylethanolamine (PE) with LC3-I is catalyzed by ATG7 (E1-like) and ATG3 (E2-like). Both conjugation systems can contribute to autophagosome formation.

The autophagy pathway is coregulated by energy depletion through activation of AMPK, which senses cellular AMP levels (3, 18, 24). AMPK can activate autophagy via the direct phosphorylation of ULK1 at Ser 317 and Ser 777 (24) and can also phosphorylate Beclin-1 (Thr 388), leading to PI3KC3 activation (28). Enhanced activity of the Beclin-1/PI3KC3 complex generates phosphatidylinositol-3-phosphate (PI3P), a second messenger molecule that regulates autophagosomal nucleation (10, 26, 29). PI3P recruits additional proteins to the site of autophagosome nucleation. These include PI3P-binding effector proteins such as the WD-repeat protein interacting with phosphoinositide (WIPI) proteins (30) and the double FYVE-containing protein 1 (DFCP1) (31). Transmembrane proteins such as mammalian Atg9 and vacuole membrane protein 1 (VMP1) are also required for autophagosome assembly (29). Recent studies have uncovered a novel role for ATG2A in the transfer of endoplasmic reticulum (ER)–derived glycerophospholipids to the autophagosome membrane (32).

Regulation of Autophagosome Elongation and Fusion

Two ubiquitin-like conjugation systems contribute to the elongation and maturation of the autophagosome: the ATG12–ATG5 conjugation system and the microtubule-associated protein light chain 3 (LC3) conjugation system (4, 10, 29). Although essential for yeast autophagosome formation, these systems may not be indispensable for autophagosome formation in mammals, but they are crucial for degradation of the autophagosome inner membrane (33).

ATG12, a ubiquitin-like protein, is conjugated to ATG5 by ATG7 (E1-like) and ATG10 (E2-like) enzymes. ATG12–ATG5 recruits ATG16L to form a complex that regulates autophagosome elongation (29). The ubiquitin-like protein LC3, the mammalian homologue of yeast Atg8, mediates autophagosome formation (29). The proform of LC3 is cleaved by the Atg4B cysteine protease to generate LC3-I. LC3 isoforms (e.g., LC3B), and other mammalian Atg8 homologues are conjugated with the phospholipid phosphatidylethanolamine that is catalyzed by Atg7 (E1-like) and Atg3 (E2-like) enzymes (29). In mammals, the conversion of LC3-I (unconjugated form) to LC3-II (autophagosomal membrane–associated phosphatidylethanolamine-conjugated form) is regarded as an indicator of autophagosome formation (10). LC3-II is retained in the membrane of mature autophagosomes until the autophagosome-lysosome fusion step, when it is degraded by lysosomal activity or converted to LC3-I at the outer membrane by Atg4B (34).

Other recent developments have shed light on the molecular processes regulating autophagosome-lysosome fusion events (1, 35). This process involves dyneins and specific SNARE proteins: the autophagosomal Qa-SNARE syntaxin-17 and its interaction with lysosomal/endosomal R-SNAREs, SNAP-29, tethering factors such as the homotypic fusion and vacuole protein sorting (HOPS) tethering complex, and the small GTPase RAB7 (1, 35).

SELECTIVE AUTOPHAGY

Mitophagy

Mitophagy refers to a selective autophagy mechanism for the degradation of mitochondria (8). The regulation of mitophagy involves a canonical pathway dependent on the activation of the transmembrane Ser/Thr kinase PINK1 [phosphatase and tensin homolog deleted in chromosome 10 (PTEN)-induced putative kinase 1]. PINK1 is stabilized on damaged or depolarized mitochondria (8, 36). PINK1 recruits the E3 ubiquitin ligase Parkin (PRKN, also known as PARK2) to the mitochondria, which ubiquitinates outer mitochondrial membrane proteins, including mitofusins (36–37). PINK1 may directly phosphorylate ubiquitin to facilitate Parkin activation and recruitment (38).

Ubiquitin-modified mitochondrial proteins are recognized and targeted to autophagosomes by the autophagic cargo adaptor proteins. Of these, p62/SQSTM1 (p62) (29), NDP52, or optineurin has been proposed to facilitate mitochondria recruitment to the autophagosome via the intrinsic LC3-interacting region (LIR) (39) or directly assist in autophagosome assembly through interactions with Atg8 homologs (40). Mutations in the *PINK1* and *PRKN* genes are associated with accumulation of dysfunctional mitochondria and recessive familial forms of Parkinson's disease (41). An alternative mode of mitophagy regulates the turnover of mitochondria in erythrocytes and reticulocytes and requires the BH3-only protein, Nix (Bnip3L). Nix localizes to the outer mitochondrial membrane and directly interacts with mammalian Atg8 homologs via an LIR motif (42).

Aggrephagy

The process of aggrephagy refers to the selective autophagic degradation of protein aggregates, which may be significant in kidney diseases (7), especially those conditions implicating enhanced activation of the ER stress pathway (43–44). p62 participates in direct protein–protein interactions with ubiquitinated proteins via its ubiquitin-associated domain, and with LC3 localized to the isolation membrane via an LIR motif, thus facilitating the sequestration of ubiquitinated proteins in autophagosomes (45). In addition to p62, the selective autophagy adaptor NBR1 and other adaptor proteins are required for the formation of Ub-positive protein aggregates, which facilitate their sequestration and removal by aggrephagy (7, 46).

REGULATION OF AUTOPHAGY BY OXIDATIVE STRESS

Oxidative stress or injury, typically associated with mitochondrial dysfunction or exposure to xenobiotics, is a common component of kidney diseases. The regulation of autophagy by oxidative stress has been implicated in kidney disorders, including AKI, such as ischemia/reperfusion (I/R) injuries, and CKD, including diabetic nephropathy (47).

Oxidative stress occurs when the generation of reactive oxygen species (ROS) exceeds endogenous cellular antioxidant defenses. Excess production of ROS, which include superoxide (O_2^-), its dismutation product hydrogen peroxide (H_2O_2), and the highly reactive hydroxyl radical, generated from partial reduction of oxygen, may lead to progressive damage to lipids, proteins, and nucleic acids. In contrast, physiological levels of ROS have been implicated in the regulation of cellular signal transduction pathways, leading to adaptive responses. Mitochondria dysfunction may lead to increased production of mitochondria-derived ROS (mtROS) (47–48).

The regulation of autophagy by pro-oxidants has been shown in various cultured cells, primarily by application of H_2O_2 and ROS-generating compounds (49). For example, application of mitochondrial electron transport chain inhibitors, such as rotenone, which inhibits complex 1, increased autophagy activation in human embryonic kidney fibroblasts by increasing mtROS production (50). Carbon monoxide, which also stimulates mtROS production, is a potent activator of autophagy (51). NADPH oxidases (NOX 1–5) represent a major source of cytosolic ROS production. NOX4, a mitochondrial-localizing NOX isoform, was required for propagation of injury in sepsis-induced AKI in mice (52).

REGULATION OF AUTOPHAGY BY ENDOPLASMIC RETICULUM STRESS

The ER is a vital organelle that regulates the posttranslational processing, folding, and trafficking of nascent polypeptides and regulates intracellular Ca^{2+} flux. Various noxious stimuli

(e.g., hypoxia, Ca^{2+} ionophores, oxidative stress, and inhibitors of protein glycosylation such as tunicamycin) can disrupt this organelle, causing ER stress associated with the accumulation of misfolded protein aggregates (53–54). The unfolded protein response (UPR) occurs as an adaptive response to ER stress and involves upregulated synthesis of ER chaperone proteins (i.e., Bip/GRP78), cessation of general protein translation, and the activation of an ER-associated protein degradation system (54). The UPR is regulated by canonical pathways involving inositol-requiring kinase 1 (IRE1), activating transcription factor 6 (ATF6), and protein kinase R-like endoplasmic reticulum kinase (PERK) (54). ER stress may also promote apoptosis, via activation of caspases (i.e., caspase-12, -9, -8) and the transcription factor C/EBP homologous protein (CHOP) (54).

The relationships between the ER stress-induced UPR and the activation of autophagy have been demonstrated in various cultured cells (55), including kidney tubular epithelial cells (56). The ER stress-activating compound tunicamycin induced the UPR response and activated autophagy in renal proximal tubular cells, as measured by LC3-II accumulation (56). Tunicamycin preconditioning also protected against kidney I/R injury in vivo by inducing the expression of ER chaperones and autophagy proteins (56).

In kidney diseases, ER stress can have both adaptive and maladaptive consequences as a stress response to acute kidney injuries and hypoxia (57). ER stress has been implicated in AKI, CKD, and age-related kidney dysfunction (43–44). Hypoxia is a known activator of the UPR by upregulation of glycogen synthase kinase 3β (GSK3β), which leads to the induction of PERK signaling (58). Activating transcription factor 4 (ATF4), a component of PERK pathway signaling, is induced under severe hypoxia and is required for ER stress and hypoxia-induced autophagy by regulating the transcription of LC3B (59). Hypoxia can induce autophagy in cultured renal proximal tubular cells and in renal I/R injury as an adaptive response to injury (60).

Activation of the ER stress response occurs in renal tubular epithelial cells exposed to high protein concentrations and in vivo by experimental proteinuria (61). Thus, autophagy activation during ER stress may represent a mechanism for the selective degradation of protein aggregates. Further studies will be required to define the relationships between the ER pathway and autophagy in the context of kidney diseases.

AUTOPHAGY AND INFLAMMATION

Inflammation is a common component of kidney disorders, including response to various forms of AKI and CKD (47). Autophagy has been broadly implicated as a mediator of inflammatory disorders, including inflammatory bowel disease and various autoimmune disorders (1, 16). Genetic variations in autophagy-associated genes (e.g., *Atg5*, *Ulk1*) have been associated with susceptibility to inflammatory diseases (16). Autophagy can also exert an adaptive role in inflammatory disorders by regulating inflammatory processes, such as interferon production or inflammasome activation. Furthermore, autophagy can reduce pathogen-triggered inflammation by countering pathogen/microbial infections via selective autophagy (xenophagy) (16–17). Mitochondrial dysfunction plays a key role in the regulation of specialized inflammatory processes, such as activation of the nucleotide-binding oligomerization domain (NOD)-leucine-rich repeat (LRR) and pyrin domain–containing protein 3 (NLRP3) inflammasome (62–63). Autophagy can dampen inflammation by downregulating NLRP3 inflammasome-associated cytokine production and maturation [e.g., interleukin (IL)-1β, IL-18]. These effects were reliant on the autophagy-dependent preservation of mitochondrial function and reduction of mtROS formation by removal of depolarized mitochondria (63). Macrophages deficient in major autophagy proteins (LC3B, Beclin-1) displayed increased NLRP3/caspase-1-dependent cytokines in response to proinflammatory

stimuli (63). A direct role of autophagy in inflammasome regulation has been proposed, via the autophagy-directed turnover of ubiquitin modified inflammasome regulatory proteins (64).

