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Autophagy in kidney homeostasis and disease

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Abstract

Autophagy is a conserved lysosomal pathway for the degradation of cytoplasmic components. Basal autophagy in kidney cells is essential for the maintenance of kidney homeostasis, structure and function. Under stress conditions, autophagy is altered as part of the adaptive response of kidney cells, in a process that is tightly regulated by signalling pathways that can modulate the cellular autophagic flux — mammalian target of rapamycin, AMP-activated protein kinase and sirtuins are key regulators of autophagy. Dysregulated autophagy contributes to the pathogenesis of acute kidney injury, to incomplete kidney repair after acute kidney injury and to chronic kidney disease of varied aetiologies, including diabetic kidney disease, focal segmental glomerulosclerosis and polycystic kidney disease. Autophagy also has a role in kidney ageing. However, questions remain about whether autophagy has a protective or a pathological role in kidney fibrosis, and about the precise mechanisms and signalling pathways underlying the autophagy response in different types of kidney cells and across the spectrum of kidney diseases. Further research is needed to gain insights into the regulation of autophagy in the kidneys and to enable the discovery of pathway-specific and kidney-selective therapies for kidney diseases and anti-ageing strategies.

Autophagy is a lysosomal degradation pathway that breaks down cytoplasmic components for clearance and reuse. Depending on the type of cargo to be degraded and the route of cargo delivery to lysosomes, autophagy can be classified as macroautophagy, microautophagy or chaperone-mediated autophagy. Macroautophagy refers to the engulfment of large cytoplasmic materials by autophagosomes, which then fuse with

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lysosomes for degradation[1]. Microautophagy involves the direct sequestration of small cytosolic components into an inward invagination of the lysosomal membrane without the formation of autophagosomes[2]. In chaperone-mediated autophagy, proteins containing the KFERQ motif are identified by cytosolic heat shock cognate 71 kDa protein (encoded by *HSPA8*) and then translocated across the lysosomal membrane via interactions with lysosome-associated membrane glycoprotein 2A (LAMP2) for delivery into the lysosomal lumen through multimerization of LAMP2 with other cofactors[3].

Macroautophagy (hereafter termed autophagy) has a variety of physiological and pathophysiological roles[1,4,5]. Basal autophagy removes potentially dysfunctional organelles and long-lived proteins to maintain cellular homeostasis, whereas autophagy induced in response to environmental and intracellular stress might serve as an adaptive response to ensure cell survival. However, autophagy might also contribute to cellular dysfunction and organ damage.

In this Review, we provide an overview of the cellular and molecular basics of autophagy, analyse the physiological roles of basal autophagy in the major types of resident kidney cells and summarize current evidence on the regulation and pathological roles of autophagy in glomerular and tubulointerstitial diseases. The therapeutic potential and challenges of targeting autophagy for the prevention and treatment of kidney diseases are also discussed.

Molecular basics of autophagy

Autophagy is a multistep process that involves initiation, nucleation, expansion, fusion and degradation (FIG. 1). Several distinct complexes that are composed of autophagy-related proteins (ATGs) function co-ordinately with membrane trafficking components at different steps of autophagosome biogenesis (FIG. 1). The complex formed by serine/threonine protein kinases ULK1, ULK2 and other protein partners is a major component in the initiation of autophagy[6-8], whereas the class III phosphoinositide 3-kinase (PI3K) complex regulates vesicle nucleation and phagophore formation[9,10]. Two ubiquitin (Ub)-like conjugation systems, the ATG12–ATG5–ATG16L system and the microtubule-associated protein 1 light chain 3 (MAP1LC3; also known as LC3) system control the expansion and completion of the autophagosome[11] (FIG. 1). The regulation of autophagosome–lysosome fusion to form autolysosomes is less well understood. Cytoskeleton components and cytoskeleton-related motor proteins mediate the movement of autophagosomes to lysosomes for tethering, followed by soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE)-mediated fusion[12]. Notably, autolysosomes are not permanent and disintegrate once autophagy is terminated. This process, called autophagic lysosome reformation (ALR), allows recycling of lysosomal membrane proteins and regeneration of lysosomes from autolysosomes through reformation tubules and vesicles.

In addition to non-selective cytosolic bulk degradation, autophagy can also selectively remove specific cargoes, such as mitochondria by mitophagy, lysosomes by lysophagy, protein aggregates by aggrephagy and lipid droplets by lipophagy[13]. Mitophagy is the best-described form of selective autophagy and is predominantly mediated by LC3-associated autophagy receptors via both Ub-dependent and Ub-independent pathways (FIG. 2).

Regulatory signalling pathways

Autophagy is tightly regulated to enable cells to adapt to or counteract cellular stress. Several studies have highlighted a complex signalling network that senses changes in nutrient and/or energy status to either stimulate or inhibit autophagy. Intracellular stress, which can be induced by reactive oxygen species (ROS), endoplasmic reticulum (ER) stress, hypoxia, DNA damage and immune signalling, has also been demonstrated as a potential stimulator of autophagy (reviewed elsewhere[14]).

Nutrient and/or energy signalling.—Autophagy induced by nutrient and/or energy deprivation is primarily regulated by signalling pathways that include the serine/threonine protein kinase mammalian target of rapamycin (mTOR), AMP-activated protein kinase (AMPK) and sirtuins (FIG. 3). mTOR, in combination with cofactors and regulators, forms the rapamycin-sensitive mTOR complex 1 (mTORC1) and the rapamycin-insensitive mTORC2 (REF.[15]). mTORC1 is a master regulator of autophagy that is activated by amino acids or growth factors, such as insulin and insulin-like growth factor-1, via upstream pathways[15]. Hamartin (encoded by *TSC1*)–tuberin (encoded by *TSC2*) complexes negatively regulate mTORC1 by inhibiting GTP binding protein RHEB[15]. Under nutrient-rich conditions, active mTORC1 suppresses autophagy by phosphorylating ULK1 and ATG13 to inhibit the ULK1–ULK2 complex. Active mTORC1 also stimulates ribosome biogenesis and mRNA translation by phosphorylating two downstream targets, ribosomal protein S6 kinase β1 (S6K1; also known as p70S6K) complexed with S6 ribosomal protein (S6RP) and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1)[15,16], which supports cell growth and proliferation. Under starvation, mTORC1 is suppressed and dissociates from the ULK1–ULK2 complex, allowing ULK1 to be activated by AMPK to induce autophagy[17] (FIG. 3). Well-known inhibitors of mTORC1 include sirolimus (also known as rapamycin) and rapamycin analogues (also known as rapalogs), such as everolimus (also known as RAD001) and temsirolimus[18]. Unlike mTORC1, the function of mTORC2 in autophagy is controversial[19,20].

As mentioned above, AMPK positively regulates autophagy. Under conditions of energy depletion, AMPK is activated by a high AMP:ATP ratio through upstream kinases including serine/threonine-protein kinase STK11 (also known as LKB1), calcium/calmodulin-dependent protein kinase 2 (CAMKK2), and mitogen-activated protein kinase kinase kinase 7 (also known as TAK1)[21-23] (FIG. 3). Active AMPK induces autophagy through direct phosphorylation of ULK1, or through the phosphorylation of tuberin or regulatory-associated protein of mTOR (RAPTOR; encoded by *RPTOR*) to inhibit mTORC1 (REFS[24-26]) (FIG. 3). Notably, although they have opposing roles in the regulation of ULK1, both AMPK and mTORC1 can be phosphorylated and inhibited by ULK1, which creates a complex crosstalk[27,28]. Several drug agents can directly or indirectly activate AMPK, including metformin, 5-aminoimidazole-4-carboxamide riboside (AICAR), A-769662 and compound 911 (REF.[29]).

Sirtuins (SIRT1 to SIRT7) are NAD^+-dependent class III histone deacetylases. Under conditions of energy depletion, SIRT1 is activated by increased NAD^+ levels. Active SIRT1 can stimulate autophagy through the deacetylation of ATG proteins[30,31] or of the

transcription factors forkhead box protein O1 (FOXO1) and FOXO3a, which transactivates autophagy genes[32]. In addition, SIRT1 crosstalks with the mTOR and AMPK pathways by interacting with hamartin–tuberin and deacetylating LKB1, thus regulating different mechanisms involved in energy metabolism and cell survival, including autophagy[33-35] (FIG. 3). Resveratrol and SRT compounds such as SRT1720 activate SIRT1 (REF.[36]).

Pathways indirectly associated with nutrient and/or energy signalling.— Pathways that are indirectly associated with those involved in nutrient and/or energy signalling have also been implicated in the regulation of autophagy during nutrient shortage (FIG. 3). For example, active mitogen-activated protein kinase 8 (MAPK8; also known as JNK1)[37] and death-associated protein kinases (DAP kinases) promote the induction of autophagy via beclin 1 (REF.[38]) (FIG. 3). Phosphorylation of eukaryotic translation initiation factor 2 subunit-α (eIF2α)[39], activation of inhibitor of NF-κB kinase (IKK)[40], nuclear translocation of transcription factor EB (encoded by *TFEB*)[41] and nuclear export of zinc-finger protein with KRAB and SCAN domains 3 (encoded by *ZKSCAN3*)[42] are also involved in starvation-induced autophagy (FIG. 3).

Autophagy in kidney homeostasis

Autophagy is reportedly dispensable for kidney development[43,44], but in adult kidneys, basal autophagy in resident kidney cells, including podocytes, proximal tubule epithelial cells (PTECs), glomerular mesangial cells (GMCs) and glomerular endothelial cells (GECs), seems to be crucial for maintaining kidney integrity and normal physiology (TABLE 1). In GMCs, for example, in vitro studies showed that autophagy regulates the turnover of collagen I[45] and is cytoprotective in response to serum deprivation[46]. However, the role of autophagy in GMCs in vivo awaits further investigation.

Podocytes

In transgenic mice with GFP-tagged LC3 (LC3–GFP), abundant punctate distribution of LC3–GFP was observed in podocytes, but staining was minimal in kidney tubules and in other glomerular cells under physiological conditions[44], which suggests that podocytes have a high level of basal autophagy. Moreover, blockade of autophagosomal degradation with the lysosomal inhibitor chloroquine induced a rapid accumulation of LC3–GFP puncta in podocytes, suggesting that these cells had a normal autophagic flux[44]. In doxycycline-controlled podocyte-specific *Atg5*-knockout mice, the acute induction of *Atg5* deletion in 12-week-old mice triggered a rapid onset of albuminuria, indicating that basal autophagy is crucial for the maintenance of podocyte function[44]. By contrast, up to 2–4 months after birth, kidney histology, glomerular ultrastructure and albuminuria were indistinguishable between mice with a constitutive podocyte-specific *Atg5* deletion and their wild-type littermates[44]. Increased proteasome activity seemed to compensate for the constitutive loss of autophagy in these mice. Moreover, mice with lysosomal dysfunction in podocytes, induced by inducible podocyte-specific deletion of the genes encoding mTOR, prorenin or PI3K catalytic subunit type 3 (also known as VPS34) developed severe glomerulosclerosis and proteinuria[47-50], highlighting the importance of an intact autophagic flux for the maintenance of podocyte homeostasis.

Although podocytes have high basal levels of autophagy, high mTORC1 activity is also required for these cells to maintain their function[51,52]. This seemingly contradictory finding suggests a potential interplay between these two pathways in podocytes. An early study suggested that podocytes form TOR–autophagy spatial coupling compartments (TASCCs), which are distinct cytoplasmic compartments enriched for mTORC1 that include autolysosomes but largely exclude autophagosomes[53]. Therefore, TASCCs provide cells with a mechanism for simultaneous activation of anabolic and catabolic processes, which facilitates mTOR-controlled synthesis of secretory proteins with sources of energy or building blocks provided by constitutive autophagy. Moreover, the products of autolysosomal degradation can reactivate mTORC1 within TASCCs, which might, in turn, initiate ALR[54]. Podocyte-specific *Mtor*-knockout mice accumulated autophagosomes and autolysosomes within podocytes, and developed severe proteinuria and kidney failure shortly after birth, suggesting that mTOR-associated ALR might be important for podocyte health[49].