Based on these observations, autophagy is predicted to contribute to moderating inflammation associated with kidney dysfunction, in a context-specific fashion, as discussed in the following sections.

AUTOPHAGY IN KIDNEY DISEASE

Autophagy is required for normal kidney functions (65). Evidence of the critical role of autophagy in kidney disease is centered on observations of the emergence of kidney pathologies in mice bearing genetic deletions of key autophagy regulator proteins. For instance, targeted deletion of *Atg5* or *Atg7* in kidney epithelium resulted in CKD in mice, associated with podocyte and tubular dysfunction, glomerular and tubulointerstitial injury, and progressive organ failure (66). Kidney tissues from these mice exhibited elevated ER stress, mitochondrial dysfunction, and mtROS production (66). These data show that autophagy deficiency in the kidney epithelium of mice can mimic the pathological features observed in the kidneys of patients with idiopathic focal segmental glomerular sclerosis (FSGS) (66).

Autophagy plays an important role in the maintenance of the homeostasis and function of podocytes (65, 67). Podocyte-specific conditional deletion of *Atg5* in mice promoted the development of age-dependent glomerulopathy that was accompanied by an accumulation of oxidized and ubiquitinated proteins, heightened ER stress, podocyte loss, and proteinuria (65). These findings suggest that autophagy acts as an important safeguard against age-dependent glomerular disease and decline of kidney function (65).

Recent genetic studies also suggest that autophagy serves as an important basal antioxidant defense of the renal vasculature (68). Endothelial and hematopoietic cell-specific conditional *Atg5*-deficient mice (*Atg5* cKO) displayed renal vascular abnormalities. These mice died at 12 weeks but were rescued by bone marrow transplantation from normal donors. Transplanted *Atg5* cKO mice developed mesangiolysis and glomerulosclerosis, which were reversible by antioxidant therapy. These studies suggest that autophagy provides antioxidant protection of the glomerular vasculature (68).

ACUTE KIDNEY INJURY

The injury and death of tubular epithelial cells are primary features of AKI, which may progress to CKD in repeated or unrepaired injury. Various insults such as renal I/R, sepsis, or exposure to nephrotoxins contribute to nutrient depletion and oxidative stress–induced activation of autophagy (47). Accumulating evidence in various rodent models, such as I/R-induced, sepsis/endotoxemia-induced, and nephrotoxin-induced AKI, strongly suggests that autophagy generally protects the kidney from injury, though contrasting findings have also been reported (47).

Nephrotoxin (Cisplatin)-Induced Acute Kidney Injury

The chemotherapeutic agent cisplatin, which induces AKI, can induce autophagy in renal tubules, as evidence by increased autophagosome formation (69). Inhibition of autophagy using pharmacological inhibitors (3-MA or bafilomycin) or Beclin-1 knockdown increased tubular cell apoptosis in response to cisplatin (69). Conflicting studies have reported that cisplatin-induced increases in autophagy may aggravate renal damage and apoptosis (70). However, mice with conditional deletion of *Atg5* in kidney proximal tubules (pt*Atg5*-KO) were susceptible to cisplatin-induced AKI

and displayed increased DNA damage, protein aggregation, apoptosis, and ROS production in the kidney relative to wild-type mice. These experiments concluded that autophagy is renoprotective in cisplatin-induced AKI (71).

Metformin, a therapeutic agent used for type 2 diabetes mellitus, is an AMPK activator that has been shown to protect against gentamicin-induced nephrotoxicity in rats (72). Pretreatment with metformin markedly attenuated cisplatin-induced AKI and reduced tubular cell apoptosis and inflammatory cell accumulation in mouse kidneys. Metformin preconditioning increased phospho(p)-AMPKα expression and autophagy induction in the kidneys after cisplatin challenge. In cultured rat kidney tubular cells (NRK-52E), metformin stimulated the expression of p-AMPKα, induced autophagy, and inhibited cisplatin-induced apoptosis. Inhibition of AMPKα activation or autophagy induction abolished the protective effect of metformin in cisplatin-induced cell death. These results suggest that metformin protects against cisplatin-induced tubular cell apoptosis and AKI by activating AMPKα and autophagy in kidney tubular cells (73).

Recently, a novel protective role for the mitochondrial biogenesis regulator PGC-1α was uncovered in mice subjected to cisplatin-induced AKI. The authors attributed the protective effects of PGC-1α to the novel modulation of lysosomal biogenesis via transcription factor EB (TFEB) (74).

Sepsis-Induced Acute Kidney Injury

Sepsis, characterized by systemic inflammation, is a major cause of AKI in intensive care unit patients. Recent studies implicate a role for autophagy in sepsis and its associated AKI.

Lipopolysaccharide (LPS), a mediator of gram-negative bacterial sepsis, induced autophagy in renal tubular epithelial cells in vivo and in vitro (75–76). Enhanced autophagy by increased LC3-II and Beclin-1 expression and autophagosome formation was also observed in renal cortex after LPS challenge (77). Pharmacological inhibition of autophagy with chloroquine worsened LPS-induced AKI (76). Opposing findings were reported using the autophagy inhibitor 3-MA, which ameliorated LPS-induced AKI (77). The reasons for discrepancies in studies using chemical modulators of autophagy remain unclear, such that genetic validation studies may be required for conclusive interpretation of autophagy involvement.

Mice genetically deficient for *Atg*7 specifically in renal proximal tubular epithelial cells (pt*Atg*7-KO) were susceptible to LPS-induced AKI, relative to wild-type mice (75–76). The pt*Atg*7-KO mice developed more severe kidney dysfunction and parenchymal injury, with evidence of elevated IL-6 and STAT activation in kidney tissue in response to LPS relative to wild-type mice (75). In vitro experiments demonstrated impaired autophagy and enhanced IL-6 production in response to LPS in isolated *Atg*7-KO renal tubular epithelial cells (75).

Howell et al. (78), utilizing a murine model of LPS-induced AKI, suggested that failure to recover renal function in older adult mice is due to impaired autophagy. The administration of the mTOR inhibitor temsirolimus after established endotoxemia protected against the development of AKI in response to LPS challenge by inducing autophagy. These results suggest that autophagy is activated and confers protection in endotoxic kidney injury (75–76).

A general protective role of autophagy in sepsis was revealed by studies using the cecal ligation and puncture (CLP) model of polymicrobial sepsis, which reported induction of autophagy in multiple organs after CLP. The autophagy inhibitor chloroquine increased susceptibility of mice to the lethal effects of CLP (79). In mice subjected to CLP, autophagy was enhanced at early time points (i.e., 6 h) in kidney tissue, as suggested by increased LC3-II and autophagosome accumulation, followed by decline at later time points (i.e., 24 h). Further induction of autophagy by rapamycin improved renal function and ameliorated tubular epithelial injury in CLP-treated mice (80). In studies using a rat model of CLP-induced sepsis, markers of autophagy (i.e., LC3-II)

were increased in the kidney proximal tubules at early time points after CLP, followed by decline in the later stages of sepsis-induced AKI. These studies demonstrated that knockdown of *Atg7* enhanced TNF-α-induced cell death, whereas treatment with the autophagy activator rapamycin inhibited it in renal tubular cells in vitro (81).

Recent studies implicate sirtuin 3 (Sirt3)-dependent AMPK activation in the upregulation of autophagy in sepsis-induced AKI (82). In the CLP model, Sirt3 knockout mice were susceptible to sepsis-induced AKI and displayed downregulation of AMPK and autophagy in the kidney (82). These studies suggest that enhanced autophagy provides a general renoprotective mechanism in AKI by reducing apoptosis, inflammation, and oxidative stress.

Ischemia/Reperfusion–Induced Acute Kidney Injury

I/R injury results from a sudden temporary decrease in blood flow to the organ and is a frequent cause of AKI. In a rat model of renal I/R injury, the expression of autophagy proteins (i.e., Beclin-1, LC3) increased in proximal and distal epithelial cells after I/R (83). Overexpression of the anti-apoptotic protein Bcl-X_L reduced the expression of both proapoptotic and autophagy markers and ameliorated AKI in this model (83). Increased autophagosome formation was also observed in renal proximal tubular epithelial cells in vivo in response to renal I/R injury in the mouse and, in vitro, in response to injurious stimuli such as hypoxia (84). Inhibitors of autophagy such as chloroquine and 3-MA aggravated renal I/R injury and enhanced tubular apoptosis (85–86).

The kidney proximal tubule-specific targeted genetic deletion of *Atg5* or *Atg7* (pt*Atg5*-KO, pt*Atg7*-KO) resulted in increased susceptibility to I/R-induced renal injury and apoptosis (87–89). In pt*Atg5*-KO mice, I/R injury increased the accumulation of p62 and ubiquitin-positive cytoplasmic inclusions and compromised renal function as shown by greater elevations in blood urea nitrogen and serum creatinine levels (89).

Mitochondrial injury may contribute to the propagation of AKI through incompletely understood mechanisms. Recent studies have evaluated the specific role of mitophagy in I/R-induced AKI. Mitophagy, dependent on PINK1 and PRKN, was induced in renal proximal tubular cells in both in vitro and in vivo models of ischemic AKI. Mice genetically deficient in *PINK1* or *PRKN*, as well as *PINK1/PRKN* double-knockout mice, were susceptible to ischemic AKI (90). Furthermore, genetic deficiency in PINK1 or PRKN enhanced mitochondrial dysfunction, ROS production, and inflammatory responses. These results suggest that PINK1/PRKN-mediated mitophagy plays an important role in mitochondrial quality control, tubular cell survival, and renal function during AKI.

Accumulating evidence supports a strong link between AKI and progression to CKD. Recent studies have shown that I/R activated autophagy in renal tubules. The stress-responsive transcription factor FoxO3 was activated in kidney tubular epithelial cells in response to hypoxia, resulting from the inhibition of FoxO3 prolyl hydroxylation and the activation of hypoxia-inducible factor (HIF)-1α (91). Tubule-specific deletion of *Hif-1α* decreased hypoxia-induced FoxO3 activation and aggravated tubular injury and interstitial fibrosis following ischemic injury. Tubule-specific deletion of *FoxO3* also aggravated renal injury and promoted AKI to CKD transition, with impaired autophagy and increased oxidative injury in renal tubules (91). These studies suggest that FoxO3 can be activated for hypoxia adaptation in kidney and thereby protects against CKD development.

CHRONIC KIDNEY DISEASE

CKD, characterized by progressive loss of kidney function, is a significant healthcare burden (92). Development of kidney fibrosis is a hallmark of CKD and represents the final common response

to injury (93). Transforming growth factor-beta 1 (TGF-β1) is a cardinal profibrogenic factor for the development of kidney fibrosis (93) and can mediate fibroblast activation (94). Accumulating studies have implicated a central role for autophagy in the development of CKD, including diabetic nephropathy, obstructive nephropathy, immunoglobulin A (IgA) nephropathy, and adriamycin-induced nephropathy (95–97). In these conditions, autophagy may be induced as an endogenous protective mechanism in both renal tubular epithelial cells and podocytes, and impairment of the autophagic process leads to progressive CKD (98).

Kidney Fibrosis

Increased autophagy has been observed in renal tubular epithelial cells of obstructed kidneys after unilateral ureteral obstruction (UUO), a well-established experimental model of kidney fibrosis (99–102). Mice genetically deficient in the autophagy proteins LC3B (*Map1lc3b*$^{-/-}$) or Beclin-1 (*Becn1*$^{+/-}$) displayed increased collagen deposition and increased mature TGF-β1 levels in obstructed kidneys after UUO. These studies demonstrated that, in kidney tubular epithelial cells, the levels of mature TGF-β1 are regulated by autophagic degradation to suppress UUO-induced kidney fibrosis (99). We have also described a novel antifibrotic role of autophagy in the degradation of collagen (103).

Conditional deletion of *Atg5* in proximal tubular epithelial cells (pt*Atg5*-KO) promoted cell cycle arrest at the G_2/M phase and augmented tubulointerstitial fibrosis in mouse kidneys after UUO (101). In *Atg5*-KO primary proximal tubular epithelial cells, angiotensin-II stimulation induced cell cycle G_2/M arrest and increased collagen production. These studies identified a cytoprotective effect of ATG5 in kidney proximal tubules. Furthermore, pt*Atg5*-KO mice displayed increases in NF-κB-mediated proinflammatory cytokine production in response to UUO, suggesting an anti-inflammatory effect of ATG5 (102).

Recent studies also examined the importance of autophagy in distal tubules for protection against kidney fibrosis. Experiments using green fluorescent protein (GFP)-LC3 transgenic mice revealed increased GFP-LC3 puncta, indicative of elevated autophagosome formation, in distal tubular cells of the obstructed kidneys after UUO (104). Moreover, conditional deletion of *Atg7* in distal tubular epithelial cells (dt*Atg7*-KO) augmented tubulointerstitial fibrosis and epithelial-mesenchymal transition–like phenotype change in mouse kidneys after UUO and involved activation of the TGF-β pathway. The dt*Atg7*-KO mice also displayed increased mitochondrial dysfunction, increased accumulation of p62-positive protein aggregates, and increased apoptosis. These changes were associated with increased activation of the NLRP3 inflammasome, including caspase-1 activation and increased IL-1β production (104). Taken together, these findings suggest that autophagy in distal tubular epithelial cells protects against renal tubulointerstitial fibrosis by regulating TGF-β and inflammasome-dependent IL-1β production. Recent studies have also identified Atg5 as a regulated target of micro-RNA (miR)-376b. Inhibition of miR-376b augments Atg5-dependent macrophage autophagy and provides protection against adenine diet–induced kidney fibrosis in FVB/N mice (105).

The above findings provide evidence supporting the notion that autophagy is protective against kidney fibrosis. Livingston et al. (106) reported contrasting findings that proximal tubule–specific deletion of *Atg7* (pt*Atg7*-KO) ameliorated tubulointerstitial fibrosis in the UUO model. The authors proposed a profibrotic function of persistent activation of autophagy in this model. The reasons for the opposing results remain unclear. Clearly, the role of autophagy in CKD is complex, and further investigations are warranted to develop optimal therapeutic approaches targeting autophagy in CKD.

Podocytopathies

Podocytes are terminally differentiated cells with specialized functions that play an important role in regulating the renal glomerular filtration barrier. Podocyte dysfunction leads to proteinuric kidney disease (95). Podocyte injury and loss attributed to genetic, toxic, immunologic, or metabolic insults underlie the most common glomerular diseases leading to proteinuria (107). Podocyte depletion triggers the development and progression of glomerulosclerosis (65).

Podocytes are long-lived cells that display a constitutively high level of autophagy (65). Inhibition of autophagy by 3-MA or Beclin-1 knockdown resulted in abnormal podocyte morphology (108). Autophagy was substantially increased in glomeruli from mice with induced proteinuria and in glomeruli from patients with acquired proteinuric diseases (65). Mice with podocyte-specific deletion of *Atg5* (podo*Atg5*-KO) exhibited increased susceptibility to experimental bovine serum albumin overload-induced proteinuria (65). Similarly, podo*Atg5*-KO mice subjected to puromycin aminonucleoside (PAN) treatment, a model of nephrosis and proteinuria, were susceptible to glomerular disease, with evidence of podocyte loss and foot process effacement (65). These studies concluded that basal and inducible autophagy are crucial homeostatic mechanisms to maintain podocyte integrity and protect against glomerular injury (65). Similar findings were reported with pharmacological manipulation of autophagy. In PAN-treated rats, administration of chloroquine or 3-MA increased podocyte injury and foot process effacement and proteinuria and reduced the expression of podocyte markers (109). Genetic interference of Beclin-1 or treatment with the autophagy-inhibiting compounds chloroquine or 3-MA enhanced PAN-induced podocyte apoptosis in vitro (109). Furthermore, the autophagy-activating compound rapamycin protected against these changes in vitro and in vivo (109).

Podocyte injury and loss result in podocyte depletion that underlies the development of FSGS. Mice subjected to adriamycin-induced nephropathy, an experimental model of FSGS, developed podocyte injury and apoptosis and increased proteinuria. GFP-LC3 transgenic mice displayed higher LC3 puncta in podocytes as the result of adriamycin challenge, indicative of increased autophagy in podocytes (67). podo*Atg7*-KO mice were highly susceptible to podocyte injury and glomerulopathy in response to adriamycin-induced kidney injury (67). These podo*Atg7*-KO mice displayed increased proteinuria and podocyte foot process effacement relative to wild-type mice in response to adriamycin. In an experimental model of protein overload-induced proteinuria, the Nix (Bnip3L)-dependent mitophagy pathway was impaired in renal tubular epithelium. Genetic overexpression of Nix ameliorated tubular epithelial cell injury and mitochondrial dysfunction in response to experimental proteinuria (110).

In studies of human diseases, morphological changes in mitochondria were observed in kidney biopsies from patients with FSGS (111). These patients displayed decreased Beclin-1 expression and reduced autophagosome numbers in podocytes compared to patients with minimal change disease (109). Nix expression was reduced in the kidneys of patients with proteinuria and correlated with decline in glomerular filtration rate (GFR) (110). Taken together, these data support important roles for autophagy in the pathophysiology of human glomerular disorders.

Diabetic Nephropathy

Diabetic nephropathy is a devastating complication of diabetes mellitus and the leading cause of progressive CKD and end-stage renal disease. Diabetic nephropathy, characterized by the development of albuminuria and a progressive decline in GFR in association with glomerulosclerosis, is a podocytopathy, together with other glomerular cells (mesangial and endothelial) participating in the development of diabetic kidney disease. Podocyte apoptosis coincides with onset of albuminuria and leads to podocytopenia in diabetic nephropathy (112). Accumulating studies suggest that

the metabolic dysfunction and downregulation of autophagy via regulation of key metabolic regulators, such as mTOR and AMPK, contribute to the pathogenesis of diabetic nephropathy (113). The activation of mTOR (a suppressor of autophagy) was correlated with increased glomerular injury in diabetic nephropathy patients, including glomerular hypertrophy and hyperfiltration (114).

In mouse models, indirect activation of mTORC1, by podocyte-specific genetic deletion of a key inhibitor *Tsc1*, promoted mesangial expansion and fibrosis, podocyte injury and foot process effacement, and proteinuria (115). These pathological changes in *Tsc1*-deficient mice were reversible by pharmacological application of rapamycin, an inducer of autophagy (115). Genetic ablation of mTORC1 (by podocyte-specific homozygous deletion of Raptor) in mice resulted in early proteinuria and progressive glomerulosclerosis in the absence of pathogenic stimuli (114). Loss of podocyte function was further aggravated by overlapping genetic deletion of mTORC2 (114).

In a streptozotocin (STZ)-induced mouse model of type 1 diabetes, mTORC1 hyperactivation was observed in podocytes. Mice with monoallelic mTORC1 were also protected against an STZ-induced experimental model of diabetic nephropathy by permitting autophagy (114). Mice with hemizygous deletion of Raptor in podocytes on *db/db* background (Raptor$^{+/-}$*db/db*) displayed reduced glomerular injury, foot process effacement, glomerular basement membrane thickening, and reduced proteinuria at 40 weeks of age relative to *db/db* mice (115). Taken together, these studies suggest that mTORC1 activity is required for basal podocyte homeostasis, but excess mTORC1 activity may also contribute to podocyte dysfunction and aggravate pathological conditions such as diabetic nephropathy, presumably by inhibiting autophagy (114).