Another study revealed that AMPK, rather than mTORC1 signalling, controlled the high basal levels of autophagy in podocytes. The researchers showed that mice with podocyte-specific mTORC1 hyperactivation (induced by podocyte-specific deletion of *Tsc1*) and mTORC1 hypoactivation (induced by podocyte-specific deletion of *Rptor*) had high basal levels of autophagy that were comparable to those of wild-type mice[55]. These findings suggested that basal podocyte autophagy was independent of mTORC1 activity. In glomeruli, podocyte-specific AMPK activity was increased in mice with mTORC1 hyperactivation and suppressed in mice with mTORC1 hypoactivation[55]. AMPK activity might counteract the effects of mTORC1 activation to stabilize the levels of autophagy in these mice. Short-term (3 days) and long-term (3 weeks) treatment with AICAR resulted in similarly high levels of podocyte autophagy in mice with podocyte-specific mTORC1 hyperactivation or hypoactivation and in wild-type mice, compared with mice without AICAR treatment, suggesting that autophagy induction by AMPK is mTORC1-independent in podocytes[55]. Treatment with rapamycin for 3 days inhibited mTORC1 activity and concurrently activated AMPK in podocytes, which led to autophagy induction, whereas 3 weeks of treatment with rapamycin inactivated AMPK and counteracted the pro-autophagic effect achieved by mTORC1 inhibition and thus autophagy levels returned to the baseline observed in untreated mice[55]. These findings suggest that the inhibitory effect of mTORC1 on podocyte autophagy is limited to a short-term adaptive response and that AMPK signalling is the main regulatory pathway in the control of podocyte autophagy, which would allow concurrent high basal autophagy and high mTORC1 activity in podocytes[55]. Tyrosine protein kinase JAK2 was also reported to modulate basal autophagy in podocytes by facilitating the translocation of signal transducer and activator of transcription 1 (STAT1) to the nucleus, where it binds to the *Tfeb* promoter to induces its expression[56].

Proximal tubule epithelial cells

Kidney PTECs have relatively low levels of autophagy under physiological conditions[43]. However, in mice, selective deletion of *Atg5* or *Atg7* in proximal tubules resulted in progressive kidney damage and premature kidney ageing, as demonstrated by an accumulation of damaged mitochondria, sequestosome 1 and Ub-positive aggregates, as well as elevated tubular cell apoptosis and kidney tubulointerstitial fibrosis compared with wild-

type controls[43,57]. By contrast, the kidneys of mice with *Atg5* deletion in distal tubules or collecting ducts were histologically similar to those of wild-type mice[43]. These findings suggest that low levels of basal autophagy are essential to the maintenance of PTEC function under normal conditions and that proximal tubules rely on basal autophagy more than other tubular segments.

Using the mito-quality control (mito-QC) mitophagy reporter mouse model, we examined the level of basal mitophagy in the kidney[58]. Mito-QC mice express a tandem mCherry–GFP protein fused to the outer mitochondrial membrane mitochondrial fission 1 protein (encoded by *FIS1*); the differential pH sensitivities between mCherry and GFP enable tracking of the delivery of mitochondria to lysosomes to form mitolysosomes[59]. Interestingly, under control conditions mice had a high level of mitophagy in some cortical proximal tubules, where mitolysosomes were predominantly observed near the apical membrane[58]. A high rate of mitochondrial turnover might be required to meet the high energy demand of the large number of ATP-dependent transporters that are involved in reabsorption and excretion at the apical membrane of kidney proximal tubules. By contrast, mitochondrial turnover was minimal in S3 segment proximal tubules under control conditions. Mitophagy was also observed at much lower levels in glomeruli compared with cortical proximal tubules[58].

Glomerular endothelial cells

In mice with a GEC-specific *Atg5* deletion (*Atg5*$^{fl/fl}$; cadherin 5 (*Cdh5*)–Cre), researchers observed slight dilation of glomerular capillaries, podocyte foot process effacement and loss of glomerular endothelial fenestrations in the glomerular capillary loops from 10 weeks of age; however, functional defects, as assessed by urinary creatinine and blood urea nitrogen (BUN), were not apparent up to 20 weeks of age[60]. Similar changes in the capillary loops and glomerular endothelial fenestra were observed at 4 weeks of age in another conditional *Atg5*-knockout mouse model (*Atg5*$^{fl/fl}$; angiopoietin 1 receptor (*Tek*)–Cre) in which *Atg5* loss occurred in both endothelial and haematopoietic cells[61]. However, at 8 weeks of age, the capillaries of *Tek–Atg5*$^{-/-}$ mice had a lobular pattern, with thickening of the capillary loops and mesangial matrix expansion, and they died by 12 weeks of age, potentially owing to severe haematopoietic injury. Of note, 6-month-old *Tek–Atg5*$^{-/-}$ and wild-type mice that had received a bone marrow transplant from wild-type donors at 4 weeks of age, which would have rescued the loss of *Atg5* in the haematopoietic compartment of *Tek–Atg5*$^{-/-}$ mice, had similar levels of urinary albumin and plasma urea nitrogen[61]. However, at 12 months of age, these transplanted *Tek–Atg5*$^{-/-}$ mice developed mesangiolysis and glomerulosclerosis accompanied by kidney dysfunction, as assessed by plasma creatinine and BUN, which suggests that autophagy deficiency in GECs but not in haematopoietic cells contributed to the glomerular defects observed in *Tekα Atg5*$^{-/-}$ mice. Moreover, administration of the ROS scavenger *N*-acetyl-L-cysteine (NAC) protected against the glomerular lesions in *Tek–Atg5*$^{-/-}$ knockout mice[61]. This observation suggests that endothelial autophagy might protect glomeruli from oxidative stress and maintain the integrity of glomerular capillaries. The differences in glomerular defects observed between *Tek–Atg5*$^{-/-}$ and *Cdh5–Atg5*$^{-/-}$ mice might be attributable to the different activity and/or site specificity of the promoters used to drive Cre recombinase in these two mouse models[60,61].

Autophagy in kidney ageing

Ageing is associated with structural and functional changes in the kidney that potentially compromise its capacity for repair and increase its susceptibility to damage — such effects might underlie the high incidence of chronic kidney disease (CKD) in elderly individuals[62]. One study reported that ageing in mouse tissues, including kidney tissues, was characterized by suppression of autophagy by the run domain beclin-1-interacting and cysteine-rich domain-containing protein (rubicon)[63]. Accordingly, the expression of the autophagy proteins LC3-I and ATG7 was dramatically reduced in the kidneys of 24-month-old rats compared with those of 3-month-old animals, and this decrease was accompanied by an accumulation of sequestosome 1, Ub-positive protein aggregates and damaged mitochondria, as well as the induction of oxidative stress[64].

Autophagy deficiency in podocytes also seems to promote ageing. At 20–24 months of age, mice with a podocyte-specific *Atg5*-knockout displayed more obvious features of cellular ageing such as mitochondrial damage, ER stress and accumulation of oxidized and ubiquitylated protein aggregates and lipofuscin compared with age-matched wild-type controls[44]. Proteasome activity increased in 8-month-old $Atg5^{-/-}$ mice compared with wild-type controls, and this increase seemed to clear protein aggregates and compensate for the loss of autophagy. However, this increase in proteasome activity was significantly reduced in older mice compared with younger *Atg5*-knockouts[44]; podocyte loss and progressive glomerulosclerosis were also observed in older mice. Another study, in which researchers used a mouse model of podocyte-specific deletion of the lysosomal aspartic protease cathepsin D (*Ctsd*), further demonstrated the importance of lysosomal activity in podocyte maintenance during ageing[65]. Defective autolysosome degradation in podocytes triggered the accumulation of toxic lipofuscins, protein aggregates and other harmful cytoplasmic materials, eventually leading to apoptotic podocyte death. As a result, these podocyte-specific $Ctsd^{-/-}$ mice developed proteinuria at 5 months of age and kidney failure by 20–22 months of age[65].

The mechanisms underlying the ageing-related reduction of autophagy in podocytes were further investigated. One study reported that podocyte SIRT1 expression was lower in 26–28-month-old mice than in 3-month-old mice and that podocyte-specific knockdown of *Sirt1* aggravated age-associated kidney injury in ageing mice[66]. Another report indicated that ageing was accompanied by a reduction in the expression of CCAAT/enhancer-binding protein-α (CEBPα) and that podocyte-specific deletion of CEBPα accelerated kidney ageing, as indicated by the increased accumulation of senescence markers and ageing-induced kidney lesions in *Cebpa*-knockout mice compared with wild-type controls[67]. In vitro, exposure to adriamycin induced premature senescence in podocytes, which was accompanied by a reduction in phospho–AMPK and an increase in phospho–mTOR — overexpression of CEBPα restored the dysregulated activity of AMPK and mTOR[67]. These findings suggest that ageing-related changes in the expression of proteins such as SIRT1 and CEBPα might lead to reduced autophagy in podocytes and promote kidney ageing.

Unlike podocytes, compared with 2-month-old mice, PTECs of 24-month-old mice have a higher basal autophagic flux, but their response to starvation-induced autophagy is

blunted[68]. In a mouse model of tamoxifen-inducible proximal tubule-specific *Atg5* deletion, sequestosome 1 and Ub-positive aggregates accumulated at higher levels in aged kidneys than in those from younger mice, suggesting that aged kidneys are more reliant on autophagy for the removal of damaged proteins and organelles[68]. The researchers also demonstrated that disruption of autophagy in proximal tubules for 24 months caused significant kidney dysfunction, tubular atrophy and tubulointerstitial fibrosis, which were accompanied by an aggravation of ageing-related mitochondrial dysfunction as well as damage to mitochondrial and nuclear DNA. Furthermore, the researchers reported dysregulation of nutrient sensing pathways in the proximal tubules of aged mice, including an ageing-related decline in SIRT1 levels and the failure to further activate AMPK in response to starvation and mTOR hyperactivation[68]. Moreover, in doxycycline-inducible kidney tubule-specific *Atg5*-knockout mice treated with doxycycline from 2 months of age for 2 weeks, ageing-related markers, including accumulation of sequestosome-1 and ubiquitin-positive inclusion bodies, as well as concentric membrane bodies, appeared in renal tubular cells 4–5 months after induction of *Atg5* loss in tubular segments[43]. These findings further suggested that autophagy impairment accelerated the ageing process and tubular damage in young mice.

Autophagy in acute kidney injury

Acute kidney injury (AKI) is common and is characterized by a rapid decline of kidney function. Research has demonstrated that autophagy has an important role in the development of AKI — autophagy-mediated removal of protein aggregates and damaged organelles to maintain cellular homeostasis might also protect cells and tissues against injury. Induction of autophagy in kidney tubular cells, particularly in PTECs, has been documented in rodent models of AKI induced by kidney ischaemia–reperfusion injury (IRI), by nephrotoxic drugs such cisplatin, by sepsis and other AKI risk factors[69].

Earlier studies, which used mostly pharmacological approaches to inhibit autophagy, reported contradictory findings, but the subsequent use of mouse models with selective deletion of autophagy genes in proximal tubules has provided further insight into the role of autophagy in AKI. For example, selective *Atg5* or *Atg7* deletion in proximal tubules dramatically sensitized the kidney to kidney IRI, as demonstrated by more severe kidney dysfunction and tubular cell apoptosis in these mice compared with wild-type controls[44,57,70]. Similarly, proximal tubule-specific *Atg7* or *Atg5*-knockout mice were more sensitive to cisplatin-induced AKI than control mice[70,71]. These and other related studies provide compelling evidence for a protective role of tubular cell autophagy in AKI induced by kidney IRI and cisplatin (TABLE 2).

The beneficial effect of autophagy was further confirmed in two experimental models of sepsis-induced AKI caused by caecal ligation and puncture, or lipopoly-saccharide exposure[72-75]. Autophagy and/or mitophagy were also protective in contrast-induced nephrotoxicity, another clinically relevant model of AKI[76-78]. Moreover, promoting autophagy through pharmacological inhibition of mTORC1, or activation of AMPK or sirtuins, has been shown to be renoprotective during AKI[70,75,79].

Collectively, current evidence suggests that autophagy is active in kidney tubular cells and functions as a defence mechanism in AKI (FIG. 4). Notably, mTORC1 inhibits autophagy but also promotes cell growth and differentiation — mTORC1 might be essential for maintaining kidney tubular homeostasis in response to ischaemic stress[80] and mTOR inhibitors should therefore be used with caution.

In AKI, the initial insults induce intracellular stress in PTECs, including oxidative stress, ER stress, hypoxia, DNA damage, immunity-related signalling pathways and nutrient and/or energy deprivation[69], all of which are known to stimulate autophagy. However, the precise mechanisms that regulate autophagy in tubular cells in response to these stress signals are yet to be elucidated in models of AKI.

In addition, emerging studies suggest a complex crosstalk between autophagy and various pathways of cell death, including apoptosis, necrosis and necroptosis, which also contribute to the complex regulation of autophagy (reviewed elsewhere[69]). In general, autophagy and apoptosis can be induced in response to the same stimuli and these two pathways might share common regulatory molecules enabling them to regulate and modify each other to coordinately determine the fate of the cell. The interplay between autophagy and apoptosis in AKI has been observed in a few studies. In a model of ischaemic AKI, overexpression of the anti-apoptotic protein BCL2L1 in kidney tubular cells significantly reduced beclin 1 expression and suppressed autophagy compared with controls[81]. In cultured PTECs, exposure to cisplatin at different doses stimulated distinct components of the unfolded protein response (UPR) — a 10-μM dose of cisplatin promoted autophagy, whereas a 50-μM dose induced apoptosis[82]. Moreover, in PTECs treated with 50 μM of cisplatin, inhibition of autophagy enhanced the activity of caspases 3, 6 and 7, whereas key autophagy proteins, such as beclin 1, ATG5 and ATG12 were cleaved and degraded in a caspase-dependent manner[83].

One study suggested that autophagy has a major role in mediating the renoprotective effects of caloric restriction against kidney IRI in rats. In this experimental setting, preconditioning with caloric restriction stimulated autophagy in kidneys, potentially by increasing SIRT1 expression, which prevented the decrease in peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α) and endothelial nitric oxide synthase in kidney tubules, leading to renoprotection[79]. Ischaemic preconditioning (IPC) is a potential approach for tissue protection during subsequent ischaemic injury in various organs including the kidney, although it seems to be less effective in aged kidneys[84]. The loss of the beneficial effect of kidney IPC in aged kidneys is most likely due to defects in autophagy, which suggests that these two mechanisms are closely related[84]. Indeed, we demonstrated that kidney IPC promoted the clearance of damaged mitochondria via mitophagy to protect tubular cells and kidneys against ischaemic injury[58]. Moreover, in a rat model of ischaemic AKI, IPC protection was mediated by autophagy and regulated by a signalling pathway that involved a serine/threonine protein kinase SGK1–FOXO3a–hypoxia-inducible factor 1α (HIF1α) signalling axis[85]. These findings suggest that autophagy might interact with other defence mechanisms to protect the kidneys from AKI.

Selective autophagy in AKI

Growing evidence suggests that mitophagy is an important defence mechanism in AKI[86,87]. We reported a dramatic induction of mitophagy in proximal tubules during kidney IRI, which was partially abrogated in mice with a single or double knockout of the mitophagy genes *Pink1* and *Park2* (also known as *Prkn*). Importantly, compared with wild-type littermates, *Pink1* and/or *Park2*-knockout mice had more severe kidney dysfunction and tissue damage, which were accompanied by an accumulation of damaged mitochondria and increased tubular cell death, ROS generation and kidney inflammation, which suggested an important role for mitophagy in the protection of tubular cells during ischaemic AKI[88]. We subsequently demonstrated that induction of BCL-2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3)-mediated mitophagy also provided renoprotection during ischaemic AKI[89].

Consistent with these findings, mitophagy with pro-survival effects was also activated in PTECs during AKI induced by cisplatin[90,91], contrast agents[77,78] or sepsis[92]. For instance, deletion of *Pink1* or *Park2* in mice abolished contrast-induced mitophagy in kidney tubules and worsened kidney injury as assessed by kidney tubular damage, plasma creatinine and BUN, mitochondrial damage, ROS production and cell death[77]. In cultured PTECs, knockdown of *Pink1* or *Park2* prevented the induction of mitophagy in response to contrast media and this reduction in mitophagy enhanced PTEC death by activating the NLRP3 inflammasome[77]. These findings suggest that mitophagy might protect against AKI by decreasing inflammation.

Mitophagy induction in PTECs might represent a common mechanism for kidney tubular cell protection in various models of AKI. The mitophagy-mediated elimination of dysfunctional mitochondria might prevent excessive ROS accumulation and the activation of the mitochondrial apoptotic cascade, as well as inhibit the release of mitochondrial DNA and other damage-associated molecular patterns that might otherwise promote inflammation during AKI.

Lysosomes are also abundant in PTECs, where they are used for endocytosis-mediated tubular reabsorption. In a mouse model of hyperuricaemia-associated AKI induced by uric acid and oxalic acid, lysosome damage accompanied by lysophagy occurred in proximal tubules in wild-type mice[93]. Proximal tubule-specific *Atg5*-knockout impaired hyperuricaemia-induced lysophagy in kidney tubules and aggravated hyperuricaemia-induced kidney injury as assessed by plasma creatinine and BUN[93]. These findings suggest a renoprotective role of lysophagy in AKI.

Autophagy and the AKI–CKD transition

After AKI, surviving tubular cells proliferate, migrate and then differentiate to replace lost tubular cells and repair injured kidney tubules[94,95]. Tubular repair might be complete after mild AKI, but severe or episodic AKI are usually followed by incomplete or maladaptive repair that results in the development of kidney fibrosis and CKD[96,97]. Although the mechanisms that underlie the transition from AKI to CKD remain to be elucidated, emerging evidence suggests that autophagy in kidney tubules is involved in kidney repair.

In one study, researchers used autophagy-reporter mice that expressed a tandem red fluorescent protein (RFP)–enhanced GFP (EGFP)–LC3 fusion protein to investigate autophagy during IRI-induced AKI and subsequent recovery[98]. They reported that autophagy was induced in proximal tubules 1 day after reperfusion and that at day 3 autophagosomes fused with lysosomes for clearance, leading to the resolution of autophagy in those cells. The inhibition of mTOR in some proximal tubule cells led to persistent activation of autophagy and impaired proliferation, which indicated that mTOR activation contributed to the resolution of autophagy and tubular repair.

The importance of dynamic changes in autophagy to normal tubular repair after ischaemic AKI was further verified in proximal tubule-specific telomerase (*TerC* or *TerT*)-knockout mice[99]. Telomerases are involved in the elongation of telomeres and thus prevent telomere shortening. Studies demonstrated that telomere shortening induced autophagy[100] and that the induction of autophagy reduced telomerase activity[101]. Kidney IRI caused comparable kidney injury at day 1 in both wild-type and telomerase (*TerC* or *TerT*)-knockout mice, but whereas wild-type mice showed kidney recovery 2–3 days after IRI and kidney function, as assessed by plasma creatinine and BUN, was restored to normal within 14 days, knockout mice had delayed and incomplete kidney recovery. In addition, compared with wild-type mice, in which autophagy was initiated at day 1 after reperfusion, peaked at day 3 and then reduced gradually within 7–14 days, proximal tubule-specific telomerase-knockout mice showed no induction of autophagy as late as 14 days after reperfusion[99]. These results suggest that, after ischaemic AKI, timely and tightly regulated autophagy is crucial for kidney tubules to survive the initial injury, regenerate and contribute to normal kidney repair (FIG. 4).

Interstitial fibrosis is a hallmark of maladaptive repair in the transition from AKI to CKD (FIG. 4) and autophagy might also have a pro-fibrotic role according to findings from a mouse model of post-ischaemic kidney fibrosis[102]. Following ischaemic AKI, researchers observed persistent activation of autophagy and pro-senescent changes in the S3 segments of proximal tubules, as well as kidney fibrosis. *Atg5* deletion in the S3 segment inhibited tubular senescence, attenuated interstitial fibrosis and improved kidney function as assessed by transcutaneous measurement of the glomerular filtration rate, which suggested that autophagy in tubular cells contributed to post-ischaemic kidney fibrosis by promoting senescence[102]. Notably, compared with wild-type littermates, *Atg5*-knockout mice had more tubular cell death 2 h after reperfusion, but reduced tubular damage and inflammation at day 3, suggesting that autophagy enhances cell survival during the initial tubular injury but hinders normal repair during the recovery phase. The long-term pro-fibrotic effects of autophagy might be due its cytoprotective role — by protecting damaged tubular cells from cell death, autophagy enables compromised, senescent cells to persist and promote kidney interstitial fibrosis[102].

Tubular cell arrest at the G2/M phase after severe AKI also contributes substantially to kidney fibrosis by stimulating the generation and secretion of pro-fibrotic cytokines[103] — the formation of TASCCs in G2/M-arrested PTECs was a major contributor to pro-fibrotic changes in these cells[104]. Unlike in podocytes, TASCCs were undetectable in tubular cells of normal mice. However, many TASCC structures appeared in kidney tubules after severe

kidney injury induced by aristolochic acid or prolonged kidney ischaemia. Of note, TASCC formation began 7 days after injury, increased remarkably between days 14 and 21 as kidney fibrosis progressed, and was sustained at a moderate level at day 42, which was the experimental end point. Over 80% of these $TASCC^+$ tubular cells were also phospho-histone 3 $(pH3)^+$, which indicated that they were arrested at the G2/M phase of the cell cycle. Cyclin G1 promoted G2/M arrest in PTECs and upregulated TASCC formation. Notably, TASCC disruption after kidney injury reduced the synthesis and secretion of pro-fibrotic cytokines in PTECs and suppressed kidney fibrosis after severe AKI[104]. These results suggest that in G2/M-arrested tubular cells, the autophagy pathway might participate in the formation of TASCCs to facilitate a change in the secretory phenotype of PTECs and promote fibrotic maladaptive repair after AKI.

Several studies have examined the potential signalling pathways underlying the persistent activation of kidney tubular autophagy after AKI. One report demonstrated that chronic hypoxia after severe ischaemic AKI activated FOXO3 in a HIF1α-dependent manner[105]. Compared with wild-type controls, mice with a proximal tubule-specific *Foxo3* deletion failed to induce autophagy in kidney tubules after AKI. The expression of BNIP3, a known target of FOXO3 and a key component of the autophagy machinery, was also decreased in *Foxo3*-knockout mice, whereas ULK1 expression was only slightly reduced. These results suggest a potential role for the HIF1α–FOXO3 pathway in regulating tubular cell autophagy during the transition from AKI to CKD[105].

In addition to hypoxia, we reported that the induction of autophagy in proximal tubules appeared to be mediated by ER stress. In a mouse model of unilateral kidney IRI injury, ER stress and autophagy were both induced in kidney tubular cells, and attenuation of ER stress by the chemical chaperones tauroursodeoxycholic acid and 4-phenylbutyric acid attenuated autophagy and the progression of kidney fibrosis[106]. Persistent autophagy during kidney repair or AKI-to-CKD transition might involve complex mechanisms that are still poorly understood.