In cultured podocytes, exposure to high glucose concentrations induced autophagy, as assessed by increased autophagosome formation, LC3B-II accumulation, and Beclin-1 expression, which were inhibited by the antioxidant *N*-acetyl-L-cysteine (116). These also promoted autophagic flux, as determined by LC3B-II turnover assays (117). Genetic interference of Beclin-1 and LC3 inhibited autophagy and promoted podocyte apoptosis. These studies identified the stress response protein heme oxygenase-1 (HO-1) as an effector of podocyte autophagy, via AMPK-dependent Beclin-1 upregulation (118). Application of the mTOR inhibitor rapamycin improved proteinuria in STZ-induced diabetic mice by inhibiting podocyte apoptosis and increasing autophagy (119). In STZ-induced diabetic nephropathic mice, the chemical chaperone tauroursodeoxycholic acid (TUDCA), which protects against ER stress, improved podocyte injury and reduced albuminuria (108). TUDCA also ameliorated ER stress and promoted podocyte autophagy in vitro under high glucose conditions (108).

The role of podocyte autophagy in diabetic nephropathy–associated proteinuria was also investigated in a mouse model of high-fat diet–induced diabetic nephropathy. In this model, podocyte-specific conditional deletion of *Atg5* aggravated podocyte damage and glomerulosclerosis, compromised the glomerular filtration barrier, increased proteinuria, and caused lysosomal dysfunction. *Atg5* deficiency also increased podocyte sensitivity to high glucose–induced apoptosis (117, 120). The regulation of autophagy in the kidney vasculature may also influence susceptibility to diabetic nephropathy. Endothelial cell–specific *Atg5* deletion resulted in capillary rarefactions and glomerular endothelial lesions with loss of fenestrations and accelerated progression of diabetic nephropathy (117).

Emerging studies have revealed a crucial role for various histone deacetylases (HDACs) in the regulation of autophagy in diabetic nephropathy (121). Histone deacetylase 4 (HDAC4) is known to suppress podocyte-specific autophagy (122). In human studies, HDAC4 expression negatively correlated with GFR and was highly expressed in podocytes from patients with diabetic

nephropathy, whereas HDAC2 was highly expressed in renal tubules (123). Elevated HDAC4 was also observed in kidney biopsies from FSGS patients (123). In vitro, treatments associated with kidney injury, including TGF-β and high glucose conditions, promoted HDAC4 expression in podocytes. Wang et al. (123) proposed a mechanism by which HDAC4 suppresses autophagy via deacetylation and activation of STAT1, leading to proapoptotic signaling. Genetic interference of HDAC4 ameliorated kidney dysfunction in diabetic rats.

The silent information regulator 2 homolog (sirtuin) family of class III NAD^+-dependent HDACs exerts pleiotropic effects that may modulate the outcome of diabetic nephropathy, including regulation of autophagy, inflammation, and apoptosis (124). Sirt1 is associated with protection in age-related disorders, including diabetes (125). Sirt1 expression in kidney biopsies of diabetic patients negatively correlated with proteinuria. In mice, genetic deletion of *Sirt1* promoted proteinuria, and aggravated glomerular lesions associated with diabetic nephropathy. Sirt1-dependent renoprotection was also associated with the suppression of the tight junction protein claudin-1 in podocytes (125).

In *db/db* diabetic mice, application of resveratrol, a Sirt1 activator and known autophagy inducer, attenuated podocyte apoptosis and ameliorated diabetic nephropathy (112). In addition, resveratrol induced autophagy in both *db/db* mice and human podocytes. Inhibition of autophagy by 3-MA and Atg5 short hairpin RNA (shRNA) reversed the protective effects of resveratrol on podocytes. Mechanistic studies suggested that resveratrol may regulate autophagy and inhibit apoptosis in *db/db* mice and in podocytes by downregulating miR-383-5p (112). Administration of resveratrol to STZ-induced diabetic rats also protected against proteinuria and kidney fibrosis (126). In this model, increased expression of proangiogenic factors, vascular endothelial growth factor (VEGF), Flk-1, and angiopoietin 2 was also reversed by administration of the Sirt1 activator resveratrol. The regulatory effect of resveratrol on the expression of VEGF and other mediators in cultured podocytes under high glucose conditions was abolished by genetic interference of Sirt1 (126).

The expression of Sirt6, another sirtuin family member, was reduced in kidney biopsies from patients with podocytopathies and correlated with GFR. Podocyte-specific deletion of Sirt6 exacerbated podocyte injury and proteinuria in two independent mouse models of podocytopathy (STZ-induced diabetic nephropathy and adriamycin-induced nephropathy). Sirt6 negatively regulated Notch signaling by deacetylating histone H3K9, thereby inhibiting the transcription of Notch1 and Notch4, and restored podocyte autophagy under high glucose conditions in vitro. Sirt6 also reduced urokinase plasminogen activator receptor expression, which is implicated in podocyte foot process effacement and proteinuria. Consistently, Sirt6 overexpression reduced proteinuria by preserving podocyte function, reducing podocyte apoptosis, and increasing podocyte autophagic flux in adriamycin-induced nephropathy (127). These studies suggest that Sirt6 is a potential therapeutic target in proteinuric kidney disease (127).

Few studies have addressed the role of CMA in kidney disease. Diabetic nephropathy was associated with impaired CMA, as evidenced by decreased expression of CMA regulatory proteins such as LAMP2A and increased abundance of CMA substrate proteins. Impaired CMA during diabetic nephropathy may contribute to protein aggregation and renal hypertrophy (128).

Recent human biomarker studies reveal that the serum levels of the autophagy protein Beclin-1 were reduced in patients with diabetic kidney disease. Serum Beclin-1 levels were related to disease progression and correlated with albuminuria (129). However, the relationship between serum Beclin-1 levels and autophagy activity in kidney or other systemic tissues remains unclear.

Taken together, these studies implicate impaired autophagy as a pathogenic mediator of diabetic nephropathy and suggest the utilization of compounds for the restitution or promotion of

autophagy, including HDAC inhibitors and Sirt activators, as new possible therapeutic strategies to treat diabetic nephropathy (113, 121, 130).

Cigarette Smoke/Chronic Obstructive Pulmonary Disease–Associated Chronic Kidney Disease

Chronic obstructive pulmonary disease (COPD) represents a significant global healthcare burden of disease, and although CKD is known to occur in patients with COPD, the relationship between COPD and CKD has been largely underrecognized. Recent investigations have uncovered the kidney as a target for injury and comorbidity associated with COPD and the role of autophagy. Increased microalbuminuria, a marker of endothelial dysfunction, was previously reported in COPD patients (131–132). A more recent National Heart, Lung, and Blood Institute (NHLBI) pooled cohort study of 10,961 participants determined that albuminuria was associated with accelerated decline in lung function and incident COPD (133). We reported that structural renal lesions (i.e., injury to glomeruli, renal tubules, and interstitium) occurred with higher frequency in patients with COPD than in control subjects and may be linked to increased endothelial cell damage and increased tissue oxidative stress (134).

In a mouse model of experimental COPD, we demonstrated that chronic cigarette smoke (CS) exposure (6 months) was associated with increased albuminuria and evidence of podocyte foot process effacement, mild thickening of the glomerular basement membrane, and glomerular endothelial cell injury (134). It was also associated with renal tubular injury and fibrosis with increased collagen and fibronectin expression in kidney tissues (134, 135). Furthermore, mice subjected to chronic CS exposure displayed increased urinary levels of neutrophil gelatinase-associated lipocalin 2 (NGAL), a well-characterized kidney injury marker. Elevated markers of oxidative stress in mouse kidneys, including nitrotyrosine and 8-oxo-2′-deoxyguanosine, caused ultrastructural changes in mitochondria, including evidence of swelling and loss of cristae definition (135). CS extract exposure also induced mitochondrial dysfunction in kidney proximal tubular epithelial (HK-2) cells, including mtROS production and loss of mitochondrial membrane potential (135).

Prior evidence from mouse model studies suggested that autophagy and mitophagy may be maladaptive in the context of chronic CS-induced lung pathophysiology. Genetic deficiencies of key autophagy regulator proteins LC3B, Beclin-1, or PINK1 were associated with decreases in either emphysema (LC3B, PINK1) or airway cilia dysfunction (Beclin-1, PINK1) (136–138). We recently reported that chronic in vivo CS exposure increased autophagic activity in mouse kidneys by determining autophagic flux measured using LC3B (substrate) turnover assays (135). Exposure to CS extracts in vitro similarly increased autophagic flux in cultured HK-2 cells. Moreover, mice with heterozygous deletion of Beclin-1 (*Becn1*$^{+/-}$) were protected from kidney injury and kidney fibrosis induced by CS exposure and exhibited impaired basal and inducible mitochondrial turnover by mitophagy. We also found that CS exposure led to a progressive decrease in Beclin-1 expression in mouse kidneys and in cultured kidney tubular epithelial cells, associated with autophagic flux, indicating a novel autophagy-dependent turnover of Beclin-1 (135). These results suggest that Beclin-1 was initially required for CS-induced kidney injury and that progressive decline of Beclin-1 expression may represent an adaptive response to confer renoprotection through auto-attenuation of autophagy. These results identify the kidney as a target for CS-associated injury in COPD and the Beclin-1-dependent autophagy pathway as a potential therapeutic target in CKD. Interestingly, while autophagy has been shown to be largely renoprotective in various injury models, in the context of both CS-induced lung and kidney injury, autophagy activation

is deleterious. This underscores the notion that consequences of autophagy, whether adaptive or maladaptive, occur in a context-specific fashion.

POLYCYSTIC KIDNEY DISEASE

Autosomal dominant polycystic kidney disease (ADPKD) is a common heritable human disease that leads to renal failure. ADPKD arises from mutations in either PKD1 or PKD2, encoding polycystin-1 and its interacting partner polycystin-2, respectively, which are cilia regulatory proteins. ADPKD is associated with elevated mTOR activity, and consequently rapamycin was shown to inhibit cyst expansion in animal models of ADPKD (139–140). However, a sex dependency of rapamycin efficacy was also noted in a rat model of ADPKD (141). In mouse models of ADPKD, increased autophagy markers (i.e., LC3B-II accumulation and Beclin-1 expression) and autophagosome formation were found in cystic tubular cells, which correlated with HIF-1α activation (142).

Recent studies have further elucidated the role of autophagy, a downstream pathway of mTOR, as a therapeutic target for ADPKD. In zebrafish bearing a *pkd1a* mutation, a model of ADPKD, these mutants displayed cystic kidneys as well as mTOR activation and impaired kidney autophagic flux (143). In this model, inhibition of autophagy by genetic interference of Atg5 promoted cystogenesis, while activation of autophagy using Beclin-1 peptide ameliorated cyst formation. Treatment with rapamycin or mTOR-independent autophagy modulators carbamazepine and minoxidil also attenuated cyst formation and restored kidney function. These results suggest the protective effect of autophagy in ADPKD and autophagy activation as a novel therapeutic strategy for ADPKD.