Autophagy in tubulointerstitial fibrosis

The role of autophagy in kidney tubulointerstitial fibrosis has been extensively studied in experimental models of fibrosis such as unilateral ureteric obstruction (UUO) or treatment with transforming growth factor β1 (TGFβ1), but findings are controversial probably because of the multiple functions of autophagy in fibrosis (FIG. 4). In mice with tubule-specific inducible overexpression of TGFβ1, autophagy was activated along with tubular degeneration and widespread peritubular fibrosis. Of note, these degenerating cells were not positive for terminal deoxynucleotidyl transferase dUTP nick end labelling staining, which is used to identify apoptotic cells, suggesting that autophagy might drive tubular atrophy in TGFβ1-induced kidney interstitial fibrosis. Earlier studies showed that, in mice subjected to UUO, autophagy was induced along with apoptosis in obstructed kidney tubules and that both pathways promoted tubular atrophy and nephron loss[107]. We also reported that autophagy was persistently activated in obstructed kidney tubules in UUO mice[108]. Importantly, inhibition of autophagy by chloroquine, or by selective deletion of *Atg7* in proximal tubules, reduced kidney fibrosis in UUO mice compared with untreated or wild-

type controls, respectively, potentially by attenuating tubular apoptosis, macrophage infiltration and production of fibroblast growth factor[108].

In addition, in UUO mice, pharmacological inhibition of autophagy markedly reduced the accumulation of lipids in kidney tubules, which was accompanied by reduced tubular cell apoptosis and degeneration, and suppressed kidney interstitial fibrosis[109]. Interestingly, in mouse models of liver fibrosis induced by thioacetamide or carbon tetrachloride, the induction of autophagy in hepatic stellate cells led to lipid degradation and activated these cells to promote liver fibrosis[110,111]. These results suggest that the regulation of lipid metabolism by autophagy might modulate fibrinogenesis. In the kidney, autophagy might have a pro-fibrotic effect by promoting the accumulation of lipids and inducing lipotoxicity in kidney tubular cells, as well as stimulating lipolysis to fuel liver fibroblasts. One study in UUO mice and in cultured kidney fibroblast cells exposed to TGFβ1 suggested that autophagy has pro-fibrotic effects and is involved in the activation of kidney fibroblasts[112]. However, the role of autophagy-mediated lipolysis in these processes remains undetermined.

The upstream signalling pathways potentially involved in the induction of autophagy in PTECs during kidney fibrosis have also been investigated. Following UUO, FOXO3 was activated and expressed in the nuclei of autophagic proximal tubules and the induction of autophagy was inhibited in *Foxo3*-knockout mice[113]. FOXO3 activation increased the expression of multiple core ATG genes and proteins in UUO kidneys of wild-type mice, suggesting that FOXO3 might induce autophagy through the transcriptional activation of key autophagy genes[113]. Other proteins involved in the induction of autophagy in models of kidney fibrosis include CCN family member 4 (also known as WNT1) in PTECs[114] and protein kinase C α in kidney fibroblasts[112] following UUO or treatment with TGFβ1, as well as DNA damage-inducible transcript 3 protein (DDIT3; also known as CHOP) in distal tubules and collecting ducts in the UUO model[115].

By contrast, several studies have also reported that autophagy can have an anti-fibrotic role (TABLE 2). In a rat model of UUO, autophagy was induced through inhibition of the RACα serine/threonine protein kinase (also known as PKB or AKT)–mTOR pathway, but inhibition of autophagy by 3-methyladenine aggravated tubular cell apoptosis and tubulointerstitial fibrosis, suggesting that autophagy might exert an anti-fibrotic effect by suppressing tubular cell injury[116].

Fibronectin can also be degraded through autophagy in PTECs. In cultured mouse PTECs, microtubule associated protein 1 S (MAP1S) interacted with LC3 and activated autophagy, which contributed to the suppression of fibrosis by increasing fibronectin turnover[117]. *Map1s* deletion impaired autophagic clearance of fibronectin and induced kidney fibrosis in aged mice. The researchers also observed reduced expression of MAP1S and accumulation of fibronectin in kidney biopsy samples from patients with kidney fibrosis compared with normal kidney tissues distant from tumour foci collected from patients with clear cell renal cell carcinoma[117]. Another study showed that inhibiting autophagy prevented the degradation of mature TGFβ1 in UUO kidneys and in TGFβ1-treated PTECs, suggesting that autophagy might also inhibit kidney interstitial fibrosis by negatively regulating TGFβ1 (REF.[118]).

The anti-fibrotic effects of autophagy might result from its role in cell cycle regulation. In the UUO model, cell cycle G2/M arrest in proximal tubules was more severe in proximal tubule-specific *Atg5*-knockout mice than in wild-type mice; this effect was associated with an accelerated development of kidney interstitial fibrosis[119]. G2/M arrest and production of collagen I were further elevated in angiotensin II-treated ATG5-deficient primary PTECs compared with angiotensin II-treated wild-type cells. Moreover, *Atg5* overexpression elevated autophagy in angiotensin II-treated PTECs accompanied by a reduction in cells arrested in the G2/M phase and collagen 1 expression[119]. The same research group subsequently demonstrated that ATG5-mediated autophagy suppressed NF-κB signalling, which limited tubular cell inflammation in response to the kidney injury induced by UUO or angiotensin II[120]. Moreover, prohibitin 2-mediated mitophagy was induced by angiotensin II and attenuated kidney tubular cell injury by improving mitochondrial function and inhibiting NLRP3 inflammasome activation[121].

Autophagy induced in distal tubules and pericytes during UUO also protected against kidney interstitial fibrosis by suppressing TGFβ and NLRP3-inflammasome signalling[122,123]. Other studies further demonstrated that UUO-induced kidney fibrosis was more severe in *Pink1* and *Park2* single knockout mice than in wild-type littermates, suggesting a protective role for mitophagy in kidney fibrosis[124,125].

Collectively, these findings suggest that autophagy has a dual role in UUO-induced kidney fibrosis. Persistent activation of autophagy might induce tubular atrophy and thereby promote kidney fibrosis, whereas autophagy-mediated degradation of excessive collagen might prevent fibrosis. Of note, the UUO model is not ideal for the study of kidney repair, as fibrosis progresses rapidly, is irreversible and the injury phase cannot be easily distinguished from the repair phase.

Autophagy in diabetic kidney disease

Diabetic kidney disease (DKD) is one of the most common and life-threatening diabetic complications and a leading cause of CKD and kidney failure worldwide[126]. The pathological hallmark of DKD is persistent albuminuria or proteinuria accompanied by the decline of the glomerular filtration rate and pathological changes in all kidney compartments[126]. The pathogenesis of DKD involves a complex interplay of hyperglycaemia-induced metabolic alterations, haemodynamic changes and various types of intracellular stress. Collectively, these processes induce the production of a range of cytokines, chemokines and growth factors that promote cell injury, inflammation and kidney fibrosis. Dysregulated autophagy in major types of resident kidney cells has also been recognized as an important contributor to the progression of DKD (TABLE 3).

Podocyte autophagy in DKD

In DKD, alterations in podocyte autophagy seem to correlate with the duration of the exposure to hyperglycaemia, the severity of podocyte injury and glomerulopathy, and disease stage or severity of DKD. In a mouse kidney podocyte cell line, exposure to high glucose for 48 h induced autophagy, whereas exposure to high glucose for 15 days had a suppressive effect on autophagy[60]. In line with these in vitro findings, in streptozotocin

(STZ)-induced type 1 diabetic mice, podocyte autophagy was induced at 4 weeks and suppressed at 8 weeks post-STZ injection. Of note, 4 weeks after STZ injections these mice were only hyperglycaemic and did not have glomerular lesions; however, podocyte injury was present at the 8-week time point. Compared with wild-type mice, STZ-induced glomerulopathy was more severe in podocyte-specific *Atg5*-knockout mice[60]. These results suggest that exposure of podocytes to hyperglycaemia, either short-term exposure in vitro or in the early stages of diabetes, might induce autophagy for renoprotection, whereas prolonged hyperglycaemia in vitro or in the late stages of diabetes, might suppress autophagy in podocytes and contribute to the progression of DKD.

Furthermore, transmission electron microscopy revealed that in mice treated with STZ, mesangial expansion and glomerulosclerosis were more pronounced in podocyte-specific *Atg5*-knockout mice than in wild-type mice, underscoring the interactions between podocytes and mesangial cells in diabetes[60]. Kidney biopsy samples from patients with diabetes also indicated that autophagy was reduced in podocytes compared with kidney specimens from patients with mild urinary protein excretion or only haematuria, which were characterized by minimal changes in histology[127]. Another study showed that podocyte autophagy was impaired in patients with type 2 diabetes, as well as in OLETF rats (that is, rats with obesity-induced diabetes), with severe proteinuria, but not in those with no or minimal proteinuria[128]. Moreover, a high-fat diet induced minimal proteinuria in wild-type mice, but podocyte-specific *Atg5*-knockout mice developed substantial albuminuria accompanied by severe tubulointerstitial lesions and injury to podocytes, which accumulated damaged lysosomes. Interestingly, stimulating cultured podocytes with serum from patients with type 2 diabetes or OLETF rats with severe proteinuria inhibited autophagy and induced lysosomal dysfunction and apoptosis, suggesting that serum-derived factors might negatively regulate podocyte autophagy as DKD progresses[128].

The mechanisms underlying DKD-related impairment of podocyte autophagy are under intensive investigation. Dysregulation of nutrient and/or energy-sensing pathways and intracellular stress signals including ROS, ER stress and hypoxia have all been implicated in the regulation of podocyte autophagy in DKD[129,130] (FIG. 5). One study also demonstrated that advanced glycation end-products (AGEs) impaired autophagy in podocytes by inducing lysosomal dysfunction[127]. Treatment with the antioxidants resveratrol and vitamin E restored lysosomal function and rescued the impairment in autophagy, thereby attenuating AGE-induced podocyte injury. Another study suggested that AGEs suppress the autophagic flux in podocytes by activating mTORC1 and inhibiting the nuclear translocation of TFEB[131].

β-Arrestins, which were upregulated in the kidneys of patients and mice with diabetes compared with their healthy counterparts, as well as in podocytes exposed to high glucose, might also regulate podocyte autophagy in DKD. β-Arrestins interacted with ATG7 and negatively regulated ATG12–ATG5 conjugation, which suppressed autophagy in podocytes[132].

Histone deacetylase 4 (HDAC4) was also upregulated in cultured podocytes treated with high glucose, AGEs or TGFβ[133]. Under these conditions, HDAC4 suppressed podocyte

autophagy by deacetylating STAT1 and sensitized podocytes to injury. HDAC4 inhibition restored autophagy and ameliorated podocyte injury[133]. In addition, overexpression of FOXO1 in podocytes activated PINK1–parkin-dependent mitophagy, which eliminated aberrant mitochondria and ameliorated podocyte injury in STZ-induced diabetic mice, suggesting a role for FOXO1 in the regulation of mitophagy in podocytes[134]. Renal progranulin (PGRN) was identified in 2019 as a novel upstream positive regulator of FOXO1. PGRN was significantly reduced in kidneys of STZ-induced diabetic mice and in kidney biopsy samples from patients with DKD compared with control mice and healthy human tissue, respectively[135]. *Pgrn* deficiency aggravated mitochondrial damage and dysfunction in podocytes from diabetic mice and worsened podocyte injury and proteinuria, whereas exposure to recombinant PGRN promoted mitophagy and mitochondrial biogenesis, thereby attenuating podocyte injury in diabetic mice. PGRN-related mitochondrial protection was mediated via PGRN–SIRT1–PGC1α regulation of FOXO1 and could be abrogated by the inhibition of mitophagy[135].

The expression of phosphatase and tensin homologue (PTEN) was also reduced in podocytes from patients and mice with DKD compared with those from their healthy counterparts[136]. Compared with wild-type mice, overexpression of PTEN in inducible podocyte-specific *Pten*-knock-in mice reactivated autophagy and alleviated glomerular injury following STZ injection[136]. Moreover, SIRT6 was decreased in kidney biopsy samples from patients with podocytopathies, including DKD[137]. Podocyte-specific *Sirt6* deletion accelerated podocyte injury and proteinuria in both STZ-induced diabetic mice and *db/db* mice. SIRT6 had an essential role in maintaining autophagy in podocytes treated with high glucose by inhibiting NOTCH signalling through deacetylation of acetylated histone 3 lysine 9 (H3K9ac) at *Notch1* and *Notch4* promoter sites[137].

Tyrosine-protein phosphatase non-receptor type substrate 1 (SHPS1; encoded by *Sirpa* and also known as SIRPα) was another important regulator of podocyte autophagy in experimental models of nephropathy, including DKD[138]. SHPS1 was reduced in podocytes from STZ-induced diabetic mice and in cultured podocytes treated with high glucose. *Sirpa* deficiency inhibited podocyte autophagy in STZ-induced diabetic mice by suppressing AKT phosphorylation and subsequent glycogen synthase kinase 3β (GSK-3β)–β-catenin signalling — this effect exacerbated diabetes-induced proteinuria and podocyte injury compared with diabetic wild-type mice[138]. The observed autophagy impairment in diabetic podocytes seems to involve a complex interplay of various regulation mechanisms, including inhibition of autophagy due to excessive nutrient and/or energy, and activation of autophagy due to hyperglycaemia-induced intracellular stress.

PTEC autophagy in DKD

Hyperglycaemia, hyperinsulinemia and a high level of free fatty acids are major metabolic alterations observed in diabetes. Among them, hyperglycaemia inhibits autophagy in kidney tubules of diabetic animals and patients with diabetes mellitus[139,140]. Sodium–glucose cotransporter 2 (SGLT2) is the primary regulator of tubular glucose reabsorption and its expression is upregulated in diabetic kidneys. *Sglt2* deletion alleviated STZ-induced accumulation of sequestosome 1 in the kidney, suggesting that SGLT2-mediated glucose

uptake might contribute to the impairment of autophagy observed in PTECs under conditions of hyperglycaemia[141]. Pharmacological inhibition of SGLT2 might therefore contribute to kidney protection in DKD by attenuating the inhibitory effect of hyperglycaemia on autophagy.

One study reported that autophagy was impaired in high glucose-treated PTECs, diabetic kidneys of mice and kidney biopsy samples from patients with DKD[140]. Importantly, autophagy impairment in these conditions was shown to be mediated by the p53–microRNA (miR)-214–ULK1 axis, where p53 is activated in DKD to induce miR-214 in kidney tubules. Upon induction, miR-214 directly targets ULK1, leading to autophagy impairment[140]. p53 was also reported to induce miR-155 to repress SIRT1 resulting in autophagy suppression during high glucose treatment of cultured PTECs[142]. In diabetic rats and in PTECs exposed to high glucose, the upregulation of miR-22 was also shown to suppress autophagy by reducing PTEN expression in PTECs[143]. This regulation of gene expression mediated by miR-NAs might represent an integral part of the autophagy regulatory network in both podocytes and PTECs in response to hyperglycaemia. Thioredoxin-interacting protein (encoded by *TXNIP*) was also upregulated in the kidney of STZ-induced diabetic rats and in PTECs exposed to high glucose, where it activated mTORC1 and impaired autophagy[144]. The upregulation of TXNIP was also observed in patients with diabetes[144].

High glucose also caused defective mitophagy in PTECs and accelerated tubular cell senescence. Overexpression of optineurin but not of PINK1 restored mitophagy and protected PTECs against premature senescence and activation of the NLRP3 inflammasome, which attenuated tubular injury[145]. Inositol oxygenase (encoded by *Miox*) was upregulated in PTECs exposed to high glucose and in the kidney tubules of diabetic mice; mitochondrial fragmentation and reduction in mitophagy were also observed. Inhibition of inositol oxygenase reactivated mitophagy and improved mitochondrial integrity[139]. Another study demonstrated that administration of the mitochondria-targeted antioxidant MitoQ ameliorated tubular injury in diabetic mice and reduced high-glucose-induced PTEC death by partially restoring mitophagy. This effect was potentially mediated by an increase in PINK1 expression induced by nuclear factor erythroid 2-related factor 2 (REF.[140]). These findings suggest that mitophagy in kidney tubules might be beneficial in DKD.

Of interest, one study identified a regulatory relationship between histone deacetylase 6 (HDAC6) and TFEB and its contribution to dysregulated autophagy in PTECs in various experimental models of CKD, including DKD[146]. In diabetic PTECs, deacetylation of TFEB by HDAC6 suppressed the activity and nuclear localization of this transcription factor, which led to the inhibition of autophagy and tubular cell death[146]. Dysregulated autophagy under hyperglycaemic conditions was also shown to be dependent on increased ubiquitylation of lysine 63 (Lys63–Ub), which is often observed in tubular cells from patients with DKD. Pharmacological inhibition of Lys63–Ub induced by high glucose significantly rescued autophagy impairment in PTECs and consequently abrogated tubular cell apoptosis[147]. Collectively, these results suggest that hyperglycaemia inhibits autophagy in kidney tubules and that different signalling pathways are involved in this effect.

The excess protein present in the glomerular filtrate owing to diabetes-induced proteinuria seems to activate autophagy and/or mitophagy in PTECs as an adaptive response[148,149]. However, prolonged or excessive albumin uptake might eventually impair autophagy in PTECs through an mTOR-dependent mechanism and induce progressive tubular injury[150]. For example, in cultured PTECs and mice, chronic albumin overload resulted in defective mitophagy, which was associated with decreased expression of BNIP3L[151]. Moreover, hyperactivation of mTOR might contribute to obesity-associated inhibition of autophagy in PTECs of patients and mice with type 2 diabetes, as the autophagy defect observed in mice with obesity induced by a high-fat diet could be ameliorated by treating the mice with rapamycin[148]. Similarly, in diabetic Wistar fatty rats, impaired autophagy in PTECs was reversed by a very-low-protein diet and by a daily 40% restriction of the food consumption, which suppressed mTORC1 and activated SIRT1 pathways, respectively, resulting in an attenuation of DKD-related tubular injury and kidney dysfunction[152,153].

Of note, using cultured PTECs and different diabetic mouse models, researchers have suggested that type 1 and type 2 DKD, modelled by STZ injection and the *db/db* mouse models, respectively, differ in autophagic responses[154]. Autophagy in the proximal tubules seemed to be mainly regulated by insulin, instead of amino acids or glucose, which activated the mTOR pathway. Therefore, autophagy was suppressed in *db/db* mice owing to hyperactive mTOR, whereas high levels of autophagy were induced in STZ-injected mice, which eventually led to lysosomal stress. Notably, pretreatment with rapamycin caused stagnation of autophagy and exaggerated kidney IRI in STZ-injected mice, whereas rapamycin reactivated autophagy and attenuated kidney IRI in *db/db* mice[154]. These results highlight that understanding the distinct patterns of autophagy dysregulation in PTECs in different types of DKD is crucial to their therapeutic targeting.

AGEs generated in the hyperglycaemic state are generally endocytosed and degraded in the lysosomes of PTECs[155], but high-glucose conditions seem to lead to defective autophagy, lysosomal dysfunction and the accumulation of AGEs in PTECs. An overload of AGEs disrupted lysosomal function and autophagic flux in PTECs[156,157]. Lysosome-associated membrane glycoprotein 1 (LAMP1) was upregulated in response to AGE overload in *Atg5*-competent primary PTECs, but was suppressed in ATG5-deficient cells. Similarly, the upregulation of LAMP1 was also suppressed in PTEC-specific *Atg5*-knockout STZ-induced diabetic mice compared with diabetic controls. Of note, AGEs also accumulated in the PTECs, glomeruli and kidney vasculature of these *Atg5*-knockout diabetic mice, in which inflammation and kidney fibrosis increased compared with controls[156]. These findings suggest that autophagy in PTECs has a role in regulating lysosomal biogenesis — impaired autophagy in diabetes might therefore reduce the clearance of damaged lysosomes and lead to AGE accumulation, which further disrupts lysosomal function. This vicious cycle might eventually lead to irreversible injury in the kidney tubules, glomeruli and tubulointerstitium, contributing to the progression of DKD.

Autophagy in GECs and GMCs in DKD

The role of autophagy in GECs and GMCs in DKD remains largely unclear. A 2010 study suggested that autophagy might be involved in regulating the degradation of bone

morphogenetic protein and activin membrane bound inhibitor (BAMBI) in endothelial cells[158]. BAMBI was downregulated in glomeruli isolated from biopsy samples of patients with DKD compared with samples from healthy donors, and in the glomeruli of STZ-induced diabetic mice compared with wild-type controls[159]. STZ-induced diabetic mice with *Bambi* deletion displayed more severe glomerulopathy than diabetic wild-type mice[159]. BAMBI is a negative modulator of TGFβ signalling but canonical TGFβ signalling, as assessed by phosphorylation of Mothers against decapentaplegic homologue 3 (SMAD3) and expression of its target genes in glomeruli, was comparable in diabetic *Bambi*-knockout mice and diabetic wild-type mice. However, mitogen-activated protein kinase 3/1 (also known as ERK1/2) signalling and SMAD1/5 signalling in glomeruli were enhanced in diabetic *Bambi*-knockout mice compared with diabetic wild-type mice, which suggests that *Bambi* deletion enhances alternative TGFβ signalling in DKD[159]. Whether regulation of BAMBI by autophagy in endothelial cells is relevant to the development of DKD remains under investigation.

GEC-specific *Atg5*-knockout mice displayed mild lesions to the glomerular filtration barrier compared with wild-type mice under non-diabetic conditions, whereas STZ-induced diabetic mice with GEC-specific *Atg5* deletion exhibited more severe glomerular endothelial injuries, including glomerular basement membrane thickening and podocyte effacement, than diabetic wild-type mice[60]. These observations suggest that autophagy in GECs preserves both endothelial integrity and podocyte homeostasis. In vitro experiments also revealed changes in GMC autophagy during DKD[60]. The metalloproteinase inhibitor 3 gene (*Timp3*) was downregulated in the kidney of STZ-induced diabetic mice and in patients with diabetes, as well as in GMCs treated with high glucose; *Timp3* deletion exacerbated diabetic kidney injury in mice[160]. In cultured GMCs exposed to high-glucose, *Timp3* deletion suppressed autophagy by reducing the expression of FOXO1a and its target ATG genes; restoration of TIMP3 expression in GMCs from $Timp3^{-/-}$ mice by transfection with a *Timp3* adenovirus reversed the impairment in autophagy. A pro-survival role for autophagy was also demonstrated in GMCs treated with AGEs. Autophagy and mitophagy were activated in GMCs via ROS and prevented AGE-induced mitochondrial dysfunction and cell apoptosis[161]. Despite these in vitro findings, the role of autophagy in GMCs during DKD has not yet been verified in vivo.

Autophagy in FSGS

Focal segmental glomerulosclerosis (FSGS) is a fibrotic kidney disease characterized by glomerular lesions, particularly in podocytes — the formation of adhesions in the glomerular capillary tuft leads to capillary occlusion and nephron degeneration[162]. FSGS is either idiopathic or secondary to a number of other disorders, such as acquired immune deficiency syndrome and obesity; the cause of idiopathic FSGS remains unclear. Several studies indicate that podocyte autophagy has an important role in the development of FSGS.

Variants of the apolipoprotein L1 (*APOL1*) gene known to be associated with FSGS have been shown to interfere with endosomal trafficking and block autophagic flux, ultimately leading to podocyte injury[163,164]. Using puromycin aminonucleoside (PAN) treatment of differentiated podocytes and rats to model FSGS, researchers revealed a positive correlation

between levels of LC3-II and the number of autophagic vacuoles in podocytes, as well as the recovery from the damage induced by PAN nephrosis[165]. This study presented the first evidence that podocyte autophagy might counter the development of FSGS. Another report demonstrated that podocyte-specific *Atg5*-knockout mice were more susceptible to PAN-induced glomerulosclerosis than wild-type controls[44]. We also demonstrated that autophagy activation was triggered by exposure to adriamycin in mouse podocytes in vitro and in vivo[166]. In vitro, podocyte apoptosis induced by adriamycin was reduced by rapamycin but increased by chloroquine. In vivo, adriamycin induced more severe podocyte injury, glomerulopathy and proteinuria in inducible podocyte-specific *Atg7*-knockout mice than in wild-type mice.