KIDNEY CANCER

Autophagy can exert pleiotropic effects on the initiation and progression of cancer, as well as on the potential effectiveness of therapeutic interventions in this disease. Monoallelic disruption of the *Becn1* gene on chromosome 17q21 occurs in 40–75% of human breast, ovarian, and prostate tumors. Prior clinical studies have associated poor prognosis and aggressive tumor phenotypes with aberrant expression of Beclin-1 in several types of tumor (20). In mice, homozygous deletion of the *Becn1* gene results in embryonic lethality, while monoallelic loss of Beclin-1 ($Becn1^{+/-}$) results in spontaneous tumorigenesis, identifying Beclin-1 as a haploinsufficient tumor suppressor protein (144). Furthermore, frequent chromosomal aberrations of several autophagy genes, including *ATG5* and *UVRAG*, have been reported to occur in human cancers (11).

Autophagy may provide an anticarcinogenic function in primary cells by protecting against metabolic stress through both the homeostatic turnover of mitochondria and the clearance of protein aggregates. Genetic deletion of autophagy proteins causes mitochondrial dysfunction, enhanced oxidative stress, and susceptibility to proinflammatory stimuli, conditions that permit DNA damage leading to genetic instability (145).

In established tumors, autophagy may confer a survival advantage to tumor cells that are under metabolic stress from a high proliferation rate and exposure to hypoxia (145). Autophagy may either contribute to acquired tumor cell resistance to chemotherapeutics or facilitate chemotherapeutic or radiation-induced cytotoxicity in apoptosis-resistant tumor cells. Additional rigorous studies are needed to unravel the complex relationships between autophagy proteins and acquired tumor resistance (145).

Few studies have examined the autophagy pathway in kidney cancer. Autophagy was found to be downregulated in clear cell renal cell carcinomas (ccRCCs) compared with matched adjacent tissue. Clinicopathologic analyses indicated that advanced or metastatic ccRCCs were associated

with a lower expression of autophagy compared with localized ccRCCs (146). Stage, grade, and the level of LC3-II expression were significant factors for prognosis. A low level of LC3B-II was associated with poor prognosis of ccRCC (146). ccRCC is frequently associated with monoallelic loss and/or mutation of *ATG7*, and the low expression level of autophagy genes correlates with ccRCC progression. The protein levels of ATG7 and Beclin-1 are also reduced in ccRCC tumors (147).

Von Hippel Lindau (VHL) expression is significantly decreased in high-grade RCCs (148). The expression levels of VHL and LC3B were inversely correlated with various tumor grades of RCC tissues. VHL was shown to bind to LC3B via its LIR motif and thereby promote the ubiquitination of LC3B, thus facilitating its lysosomal turnover. A prototype proteasome inhibitor MLN9708 upregulated autophagy and promoted RCC cell death under conditions of VHL deficiency (148). An antitumor activity for autophagy in ccRCC was also proposed via promoting the degradation of HIF-2α, which required p62 and VHL. Impaired autophagy promoted proteasomal degradation of HIF-2α and vice versa (148). Absent in melanoma 2 (AIM2), a tumor suppressor protein, was also shown to exert antitumor activity by induction of autophagy in renal carcinoma cells and low AIM2 expression was correlated with poor prognosis in RCC patients (149).

Autophagy inhibition in combination with mTOR inhibition may be a promising anticancer regimen. Autophagy-modulating compounds such as everolimus (an mTOR inhibitor and autophagy activator) and hydroxychloroquine (an autophagy inhibitor) have been recently tested in Phase I/II clinical trials as a combination treatment for advanced RCC [NCT01510119 (**https://clinicaltrials.gov**)] based on preclinical studies showing synergistic cell death and tumor regression with the combined use of mTOR inhibitor and autophagy inhibitor (150). The study concluded feasibility as well as a >40% 6-month progression-free survival rate in the treatment group, indicating that the study efficacy endpoint had been met (150). Further research will be needed to leverage the therapeutic potential of targeting the autophagy pathway in kidney cancer.

SUMMARY

Autophagy, as a cellular homeostatic program, is regulated in acute and chronic kidney disorders (**Figure 3**), providing cytoprotective functions in various kidney compartments, including proximal and distal tubules and podocytes. Some reports also suggest that autophagy may be maladaptive in a cell type–, disease- or model-specific fashion. Autophagy may confer renoprotection during the pathogenesis of various kidney diseases through several possible mechanisms. These include the removal of toxic protein aggregates, the maintenance of a stable mitochondrial population via removal of dysfunctional mitochondria, the inhibition of inflammation, the turnover of ECM proteins, the inhibition of TGF-β-dependent fibrosis, and the maintenance of podocyte structure and function.

The development of therapeutics targeting the autophagy pathway in kidney disease faces challenges that are similar to those under consideration for diseases of other organs. Although a number of compounds have been developed to modulate autophagy in experimental settings, challenges remain in the development of lead compounds that would be suitable for clinical trials and eventual therapeutic application in humans.

Intervention strategies to induce autophagy in various disease models have included mTORC1 inhibitors such as rapamycin and its analogs. Inhibitors of autophagy such as chloroquine or hydroxychloroquine alone or in combination with mTOR inhibitors have been explored for use as therapies in cancer. Additional small molecules that modulate autophagy for potential therapeutic application include targeting peptides, trehalose, histone deacetylase inhibitors, Sirt1 activators such as resveratrol, and AMPK activators (e.g., metformin and vitamin D analogs) (19–20). An increased understanding of the complex role of autophagy in the pathogenesis of acute and chronic

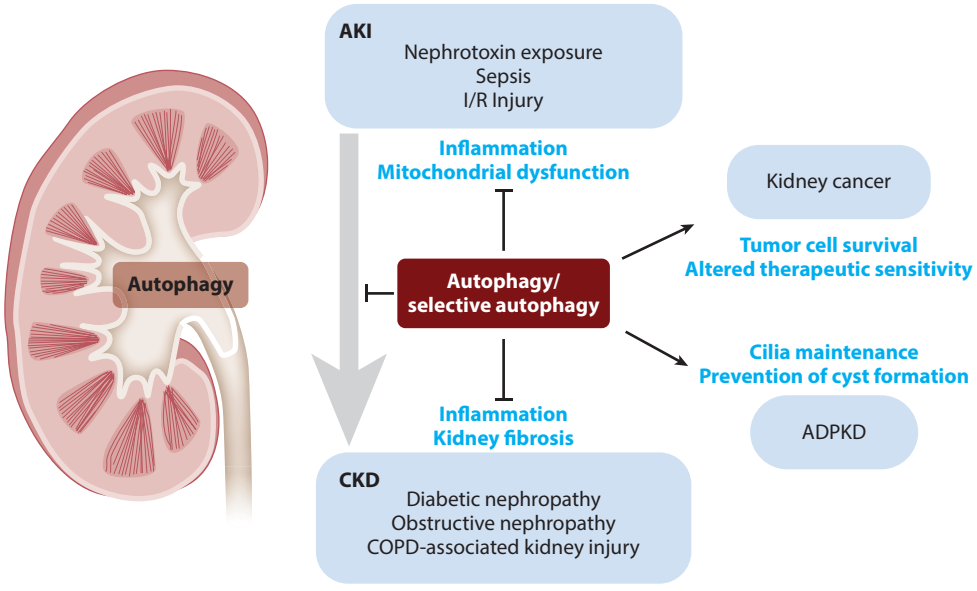

Figure 3

Autophagy in kidney disease. Autophagy and selective autophagy (i.e., mitophagy, aggrephagy, etc.) can modulate the pathogenesis of kidney diseases, including AKI and CKD. Autophagy can limit AKI and its progression to CKD in response to various insults, including nephrotoxin exposure, sepsis, and I/R injury. Autophagy may confer protection in AKI via multiple mechanisms, including the preservation of mitochondrial function and the regulation of inflammation. In addition, autophagy may be protective in various forms of CKD, including diabetic nephropathy and obstructive nephropathy, but deleterious in cigarette smoke/COPD-associated kidney injury. Autophagy provides protection in CKD via regulation of inflammation and inhibition of profibrotic processes, a hallmark of CKD. Additionally, autophagy may play protective roles in ADPKD via regulation of cilia homeostasis, and in the prevention of cysts. Finally, autophagy may play a role in kidney cancer by modulating tumor cell survival and sensitivity to therapeutic agents. Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; AKI, acute kidney injury; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; I/R, ischemia/reperfusion.

kidney disorders will hopefully further facilitate the translation of these discoveries to the development of therapies in kidney diseases in the future.

SUMMARY POINTS

1. Autophagy is a cellular homeostatic program that facilitates the lysosomal turnover of cellular constituents.
2. Autophagy can be induced in kidney tissues in models of kidney injury and disease.
3. Autophagy is generally protective in podocytes and renal tubular epithelial cells in response to injurious stimuli and constitutes an adaptive response to injury.
4. Autophagy-deficient mice exhibit increased susceptibility to the development of proteinuria and kidney fibrosis in preclinical models of CKD.
5. Autophagy provides protection in kidney disease potentially related to selective turnover of mitochondria and proteins as well as anti-inflammatory and antifibrotic effects.

6. Autophagy activation is deleterious in CS-induced kidney injury and thus consequences of autophagy, whether adaptive or maladaptive, occur in a context-specific fashion.
7. Targeting the autophagy pathway may show considerable therapeutic potential in the treatment and management of kidney disorders, including AKI, CKD, and kidney cancer.

DISCLOSURE STATEMENT

The spouse of M.E.C. is a cofounder and shareholder of Proterris, Inc. and serves on their Scientific Advisory Board.