In another study, researchers deleted *Atg5* or *Atg7* in embryonic nephron progenitor cells and demonstrated that loss of autophagy in the entire nephron was sufficient to induce albuminuria, as well as glomerular and tubulointerstitial changes, that recapitulated the pathological features of human idiopathic FSGS[167]. This phenotype was potentially due to mitochondrial dysfunction and ER stress in podocytes and kidney tubules caused by the impairment in autophagy[167]. Activation of autophagy in podocytes might therefore represent a defence mechanism in FSGS and a potential therapeutic strategy.

Autophagy in podocytes of patients with FSGS has also been investigated. Electron microscopy and biochemical analyses revealed that patients with FSGS had a significantly lower percentage of autophagosome-positive podocytes and significantly lower levels of kidney beclin 1 than patients with minimal change disease (MCD)[168]. An analysis of repeated kidney biopsies from patients with MCD further revealed that patients with reduced podocyte autophagy were more likely to progress to FSGS than patients with relatively high levels of podocyte autophagy, who were more likely to have MCD[168]. A gradual decrease in podocyte autophagy might therefore underlie the possible progression of podocytopathies from MCD to FSGS. By contrast, one study reported high *ATG3* mRNA levels in glomeruli from both patients with FSGS and patients with MCD[44]. Another study demonstrated a positive correlation between the number of autophagic vacuoles in podocytes and foot process effacement and proteinuria in patients with MCD[169]. The reasons for these discrepancies remain unknown and highlight the complexity of autophagy in human kidney cells, which complicates the use of autophagy biomarkers to predict the progression of kidney diseases.

The mechanisms underlying the activation of autophagy in FSGS podocytes remain largely unclear, but activation of mTORC1 (REFS[166,170]) and downregulation of SIRPα[138] were observed in glomeruli from both patients with FSGS and in mouse models of FSGS.

Autophagy in polycystic kidney disease

Polycystic kidney disease (PKD) is the most common genetic form of CKD and is characterized by the development and growth of multiple kidney cysts, which leads to kidney damage and can cause kidney failure[171]. Autophagic flux was impaired in kidney epithelial cells in animal models of PKD and in patients with PKD[172], suggesting that this impairment might contribute to PKD progression[173]. PKD is one of the most common

ciliopathies and these disorders are associated with decreased autophagy[174]. The presence of primary cilia seems to be required for the activation of autophagy under conditions of starvation and fluid flow, and, in turn, autophagy defects suppress ciliagenesis[175,176]. In addition, many agents that protect against PKD, such as rapamycin and the mTOR-independent agents carbamazepine and minoxidil can also restore autophagy in animal models[177-182], further suggesting that autophagy impairment has a pathogenic role in PKD. Mechanistically, aberrant mTOR activation has been linked to impaired autophagy and defective cilia in PKD[183].

Autophagy as a therapeutic target

Given the crucial role of autophagy in the development of kidney diseases and ageing, pharmacological modulation of autophagy might be a promising strategy for the prevention and treatment of kidney diseases. Pharmacological enhancement of autophagy has been beneficial in several experimental models of kidney disease, including models of AKI, DKD, PKD, FSGS and kidney ageing. Notably, the precise function of autophagy in kidney fibrosis remains controversial.

In aged mice, long-term calorie restriction restored autophagic activity through the activation of the SIRT1–FOXO3 axis, which attenuated mitochondrial oxidative damage and protected against hypoxia-induced oxidative stress and mitochondrial damage in ageing kidneys[79]. Another study demonstrated that calorie restriction enhanced autophagy and mitigated most of the age-related damage in podocytes and convoluted proximal tubules[184]. Interestingly, the preservation of kidney structure in 11- or 21-month-old mice was better in animals fed lard as a source of dietary fat than in those fed soybean or fish oils as dietary fat. Another study demonstrated that short-term inhibition of mTORC1 with a low dose of everolimus in 24-month-old rats dramatically reduced age-related kidney disease and even counter-regulated age-related gene expression changes in the kidney[185]. However, the contribution of autophagy to the anti-ageing effects of everolimus was not investigated in this study.

Inhibition of mTORC1 and activation of AMPK or SIRT1 through genetic or pharmacological approaches have also been shown to prevent the progression of DKD in animal models[186-188]. However, the side effects of long-term or complete mTORC1 inhibition in kidney cells have been well-recognized, such as the early onset of proteinuria caused by specific inhibition of mTORC1 activity in podocytes in mice and the disruption of kidney tubular homeostasis caused by specific inhibition of mTORC1 activity in kidney tubular cells in mice[51,80]. Such inhibitors should therefore be used with caution in patients with DKD. In addition, Astragaloside IV, a saponin purified from the *Astragalus* root, prevented DKD progression by stimulating autophagy in diabetic mice[189-191], whereas triptolide, the active component of the herb *Tripterygium wilfordii* Hook F, alleviated diabetic kidney fibrosis through the miR-141-3p-PTEN–AKT–mTOR pathway[188]. Long-term calorie restriction also improved glomerular damage in diabetic mice, at least partly by activating autophagy[153].

The expanding list of chemical activators of autophagy includes mTOR inhibitors and mTOR-independent agents[18,29,36,192]. mTOR inhibitors, such as rapamycin, rapamycin

analogues and mTORC1 kinase inhibitors, are already widely used as immunosuppressive agents in clinical settings[18]. mTOR-independent autophagy-inducing agents include activators of AMPK[29] and SIRT1[36], chemicals that reduce intracellular inositol or IP3 levels (such as lithium, carbamazepine and sodium valproate), the K^+-ATP channel opener minoxidil and the Tat–beclin 1 peptide, among others[193-195]. Some of these reagents have been shown to activate autophagy in kidneys in vivo with beneficial effects in animal models of AKI, DKD, FSGS or PKD[69,175,178,196]. Autophagy inhibitors, including 3-methyladenine and chloroquine, have also been widely tested in animal studies[197].

Despite these encouraging findings, clinical translation of autophagy modulators remains challenging. First, the targets of these candidate agents might regulate cellular processes beyond autophagy and their pharmacological targeting might therefore result in unexpected harmful side effects. For example, mTOR has opposite effects on autophagy and cell growth and differentiation — mTOR inhibitors might provide renoprotection by activating autophagy, but they might also inhibit cell growth and therefore hinder kidney repair[198,199]. Second, in some disease settings, autophagy might have different roles depending on the cell types involved and the disease stage. For example, autophagy activation in PTECs during AKI is renoprotective, whereas autophagy induction in leukocytes might improve cell survival and promote inflammation[200]. In addition, sustained autophagy in PTECs after AKI might compromise kidney repair[102]. Therefore, the timing, duration and magnitude of autophagy modulation should be carefully considered to maximize its protective effect. Importantly, the cytoprotective effects of autophagy modulators have thus far only been reported in animal models of kidney disease, and evidence that these findings can be translated to humans is currently lacking.

Conclusions and perspectives

Substantial evidence supports an important role for autophagy in kidney physiology and pathology. Despite this progress, many unanswered questions remain. For example, the role of autophagy in GMCs, GECs and immune cells in different kidney disease conditions, as well as the mechanisms underlying the multifaceted role of autophagy in kidney tubulointerstitial fibrosis, remain unclear. Many signalling pathways participate in the regulation of autophagy in the kidney, but their precise mechanisms and signal relay in different types of kidney cells remain largely unknown. Moreover, in many cases, autophagy might interact with other cellular processes to influence the development of kidney diseases — such interactions and the regulatory mechanisms involved must also be investigated. The role of selective autophagy, such as mitophagy, in kidney diseases and ageing is also poorly understood. In addition, current evidence for autophagy dysfunction in kidney diseases is derived from hypothesis-driven studies and evidence from unbiased research, such as omics studies[201], is still lacking. Finally, despite the obvious difficulties, future research must include the validation of preclinical findings in human samples and the testing of the potential therapeutic potential of compounds that modulate autophagy in clinical trials.

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Glossary

Vesicle nucleation
The process of mobilizing a small group of molecules to the phagophore assembly site to form a phagophore during autophagy.

Phagophore
A cup-shaped double-membrane vesicle that expands and engulfs the components destined for digestion during autophagy.

Autophagic flux
The whole process of autophagy, including autophagosome formation, maturation, fusion with lysosomes, subsequent breakdown and the release of macromolecules back into the cytosol.

Lipofuscin
A yellowish brown, autofluorescent, lipidcontaining pigment that accumulates in the cytoplasm of cells during ageing.

Ischaemic preconditioning
(IPC). An endogenous adaptive mechanism elicited by brief ischaemia that protects against a subsequent more sustained ischaemic insult.

Chemical chaperones
Small molecules that enhance protein folding and/or stability.

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Key points

- Basal autophagy is essential to the maintenance of kidney homeostasis, structure and function.
- Autophagy is suppressed in aged kidneys, which accelerates the progression of ageing and age-related kidney diseases.
- In the acute injury phase of acute kidney injury (AKI), autophagy is induced in proximal tubules and acts as a protective mechanism. During the recovery phase, regulated autophagy is crucial for tubular repair.
- Persistent activation of autophagy after AKI induces phenotypic changes in proximal tubule cells that might lead to maladaptive repair, contribute to interstitial fibrosis and promote transition from AKI to chronic kidney disease.
- Autophagy defects in kidney cells of both tubular and glomerular compartments contribute to the development of diabetic kidney disease and focal segmental glomerulosclerosis.
- Polycystic kidney disease is associated with autophagy defects in kidney tubules that might contribute to the development and formation of kidney cysts.

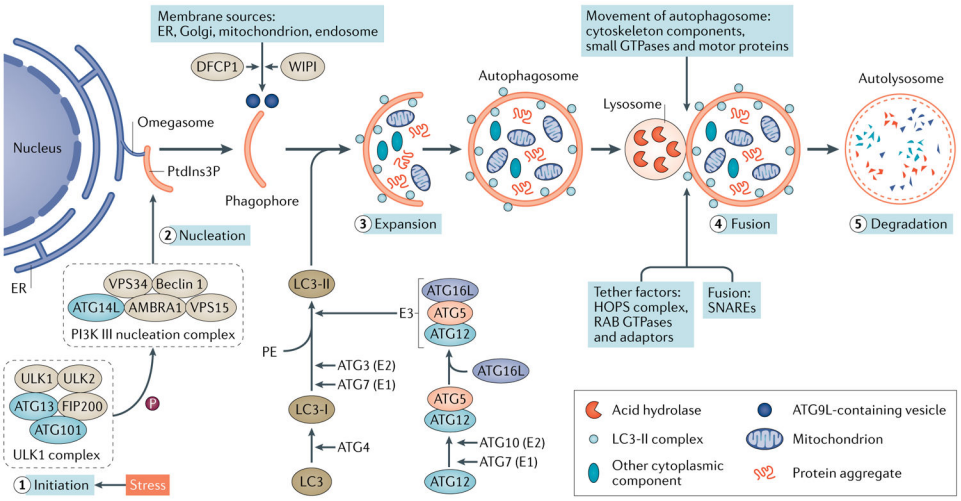

Fig. 1 | Autophagy dynamics and core machinery.

Autophagy is a muitistep process involving initiation, nucleation, expansion, fusion and degradation. Upon induction, the ULK1 complex — comprising the serine/threonine protein kinases ULK1 and ULK2, autophagy-related protein 13 (ATG13), FAK family kinase-interacting protein of 200 kDa (FIP200) and ATG101 — phosphorylates multiple substrates, including components of the phosphoinositide 3-kinase (PI3K) III nucleation complex to promote phagophore nucleation. The PI3K III complex comprises PI3K catalytic subunit type 3 (VPS34), PI3K regulatory subunit 4 (VPS15), beclin 1, beclin 1-associated autophagy-related key regulator (ATG14L) and activating molecule in beclin 1-regulated autophagy protein 1 (AMBRA1). This complex produces phosphatidylinositol-3-phosphate (PtdIns3P) at the omegasome, which recruits PtdIns3P-binding proteins for phagophore expansion. The delivery of membrane from other sources to the expanding phagophore requires PtdIns3P-binding proteins WD repeat domain phosphoinositide-interacting proteins (WIPIs) and zinc-finger FYVE domain-containing protein 1 (DFCP1), as well as cycling of the transmembrane protein ATG9L in the form of membrane vesicles and tubules. Two ubiquitin-like conjugation systems, the ATG12–ATG5–ATG16L system and the microtubule-associated protein 1 light chain 3 (LC3) system, participate in autophagosome elongation and completion. The conversion of cytosolic LC3-I into membrane-bound LC3-II is indicative of autophagy induction and autophagosome formation. The completed autophagosome then fuses with a lysosome to form an autolysosome, in which the autophagosome inner membrane and cargos are degraded by lysosomal hydrolases and eventually released for recycling. The fusion process is regulated by a large set of molecules, including cytoskeleton components and related motor proteins, tethering factors including the homotypic fusion and vacuole protein sorting (HOPS) complex, the RAB GTPases, and specific soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complexes. E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzyme; E3, ubiquitin ligase; ER, endoplasmic reticulum; PE, phosphatidylethanolamine.

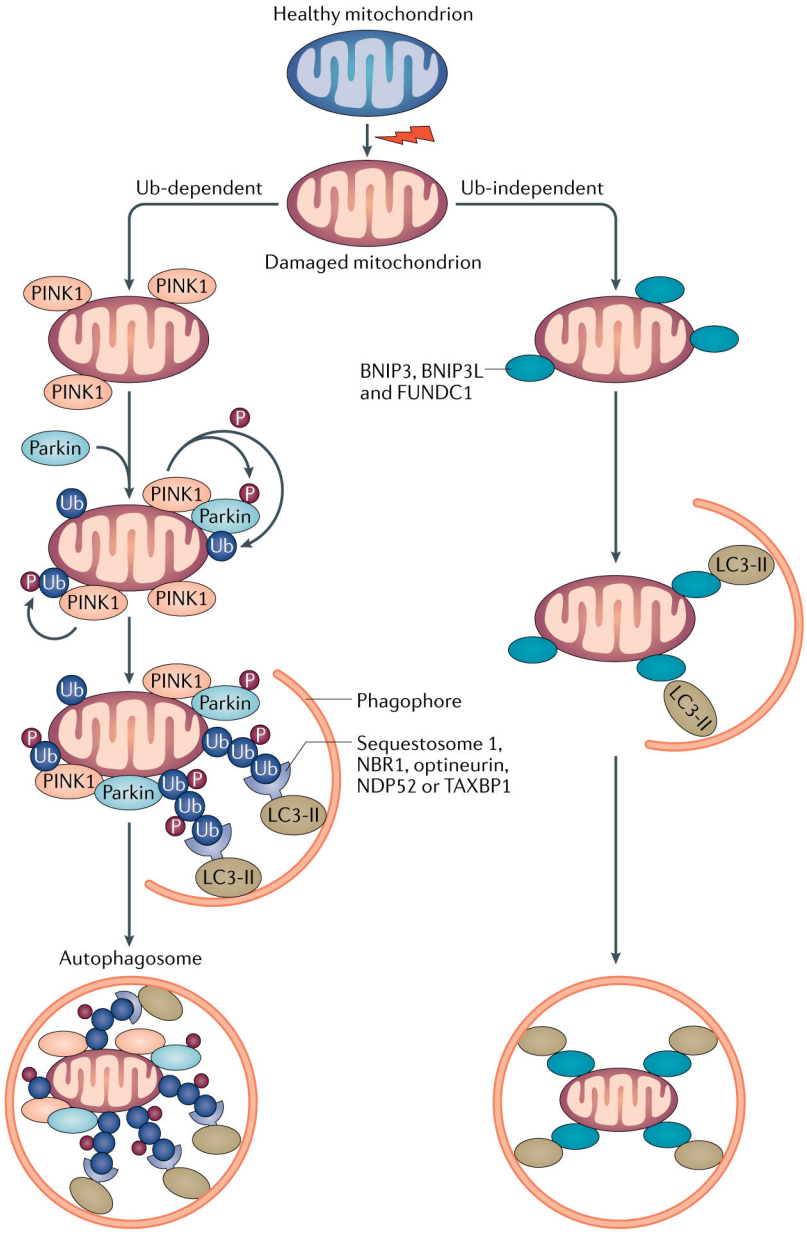

Fig. 2 |. Mitophagy pathways and cargo recognition.

Mitophagy is mainly mediated by microtubule-associated protein 1 light chain 3 (LC3)-associated autophagy receptors via both ubiquitin (Ub)-dependent and Ub-independent pathways. Ub-dependent mitophagy involves the mitochondrial serine/threonine protein kinase PINK1 and E3 Ub-protein ligase parkin (PINK1–parkin) pathway. Following loss of mitochondrial membrane potential, PINK1 accumulates on the outer mitochondrial membrane (OMM), where it recruits and phosphorylates parkin to add phospho–Ub chains on OMM proteins. Autophagy receptors, such as sequestosome 1, next to *BRCA1* protein (NBR1), optineurin, calcium-binding and coiled-coil domain-containing protein 2 (NDP52) and TAX1-binding protein 1 (TAX1BP1), which contain both Ub-binding domains and LC3-interacting regions (LIRs), bridge the ubiquitylated mitochondria to LC3-associated

autophagosomal membranes for sequestration. PINK1-mediated phosphorylation of ubiquitin might be sufficient to recruit NDP52 and optineurin and induce mitophagy independently of parkin. Ub-independent mitophagy is mediated by several OMM receptors such as BCL-2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3), BNIP3-like (BNIP3L), FUN14 domain-containing protein 1 (FUNDC1), which do not require interaction with Ub for cargo recognition. Peptidyl-prolyl *cis-trans* isomerase FKBP8 and BCL-2-like 13 (BCL2L13) are potential additional mitophagy receptors. By binding to LC3 via the LIRs at their cytosolic N terminus, mitophagy receptors link damaged mitochondria directly to autophagosomes.

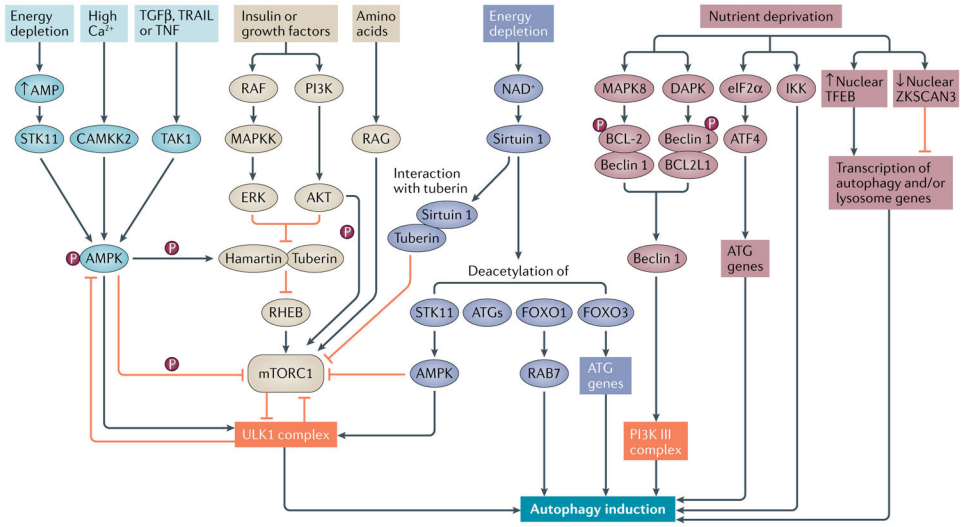

Fig. 3 | Signalling networks for the induction of autophagy.

Mammalian target of rapamycin complex 1 (mTORC1) is the major negative regulator of autophagy, whereas AMP-activated protein kinase (AMPK) and sirtuin 1 are positive regulators. mTOR is activated by amino acids via the RAG family of GTPases or by growth factors through two protein kinase pathways that involve RACα serine/threonine protein kinase (AKT) and mitogen-activated protein kinase kinase (MAPKK)–extracellular signal-regulated kinase (ERK). AKT and ERK1 or ERK2 can phosphorylate the hamartin–tuberin complex, which negatively regulates mTORC1 by inhibiting GTP-binding protein RHEB. AMPK is phosphorylated and activated by several upstream kinases, including serine/threonine protein kinase STK11, calcium/calmodulin-dependent protein kinase 2 (CAMKK2) and mitogen-activated protein kinase kinase kinase 7 (TAK1). Once activated, AMPK can directly phosphorylate serine/threonine protein kinase ULK1 to promote autophagy and/or phosphorylate hamartin–tuberin and regulatory-associated protein of mTOR (RAPTOR), which is one of the components of mTORC1, to inhibit mTORC1 and enable the induction of autophagy. Depletion of cellular energy stores increases NAD^+ levels and activates sirtuin 1. Active sirtuin 1 induces autophagy by directly deacetylating several essential autophagy proteins such as autophagy-related protein 5 (ATG5), ATG7 and microtubule-associated protein 1 light chain 3 (LC3). During starvation, sirtuin 1 can deacetylate forkhead box protein O1 (FOXO1) to upregulate the GTPase RAB7 and promote autophagic flux. Sirtuin 1 can also deacetylate FOXO3 to enhance the expression of various genes that encode ATGs such as ULK2, beclin 1, phosphoinositide 3-kinase catalytic subunit type 3 (also known as VPS34), BCL-2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3), BNIP3-like (BNIP3L), ATG12, ATG4B and LC3. Moreover, sirtuin 1 promotes autophagy by interacting with hamartin–tuberin and deacetylating STK11. mTOR-independent pathways have also been implicated in autophagic regulation under conditions of nutrient shortage. Upon starvation, mitogen-activated protein kinase 8 (MAPK8) is activated, which phosphorylates the apoptosis regulator BCL-2 to liberate and activate beclin 1 to induce autophagy. Activated MAPK8 might also phosphorylate sirtuin 1 to promote its enzymatic activity. Phosphorylation of beclin 1 by death-associated protein kinases (DAPKs) facilitates its dissociation from the anti-apoptotic protein BCL2L1, which also

promotes the induction of autophagy. Activation of eukaryotic translation initiation factor 2 subunit-α (eIF2α) and of inhibitor of NF-κB kinase (IKK) is also involved in starvation-induced autophagy. In addition, starvation stimulates the nuclear translocation of transcription factor EB (TFEB) to transactivate the expression of genes involved in autophagosome formation, lysosome biogenesis and lysosome function. By contrast, starvation stimulates the nuclear export of zinc-finger protein with KRAB and SCAN domains 3 (ZKSCAN3), which prevents its repression of ATG gene expression. ATF4, activating transcription factor 4; PI3K, phosphoinositide 3-kinase; RAF, RAF proto-oncogene serine/threonine-protein kinase.

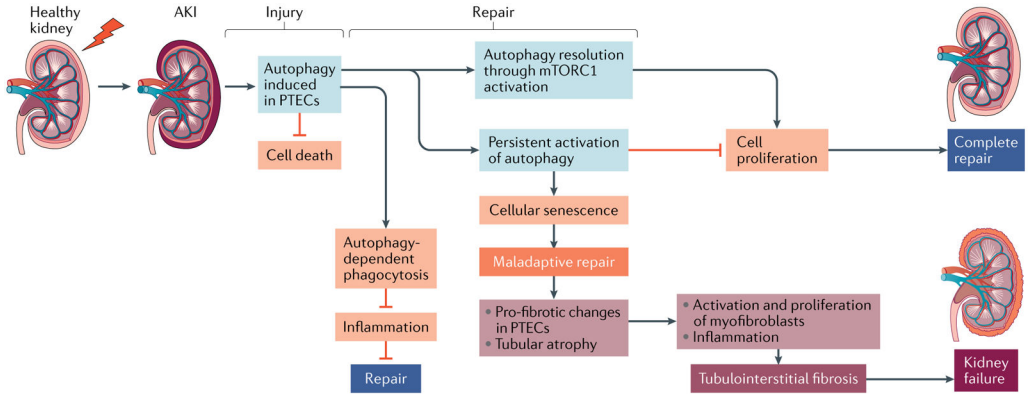

Fig. 4 |. Autophagy in AKI and kidney interstitial fibrosis.