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LITERATURE CITED

1. Levine B, Kroemer G. 2019. Biological functions of autophagy genes: a disease perspective. *Cell* 176(1–2):11–42
2. Mizushima N. 2018. A brief history of autophagy from cell biology to physiology and disease. *Nat. Cell Biol.* 20(5):521–27
3. Mizushima N, Komatsu M. 2011. Autophagy: renovation of cells and tissues. *Cell* 147:728–41
4. Yin Z, Pascual C, Klionsky DJ. 2016. Autophagy: machinery and regulation. *Microb. Cell* 3(12):588–96
5. Tekirdag K, Cuervo AM. 2018. Chaperone-mediated autophagy and endosomal microautophagy: joint by a chaperone. *J. Biol. Chem.* 293(15):5414–24
6. Oku M, Sakai Y. 2018. Three distinct types of microautophagy based on membrane dynamics and molecular machineries. *Bioessays* 40(6):e1800008
7. Lamark T, Svenning S, Johansen T. 2017. Regulation of selective autophagy: the p62/SQSTM1 paradigm. *Essays Biochem.* 61(6):609–24
8. Youle RJ, Narendra DP. 2011. Mechanisms of mitophagy. *Nat. Rev. Mol. Cell Biol.* 12(1):9–14
9. Leidal AM, Levine B, Debnath J. 2018. Autophagy and the cell biology of age-related disease. *Nat. Cell Biol.* 20(12):1338–48
10. Ravikumar B, Sarkar S, Davies JE, Futter M, Garcia-Arencibia M, et al. 2010. Regulation of mammalian autophagy in physiology and pathophysiology. *Physiol. Rev.* 90:1383–435
11. Levine B, Kroemer G. 2008. Autophagy in the pathogenesis of disease. *Cell* 132(1):27–42
12. Levine B, Klionsky DJ. 2017. Autophagy wins the 2016 Nobel Prize in Physiology or Medicine: breakthroughs in baker's yeast fuel advances in biomedical research. *PNAS* 114(2):201–5
13. Mizushima N, Yamamoto A, Matsui M, Yoshimori T, Ohsumi Y. 2004. In vivo analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker. *Mol. Biol. Cell* 15(3):1101–11
14. Galluzzi L, Baehrecke EH, Ballabio A, Boya P, Bravo-San Pedro JM, et al. 2017. Molecular definitions of autophagy and related processes. *EMBO J.* 36(13):1811–36
15. Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, et al. 2018. Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. *Cell Death Differ.* 25(3):486–541
16. Deretic V, Levine B. 2018. Autophagy balances inflammation in innate immunity. *Autophagy* 14(2):243–51
17. Deretic V, Saitoh T, Akira S. 2013. Autophagy in infection, inflammation and immunity. *Nat. Rev. Immunol.* 13(10):722–37

18. Lahiri V, Hawkins WD, Klionsky DJ. 2019. Watch what you (self-) eat: autophagic mechanisms that modulate metabolism. *Cell Metab.* 29(4):803–26
19. Galluzzi L, Bravo-San Pedro JM, Levine B, Green DR, Kroemer G. 2017. Pharmacological modulation of autophagy: therapeutic potential and persisting obstacles. *Nat. Rev. Drug Discov.* 16(7):487–511
20. Rubinsztein DC, Codogno P, Levine B. 2012. Autophagy modulation as a potential therapeutic target for diverse diseases. *Nat. Rev. Drug Discov.* 11(9):709–30
21. Galluzzi L, Pietrocola F, Levine B, Kroemer G. 2014. Metabolic control of autophagy. *Cell* 159(6):1263–76
22. Hosokawa N, Hara T, Kaizuka T, Kishi C, Takamura A, et al. 2009. Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. *Mol. Biol. Cell* 20:1981–91
23. Jung CH, Jun CB, Ro SH, Kim YM, Otto NM, et al. 2009. ULK–Atg13–FIP200 complexes mediate mTOR signaling to the autophagy machinery. *Mol. Biol. Cell* 20:1992–2003
24. Kim J, Kundu M, Viollet B, Guan KL. 2011. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat. Cell Biol.* 13:132–41
25. Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, et al. 1999. Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature* 402:672–76
26. He C, Levine B. 2010. The beclin 1 interactome. *Curr. Opin. Cell Biol.* 22:140–49
27. Itakura E, Kishi C, Inoue K, Mizushima N. 2008. Beclin 1 forms two distinct phosphatidylinositol 3-kinase complexes with mammalian Atg14 and UVRAG. *Mol. Biol. Cell* 19:5360–72
28. Zhang D, Wang W, Sun X, Xu D, Wang C, et al. 2016. AMPK regulates autophagy by phosphorylating BECN1 at threonine 388. *Autophagy* 12:1447–59
29. Yang Z, Klionsky DJ. 2010. Mammalian autophagy: core molecular machinery and signaling regulation. *Curr. Opin. Cell Biol.* 22:124–31
30. Dooley HC, Razi M, Polson HE, Girardin SE, Wilson MI, et al. 2014. WIPI2 links LC3 conjugation with PI3P, autophagosome formation, and pathogen clearance by recruiting Atg12–5-16L1. *Mol. Cell* 55(2):238–52
31. Chan EY. 2012. Regulation and function of uncoordinated-51 like kinase proteins. *Antioxid. Redox Signal.* 17:775–85
32. Valverde DP, Yu S, Boggavarapu V, Kumar N, Lees JA, et al. 2019. ATG2 transports lipids to promote autophagosome biogenesis. *J. Cell Biol.* 218(6):1787
33. Tsuboyama K, Koyama-Honda I, Sakamaki Y, Koike M, Morishita H, et al. 2016. The ATG conjugation systems are important for degradation of the inner autophagosomal membrane. *Science* 354(6315):1036–41
34. Tanida I. 2011. Autophagy basics. *Microbiol. Immunol.* 55:1–11
35. Kriegenburg F, Ungermann C, Reggiori F. 2018. Coordination of autophagosome-lysosome fusion by Atg8 family members. *Curr. Biol.* 28(8):R512–18
36. Narendra DP, Jin SM, Tanaka A, Suen DF, Gautier CA, et al. 2010. PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. *PLOS Biol.* 8:e1000298
37. Gegg ME, Cooper JM, Chau KY, Rojo M, Schapira AH, et al. 2010. Mitofusin 1 and mitofusin 2 are ubiquitinated in a PINK1/parkin-dependent manner upon induction of mitophagy. *Hum. Mol. Genet.* 19:4861–70
38. Koyano F, Okatsu K, Kosako H, Tamura Y, Go E, et al. 2014. Ubiquitin is phosphorylated by PINK1 to activate parkin. *Nature* 510:162–66
39. Lazarou M, Sliter DA, Kane LA, Sarraf SA, Wang C, et al. 2015. The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. *Nature* 524(7565):309–14
40. Padman BS, Nguyen TN, Uoselis L, Skulsuppaisarn M, Nguyen LK, et al. 2019. LC3/GABARAPs drive ubiquitin-independent recruitment of Optineurin and NDP52 to amplify mitophagy. *Nat. Commun.* 10(1):408
41. Trancikova A, Tsika E, Moore DJ. 2012. Mitochondrial dysfunction in genetic animal models of Parkinson's disease. *Antioxid. Redox Signal.* 216:896–919

42. Ding WX, Ni HM, Li M, Liao Y, Chen X, et al. 2010. Nix is critical to two distinct phases of mitophagy, reactive oxygen species-mediated autophagy induction and Parkin-ubiquitin-p62-mediated mitochondrial priming. *J. Biol. Chem.* 285:27879–90
43. Cybulsky AV. 2017. Endoplasmic reticulum stress, the unfolded protein response and autophagy in kidney diseases. *Nat. Rev. Nephrol.* 13(11):681–96
44. Maekawa H, Inagi R. 2017. Stress signal network between hypoxia and ER stress in chronic kidney disease. *Front. Physiol.* 8:74
45. Stolz A, Ernst A, Dikic I. 2014. Cargo recognition and trafficking in selective autophagy. *Nat. Cell Biol.* 16:495–501
46. Yamamoto A, Simonsen A. 2011. The elimination of accumulated and aggregated proteins: a role for aggrephagy in neurodegeneration. *Neurobiol. Dis.* 43:17–28
47. Sureshbabu A, Ryter SW, Choi ME. 2015. Oxidative stress and autophagy: crucial modulators of kidney injury. *Redox Biol*. 4:208–14
48. Di Meo S, Reed TT, Venditti P, Victor VM. 2016. Role of ROS and RNS sources in physiological and pathological conditions. *Oxid. Med. Cell. Longev.* 2016:1245049
49. Azad MB, Chen Y, Gibson SB. 2009. Regulation of autophagy by reactive oxygen species (ROS): implications for cancer progression and treatment. *Antioxid. Redox Signal.* 11(4):777–90
50. Chen Y, McMillan-Ward E, Kong J, Israels SJ, Gibson SB. 2007. Mitochondrial electron-transport-chain inhibitors of complexes I and II induce autophagic cell death mediated by reactive oxygen species. *J. Cell Sci.* 120(Part 23):4155–66
51. Lee SJ, Ryter SW, Xu JF, Nakahira K, Kim HP, et al. 2011. Carbon monoxide activates autophagy via mitochondrial reactive oxygen species formation. *Am. J. Respir. Cell Mol. Biol.* 45(4):867–73
52. Sureshbabu A, Patino E, Ma KC, Laursen K, Finkelsztein EJ, et al. 2018. RIPK3 promotes sepsis-induced acute kidney injury via mitochondrial dysfunction. *JCI Insight* 3(11):e98411
53. Bánhegyi G, Baumeister P, Benedetti A, Dong D, Fu Y, et al. 2007. Endoplasmic reticulum stress. *Ann. N. Y. Acad. Sci.* 1113:58–71
54. Schröder M, Kaufman RJ. 2005. The mammalian unfolded protein response. *Annu. Rev. Biochem.* 74:739–89
55. Li J, Ni M, Lee B, Barron E, Hinton DR, et al. 2008. The unfolded protein response regulator GRP78/BiP is required for endoplasmic reticulum integrity and stress-induced autophagy in mammalian cells. *Cell Death Differ*. 15(9):1460–71
56. Chandrika BB, Yang C, Ou Y, Feng X, Muhoza D, et al. 2015. Endoplasmic reticulum stress-induced autophagy provides cytoprotection from chemical hypoxia and oxidant injury and ameliorates renal ischemia-reperfusion injury. *PLOS ONE* 10(10):e0140025
57. Gallazzini M, Pallet N. 2018. Endoplasmic reticulum stress and kidney dysfunction. *Biol. Cell* 110(9):205–16
58. Appenzeller-Herzog C, Hall MN. 2012. Bidirectional crosstalk between endoplasmic reticulum stress and mTOR signaling. *Trends Cell Biol.* 22:274–82
59. Rzymski T, Milani M, Pike L, Buffa F, Mellor HR, et al. 2010. Regulation of autophagy by ATF4 in response to severe hypoxia. *Oncogene* 29:4424–35
60. Jiang M, Liu K, Luo J, Dong Z. 2010. Autophagy is a renoprotective mechanism during in vitro hypoxia and in vivo ischemia-reperfusion injury. *Am. J. Pathol.* 176(3):1181–92
61. El Karoui K, Viau A, Dellis O, Bagattin A, Nguyen C, et al. 2016. Endoplasmic reticulum stress drives proteinuria-induced kidney lesions via Lipocalin 2. *Nat. Commun.* 7:10330
62. Zhou R, Yazdi AS, Menu P, Tschopp J. 2011. A role for mitochondria in NLRP3 inflammasome activation. *Nature* 469(7329):221–25
63. Nakahira K, Haspel JA, Rathinam VA, Lee SJ, Dolinay T, et al. 2011. Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. *Nat. Immunol.* 12(3):222–30
64. Shi CS, Shenderov K, Huang NN, Kabat J, Abu-Asab M, et al. 2012. Activation of autophagy by inflammatory signals limits IL-1β production by targeting ubiquitinated inflammasomes for destruction. *Nat. Immunol.* 13(3):255–63

65. Hartleben B, Gödel M, Meyer-Schwesinger C, Liu S, Ulrich T, et al. 2010. Autophagy influences glomerular disease susceptibility and maintains podocyte homeostasis in aging mice. *J. Clin. Investig.* 120(4):1084–96
66. Kawakami T, Gomez IG, Ren S, Hudkins K, Roach A, et al. 2015. Deficient autophagy results in mitochondrial dysfunction and FSGS. *J. Am. Soc. Nephrol.* 26(5):1040–52
67. Yi M, Zhang L, Liu Y, Livingston MJ, Chen JK, et al. 2017. Autophagy is activated to protect against podocyte injury in adriamycin-induced nephropathy. *Am. J. Physiol. Renal Physiol.* 313(1):F74–84
68. Matsuda J, Namba T, Takabatake Y, Kimura T, Takahashi A, et al. 2018. Antioxidant role of autophagy in maintaining the integrity of glomerular capillaries. *Autophagy* 14(1):53–65
69. Periyasamy-Thandavan S, Jiang M, Wei Q, Smith R, Yin XM, et al. 2008. Autophagy is cytoprotective during cisplatin injury of renal proximal tubular cells. *Kidney Int.* 74:631–40
70. Inoue K, Kuwana H, Shimamura Y, Ogata K, Taniguchi Y, et al. 2010. Cisplatin-induced macroautophagy occurs prior to apoptosis in proximal tubules in vivo. *Clin. Exp. Nephrol.* 14:112–22
71. Takahashi A, Kimura T, Takabatake Y, Namba T, Kaimori J, et al. 2012. Autophagy guards against cisplatin-induced acute kidney injury. *Am. J. Pathol.* 180:517–25
72. Amini FG, Rafieian-Kopaei M, Nematbakhsh M, Baradaran A, Nasri H. 2012. Ameliorative effects of metformin on renal histologic and biochemical alterations of gentamicin-induced renal toxicity in Wistar rats. *J. Res. Med. Sci.* 17(7):621–25
73. Li J, Gui Y, Ren J, Liu X, Feng Y, et al. 2016. Metformin protects against cisplatin-induced tubular cell apoptosis and acute kidney injury via AMPKα-regulated autophagy induction. *Sci. Rep.* 6:23975
74. Lynch MR, Tran MT, Ralto KM, Zsengeller ZK, Raman V, et al. 2019. TFEB-driven lysosomal biogenesis is pivotal for PGC1α-dependent renal stress resistance. *JCI Insight* 5:e126749
75. Leventhal JS, Ni J, Osmond M, Lee K, Gusella GL, et al. 2016. Autophagy limits endotoxemic acute kidney injury and alters renal tubular epithelial cell cytokine expression. *PLOS ONE* 11(3):e0150001
76. Mei S, Livingston M, Hao J, Li L, Mei C, et al. 2016. Autophagy is activated to protect against endotoxic acute kidney injury. *Sci. Rep.* 6:22171
77. Wu Y, Zhang Y, Wang L, Diao Z, Liu W. 2015. The role of autophagy in kidney inflammatory injury via the NF-κB route induced by LPS. *Int. J. Med. Sci.* 12(8):655–67
78. Howell GM, Gomez H, Collage RD, Loughran P, Zhang X, et al. 2013. Augmenting autophagy to treat acute kidney injury during endotoxemia in mice. *PLOS ONE* 8(7):e69520
79. Takahashi W, Watanabe E, Fujimura L, Watanabe-Takano H, Yoshidome H, et al. 2013. Kinetics and protective role of autophagy in a mouse cecal ligation and puncture-induced sepsis. *Crit. Care* 17(4):R160
80. Sunahara S, Watanabe E, Hatano M, Swanson PE, Oami T, et al. 2018. Influence of autophagy on acute kidney injury in a murine cecal ligation and puncture sepsis model. *Sci. Rep.* 8(1):1050
81. Hsiao HW, Tsai KL, Wang LF, Chen YH, Chiang PC, et al. 2012. The decline of autophagy contributes to proximal tubular dysfunction during sepsis. *Shock* 37(3):289–96
82. Zhao W, Zhang L, Chen R, Lu H, Sui M, et al. 2018. SIRT3 protects against acute kidney injury via AMPK/mTOR-regulated autophagy. *Front. Physiol.* 9:1526
83. Chien CT, Shyue SK, Lai MK. 2007. Bcl-xL augmentation potentially reduces ischemia/reperfusion induced proximal and distal tubular apoptosis and autophagy. *Transplantation* 84:1183–90
84. Suzuki C, Isaka Y, Takabatake Y, Tanaka H, Koike M, et al. 2008. Participation of autophagy in renal ischemia/reperfusion injury. *Biochem. Biophys. Res. Commun.* 368:100–106
85. He L, Livingston MJ, Dong Z. 2014. Autophagy in acute kidney injury and repair. *Nephron. Clin. Pract.* 127:56–60
86. Jiang M, Liu K, Luo J, Dong Z. 2010. Autophagy is a renoprotective mechanism during *in vitro* hypoxia and *in vivo* ischemia-reperfusion injury. *Am. J. Pathol.* 176:1181–92
87. Jiang M, Wei Q, Dong G, Komatsu M, Su Y, et al. 2012. Autophagy in proximal tubules protects against acute kidney injury. *Kidney Int.* 82:1271–83
88. Liu S, Hartleben B, Kretz O, Wiech T, Igarashi P, et al. 2012. Autophagy plays a critical role in kidney tubule maintenance, aging and ischemia-reperfusion injury. *Autophagy* 8:826–83
89. Kimura T, Takabatake Y, Takahashi A, Kaimori JY, Matsui I, et al. 2011. Autophagy protects the proximal tubule from degeneration and acute ischemic injury. *J. Am. Soc. Nephrol.* 22(5):902–13

90. Tang C, Han H, Yan M, Zhu S, Liu J, et al. 2018. PINK1-PRKN/PARK2 pathway of mitophagy is activated to protect against renal ischemia-reperfusion injury. *Autophagy* 14(5):880–97
91. Li L, Kang H, Zhang Q, D'Agati VD, Al-Awqati Q, et al. 2019. FoxO3 activation in hypoxic tubules prevents chronic kidney disease. *J. Clin. Investig.* 129:2374–89
92. Natl. Inst. Diabetes Dig. Kidney Dis. 2019. *Kidney disease statistics for the United States*. **https://www.niddk.nih.gov/health-information/health-statistics/kidney-disease**
93. Lee SY, Kim SI, Choi ME. 2015. Therapeutic targets for treating fibrotic kidney diseases. *Transl. Res.* 165:512–30
94. Tanaka T. 2017. A mechanistic link between renal ischemia and fibrosis. *Med. Mol. Morphol.* 50(1). **https://doi.org/10.1007/s00795-016-0146-3**
95. Liu N, Shi Y, Zhuang S. 2016. Autophagy in chronic kidney diseases. *Kidney Dis.* 2:37–45
96. Lin TA, Wu VC, Wang CY. 2019. Autophagy in chronic kidney diseases. *Cells* 8(1):61
97. De Rechter S, Decuypere JP, Ivanova E, van den Heuvel LP, De Smedt H, et al. 2016. Autophagy in renal diseases. *Pediatr. Nephrol.* 31(5):737–52
98. Ding Y, Choi ME. 2015. Autophagy in diabetic nephropathy. *J. Endocrinol.* 224:R15–30
99. Ding Y, Kim SI, Lee SY, Koo JK, Wang Z, et al. 2014. Autophagy regulates TGF-β expression and suppresses kidney fibrosis induced by unilateral ureteral obstruction. *J. Am. Soc. Nephrol.* 25(12):2835–46
100. Kim WY, Nam SA, Song HC, Ko JS, Park SH, et al. 2012. The role of autophagy in unilateral ureteral obstruction rat model. *Nephrology* 17(2):148–59
101. Li H, Peng X, Wang Y, Cao S, Xiong L, et al. 2016. Atg5-mediated autophagy deficiency in proximal tubules promotes cell cycle G_2/M arrest and renal fibrosis. *Autophagy* 12(9):1472–86
102. Peng X, Wang Y, Li H, Fan J, Shen J, et al. 2019. ATG5-mediated autophagy suppresses NF-κB signaling to limit epithelial inflammatory response to kidney injury. *Cell Death Dis.* 10(4):253
103. Kim SI, Na HJ, Ding Y, Wang Z, Lee SJ, et al. 2012. Autophagy promotes intracellular degradation of type I collagen induced by transforming growth factor (TGF)-β1. *J. Biol. Chem.* 287(15):11677–88
104. Nam SA, Kim WY, Kim JW, Park SH, Kim HL, et al. 2019. Autophagy attenuates tubulointerstital fibrosis through regulating transforming growth factor-β and NLRP3 inflammasome signaling pathway. *Cell Death Dis.* 10(2):78
105. Yang S, Abdulla R, Lu C, Zhang L. 2018. Inhibition of microRNA-376b protects against renal interstitial fibrosis via inducing macrophage autophagy by upregulating Atg5 in mice with chronic kidney disease. *Kidney Blood Press. Res.* 43(6):1749–64
106. Livingston MJ, Ding HF, Huang S, Hill JA, Yin XM, et al. 2016. Persistent activation of autophagy in kidney tubular cells promotes renal interstitial fibrosis during unilateral ureteral obstruction. *Autophagy* 12(6):976–98
107. Singh L, Singh G, Dinda AK. 2015. Understanding podocytopathy and its relevance to clinical nephrology. *Indian J. Nephrol.* 25(1):1–7
108. Fang L, Zhou Y, Cao H, Wen P, Jiang L, et al. 2013. Autophagy attenuates diabetic glomerular damage through protection of hyperglycemia-induced podocyte injury. *PLOS ONE* 8:e60546
109. Zeng C, Fan Y, Wu J, Shi S, Chen Z, et al. 2014. Podocyte autophagic activity plays a protective role in renal injury and delays the progression of podocytopathies. *J. Pathol.* 234:203–13
110. Xu D, Chen P, Wang B, Wang Y, Miao N, et al. 2019. NIX-mediated mitophagy protects against proteinuria-induced tubular cell apoptosis and renal injury. *Am. J. Physiol. Renal Physiol.* 316:F382–95
111. Kawakami T, Gomez IG, Ren S, Hudkins K, Roach A, et al. 2015. Deficient autophagy results in mitochondrial dysfunction and FSGS. *J. Am. Soc. Nephrol.* 26(5):1040–52
112. Huang SS, Ding DF, Chen S, Dong CL, Ye XL, et al. 2017. Resveratrol protects podocytes against apoptosis via stimulation of autophagy in a mouse model of diabetic nephropathy. *Sci. Rep.* 7:45692
113. Kitada M, Ogura Y, Monno I, Koya D. 2017. Regulating autophagy as a therapeutic target for diabetic nephropathy. *Curr. Diab. Rep.* 17(7):53
114. Gödel M, Hartleben B, Herbach N, Shuya L, Zschiedrich S, et al. 2011. Role of mTOR in podocyte function and diabetic nephropathy in humans and mice. *J. Clin. Investig.* 121:2197–209