Acute kidney injury (AKI) induces autophagy in proximal tubule epithelial cells (PTECs) to protect against kidney injury. Following the initial injury, autophagy in PTECs undergoes dynamic changes and mammalian target of rapamycin (mTOR)-mediated autophagy resolution during the injury recovery phase facilitates tubular cell proliferation and repair. Moreover, tightly regulated autophagy is required for phagocytosis of apoptotic cells by epithelial cells, which might also inhibit inflammatory responses and thus promote kidney repair. By contrast, persistent induction of autophagy in PTECs after AKI might induce cellular senescence and suppress cell proliferation. These autophagy-mediated changes stimulate the production and secretion of pro-fibrotic cytokines, leading to maladaptive repair and tubulointerstitial fibrosis. mTORC, mTOR complex.

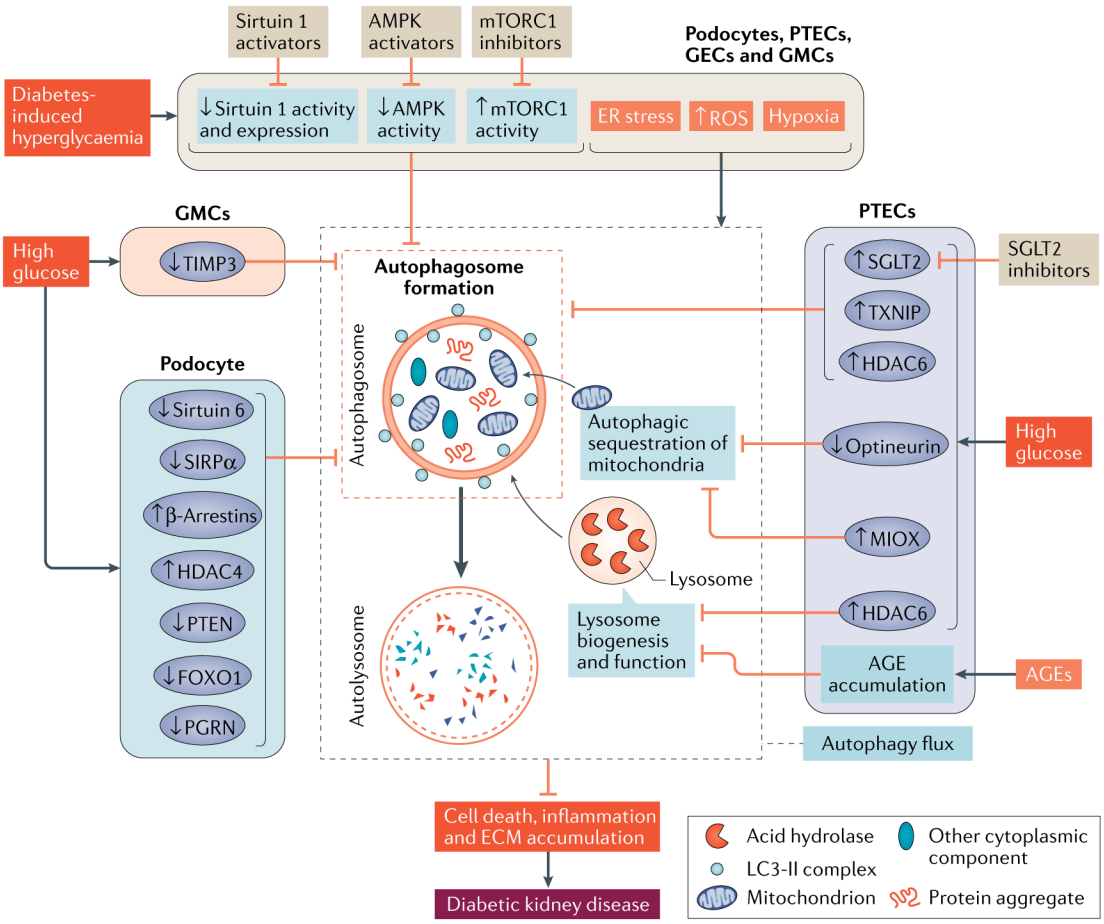

Fig. 5 | Autophagy in diabetic kidney disease.

Diabetes-associated hyperglycaemia induces cellular stress, including an increase in reactive oxygen species (ROS), endoplasmic reticulum (ER) stress and hypoxia. In the kidney, the initial adaptive response seems to include the activation of autophagy in podocytes, proximal tubule epithelial cells (PTECs), glomerular endothelial cells (GECs) and glomerular mesangial cells (GMCs). By contrast, sustained disturbance of major nutrient and/or energy sensing pathways leads to activation of mammalian target of rapamycin complex 1 (mTORC1), and inhibition of AMP-activated protein kinase (AMPK) and sirtuin 1, which suppresses autophagy in these kidney cells. In podocytes exposed to high glucose, increased expression of β-arrestins or histone deacetylase 4 (HDAC4), or reduced expression of phosphatase and tensin homologue (PTEN), forkhead box protein O1 (FOXO1), progranulin (PGRN), sirtuin 6 or tyrosine-protein phosphatase non-receptor type substrate 1 (SIRPα) might result in autophagy impairment. In PTECs, exposure to high glucose increased the expression of sodium–glucose cotransporter 2 (SGLT2), thioredoxin-interacting protein (TXNIP), HDAC6 and inositol oxygenase (MIOX), or reduced expression of optineurin, all of which might also impair autophagy. Moreover, accumulation of advanced glycation end-products (AGEs) in PTECs might cause lysosome dysfunction and consequently disrupt auto-lysosome formation and function. In GMCs, reduced expression of metalloproteinase inhibitor 3 (TIMP3) might inhibit autophagy. Collectively, intracellular stress, disturbances of nutrient-sensing pathways and changes in these signalling pathways might lead to

dysregulated autophagy and the progression of DKD. ECM, extracellular matrix; LC3, microtubule-associated protein 1 light chain 3.

Table 1 |

Effects of kidney cell-specific deletion of autophagy genes on kidney integrity and physiology

Animal model	Target kidney tissue	Kidney phenotypes	Refs
Atg5$^{fl/fl}$; *Six2*–Cre or *Atg7*$^{fl/fl}$; *Six2*–Cre	Entire nephron	Mild podocyte and tubular dysfunction within 2 months, dramatic glomerular and tubular changes resembling human FSGS by 4 months, and kidney failure by 6 months	167
Atg5$^{fl/fl}$; *Pax8*–rtTA; *TetO*–Cre	Entire tubule system	Mild increase in serum creatinine, ultrastructural alterations and accumulation of p62^{+} and ubiquitin^{+} protein aggregates in tubular cells after 5 months of induction	43
Atg5$^{fl/fl}$; *Ksp*–Cre	Distal tubules	Accumulation of oxidative stress markers, but no structural and functional changes up to 12 months	43
Atg5$^{fl/fl}$; *Nphs2*–Cre	Podocytes	Glomerulosclerosis and podocyte loss accompanied by proteinuria, ER stress and accumulation of oxidized and ubiquitylated protein aggregates at 20 months of age	44
Vps34$^{fl/fl}$; *Nphs2*–Cre or *Vps34*$^{fl/fl}$; *Pod*–Cre	Podocytes	Early-onset proteinuria, podocyte degeneration and glomerulosclerosis accompanied by disruption of autophagic flux	47,48
Atg5$^{fl/fl}$; *Cdh5*–Cre	Glomerular endothelial cells	Slightly dilated capillaries, discrete podocyte foot process effacements, loss of glomerular endothelial fenestrations and endothelial cytoplasmic thickening from 10 weeks of age	60
Atg5$^{fl/fl}$; *Tek*–Cre	Pan-endothelial cells	Mild capillary damage and ROS accumulation in glomeruli at 4 weeks of age; glomerular capillary damage and mesangial matrix expansion at 8 weeks of age	61

ER, endoplasmic reticulum; FSGS, focal segmental glomerulosclerosis; ROS, reactive oxygen species.

Table 2 |

Effects of autophagy or mitophagy defects on AKI and kidney interstitial fibrosis

Animal model	Targeted tissues	Disease model	Kidney phenotype	Refs
Atg5$^{fl/fl}$; *Kap*–Cre	Proximal tubules	Cisplatin and IRI	Increased sensitivity to AKI	57,71
Atg7$^{fl/fl}$; *Pepck*–Cre	Proximal tubules	Cisplatin, IRI and LPS	Increased sensitivity to AKI	70,74
Atg5$^{fl/fl}$; *Pax8*–rtTA; *TetO*–Cre	Entire kidney tubules	IRI	Increased sensitivity to AKI	43
Pink1 and *Park2* single or double knockout	Global	Cisplatin, IRI, contrast agent	Increased sensitivity to AKI	77,88,90
Atg7$^{fl/fl}$; *Pepck*–Cre	Proximal tubules	UUO	Reduced kidney fibrosis, fibroblast proliferation and activation, tubular atrophy, apoptosis, interstitial macrophage infiltration and production of pro-fibrotic factors	108
Atg5$^{fl/fl}$; GGT:: *Ert2*–Cre	S3 segment of proximal tubules	IRI	Reduced interstitial fibrosis and tubular senescence and superior kidney function 30 days after AKI	102
Map1lc3a knockout or hemizygous deletion of *Becn1*	Global	UUO	Increased collagen deposition and TGFβ1 levels in obstructed kidney	118
Atg5$^{fl/fl}$; *Kap*–Cre	Proximal tubules	UUO	Increased interstitial fibrosis and cell cycle arrest at the G2/M phase	119
Atg7 deletion	Distal tubules	UUO	Increased tubulointerstitial fibrosis, accumulation of damaged mitochondria, expression of NLRP3 inflammasome, IL-1β and caspase 1	123
Hif1a$^{fl/fl}$; *Pax8*–rtTA; *TetO*–Cre	Proximal tubules	IRI	Reduced autophagy in proximal tubules accompanied by increased tubular injury, interstitial fibrosis and functional damage to the kidney	105

AKI, acute kidney injury; IRI, ischaemia–reperfusion injury; LPS, lipopolysaccharide; TGF-β1, transforming growth factor β1; UUO, unilateral ureteric obstruction.

Table 3 |

Effects of kidney cell-specific deletion of autophagy genes on chronic kidney disease

Animal model	Kidney tissue with autophagy deficiency	Kidney disease model	Kidney phenotype	Ref.
Atg5$^{fl/fl}$; *Six2*–Cre or *Atg7*$^{fl/fl}$; *Six2*–Cre	Entire nephron	None	Glomerular and tubular changes resembling human FSGS by 4 months	167
Atg5$^{fl/fl}$; *Nphs2*–Cre	Podocytes	PAN, adriamycin	Increased sensitivity to glomerular diseases; increased sensitivity to diabetes-induced podocytopathy	44
		STZ-induced DKD	Leaky filtration barrier and glomerulosclerosis	60
		HFD-induced DKD	Increased sensitivity to diabetes-induced podocyte loss and proteinuria	128
Atg5$^{fl/fl}$; *Cdh5*–Cre	Glomerular endothelial cells	STZ-induced DKD	Increased sensitivity to diabetes-induced dilation of glomerular capillaries and endothelial lesions	60
Atg5$^{fl/fl}$; *Kap*–Cre	Proximal tubules	FFA/albumin overload	Increased sensitivity to proteinuria-induced tubulointerstitial lesions	148

DKD, diabetic kidney disease; FFA, free fatty acid; FSGS, focal segmental glomerulosclerosis; HFD, high-fat diet; PAN, puromycin aminonucleoside; STZ, streptozotocin.