115. Inoki K, Mori H, Wang J, Tsukasa S, Hong SK, et al. 2011. mTORC1 activation in podocytes is a critical step in the development of diabetic nephropathy in mice. *J. Clin. Investig.* 121:2181–96
116. Ma T, Zhu J, Chen X, Zha D, Singhai PC, et al. 2013. High glucose induces autophagy in podocytes. *Exp. Cell Res.* 319:779–89
117. Lenoir O, Jasiek M, Henique C, Guyonnet L, Hartleben B, et al. 2015. Endothelial cell and podocyte autophagy synergistically protect from diabetes-induced glomerulosclerosis. *Autophagy* 11:1130–45
118. Dong C, Zheng H, Huang S, You N, Xu J, et al. 2015. Heme oxygenase-1 enhances autophagy in podocytes as a protective mechanism against high glucose-induced apoptosis. *Exp. Cell Res.* 337(2):146–59
119. Xiao T, Guan X, Nie L, Wang S, Sun L, et al. 2014. Rapamycin promotes podocyte autophagy and ameliorates renal injury in diabetic mice. *Mol. Cell Biochem.* 394(1–2):145–54
120. Tagawa A, Yasuda M, Kume S, Yamahara K, Nakazawa J, et al. 2016. Impaired podocyte autophagy exacerbates proteinuria in diabetic nephropathy. *Diabetes* 65:755–67
121. Li X, Li C, Sun G. 2016. Histone acetylation and its modifiers in the pathogenesis of diabetic nephropathy. *J. Diabetes Res.* 2016:4065382
122. Wei Q, Dong Z. 2014. HDAC4 blocks autophagy to trigger podocyte injury: non-epigenetic action in diabetic nephropathy. *Kidney Int.* 86:666–68
123. Wang X, Liu J, Zhen J, Zhang C, Wan Q, et al. 2014. Histone deacetylase 4 selectively contributes to podocyte injury in diabetic nephropathy. *Kidney Int.* 86(4):712–25
124. Wang W, Sun W, Cheng Y, Xu Z, Cai L. 2019. Role of sirtuin-1 in diabetic nephropathy. *J. Mol. Med.* 97(3):291–309
125. Hasegawa K, Wakino S, Simic P, Sakamaki Y, Minakuchi H, et al. 2013. Renal tubular Sirt1 attenuates diabetic albuminuria by epigenetically suppressing Claudin-1 overexpression in podocytes. *Nat. Med.* 19(11):1496–504
126. Wen D, Huang X, Zhang M, Zhang L, Chen J, et al. 2013. Resveratrol attenuates diabetic nephropathy via modulating angiogenesis. *PLOS ONE* 8:e82336
127. Liu M, Liang K, Zhen J, Zhou M, Wang X, et al. 2017. Sirt6 deficiency exacerbates podocyte injury and proteinuria through targeting Notch signaling. *Nat. Commun.* 8(1):413
128. Sooparb S, Price SR, Shaoguang J, Franch HA. 2004. Suppression of chaperone mediated autophagy in the renal cortex during acute diabetes mellitus. *Kidney Int.* 65:2135–44
129. Naguib M, Rashed LA. 2018. Serum level of the autophagy biomarker Beclin-1 in patients with diabetic kidney disease. *Diabetes Res. Clin. Pract.* 143:56–61
130. Wakino S, Hasegawa K, Itoh H. 2015. Sirtuin and metabolic kidney disease. *Kidney Int.* 201588(4):691–98
131. Casanova C, de Torres JP, Navarro J, Aguirre-Jaíme A, Toledo P, et al. 2010. Microalbuminuria and hypoxemia in patients with chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 182(8):1004–10
132. Romundstad S, Naustdal T, Romundstad PR, Sorger H, Langhammer A. 2014. COPD and microalbuminuria: a 12-year follow-up study. *Eur. Respir. J.* 43(4):1042–50
133. Oelsner EC, Balte PP, Grams ME, Cassano PA, Jacobs DR, et al. 2019. Albuminuria, lung function decline, and risk of incident chronic obstructive pulmonary disease. The NHLBI Pooled Cohorts Study. *Am. J. Respir. Crit. Care Med.* 199(3):321–32
134. Polverino F, Laucho-Contreras ME, Petersen H, Bijol V, Sholl LM, et al. 2017. A pilot study linking endothelial injury in lungs and kidneys in chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 195(11):1464–76
135. Pabón MA, Patino E, Bhatia D, Rojas-Quintero J, Ma KC, et al. 2018. Beclin-1 regulates cigarette smoke-induced kidney injury in a murine model of chronic obstructive pulmonary disease. *JCI Insight* 3(18):99592
136. Lam HC, Cloonan SM, Bhashyam AR, Haspel JA, Singh A, et al. 2013. Histone deacetylase 6-mediated selective autophagy regulates COPD-associated cilia dysfunction. *J. Clin. Investig.* 123(12):5212–30
137. Mizumura K, Cloonan SM, Nakahira K, Bhashyam AR, Cervo M, et al. 2014. Mitophagy-dependent necroptosis contributes to the pathogenesis of COPD. *J. Clin. Investig.* 124(9):3987–4003

138. Chen ZH, Lam HC, Jin Y, Kim HP, Cao J, et al. 2010. Autophagy protein microtubule-associated protein 1 light chain-3B (LC3B) activates extrinsic apoptosis during cigarette smoke-induced emphysema. *PNAS* 107(44):18880–85
139. Boletta A. 2009. Emerging evidence of a link between the polycystins and the mTOR pathways. *PathoGenetics* 2(1):6
140. Zafar I, Ravichandran K, Belibi FA, Doctor RB, Edelstein CL. 2010. Sirolimus attenuates disease progression in an orthologous mouse model of human autosomal dominant polycystic kidney disease. *Kidney Int*. 78(8):754–61
141. Belibi F, Ravichandran K, Zafar I, He Z, Edelstein CL. 2011. mTORC1/2 and rapamycin in female Han:SPRD rats with polycystic kidney disease. *Am. J. Physiol. Renal Physiol.* 300(1):F236–44
142. Belibi F, Zafar I, Ravichandran K, Segvic AB, Jani A, et al. 2011. Hypoxia-inducible factor-1α (HIF-1α) and autophagy in polycystic kidney disease (PKD). *Am. J. Physiol. Renal Physiol.* 300(5):F1235–43
143. Zhu P, Sieben CJ, Xu X, Harris PC, Lin X. 2017. Autophagy activators suppress cystogenesis in an autosomal dominant polycystic kidney disease model. *Hum. Mol. Genet.* 26(1):158–72
144. Qu X, Yu J, Bhagat G, Furuya N, Hibshoosh H, et al. 2003. Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. *J. Clin. Investig.* 112:1809–20
145. White E. 2012. Deconvoluting the context dependent role for autophagy in cancer. *Nat. Rev. Cancer* 12:401–10
146. Deng Q, Wang Z, Wang L, Zhang L, Xiang X, et al. 2013. Lower mRNA and protein expression levels of LC3 and Beclin1, markers of autophagy, were correlated with progression of renal clear cell carcinoma. *Jpn. J. Clin. Oncol.* 43(12):1261–68
147. Liu XD, Yao J, Tripathi DN, Ding Z, Xu Y, et al. 2015. Autophagy mediates HIF2α degradation and suppresses renal tumorigenesis. *Oncogene* 34(19):2450–60
148. Kang HM, Noh KH, Chang TK, Park D, Cho HS, et al. 2019. Ubiquitination of MAP1LC3B by pVHL is associated with autophagy and cell death in renal cell carcinoma. *Cell Death Dis.* 10(4):27
149. Chai D, Shan H, Wang G, Li H, Fang L, et al. 2018. AIM2 is a potential therapeutic target in human renal carcinoma and suppresses its invasion and metastasis via enhancing autophagy induction. *Exp. Cell Res.* 370(2):561–70
150. Haas NB, Appleman LJ, Stein M, Redlinger M, Wilks M, et al. 2019. Autophagy inhibition to augment mTOR inhibition: a phase I/II trial of everolimus and hydroxychloroquine in patients with previously treated renal cell carcinoma. *Clin. Cancer Res.* 25(7):2080–87