# Autophagy and the kidney: health and disease

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## ABSTRACT

Macroautophagy is a highly evolutionally conserved degradation process by which cytosolic materials (including macromolecules such as proteins and lipids) and damaged organelles are broken down to their basic components. The role of autophagy is not only the elimination of materials, but it also serves as a dynamic recycling system that produces new components and energy for cellular renovation and homeostasis. The association of autophagy with the organ physiology and pathogenesis of various disorders such as neurodegenerative diseases, cancer, infection and inflammatory bowel disease has been revealed in recent years. Autophagy also plays an essential role in cellular homeostasis in kidney and counteracts age-related stress and kidney diseases. Here, we critically review the current evidence regarding autophagy in the kidney, in particular as assessed with tissue- or cell lineage-specific autophagy-deficient mice. Better insight into the mechanisms underlying renoprotective roles of autophagy will pave the way toward novel therapies for kidney diseases.

**Keywords:** aging, lysosome, metabolism, podocyte, proximal tubular cells

## INTRODUCTION

Autophagy, derived from the Greek ‘auto’ meaning self and ‘phagos’ meaning to eat, is defined as a catabolic pathway involving the degradation of cellular components via the lysosomal machinery [1, 2]. Extracellular materials are usually delivered to lysosomes via endocytosis. In contrast, cytoplasmic materials including proteins, lipids, glycogens and organelles are delivered to lysosomes through autophagy. Autophagy is a tightly regulated intracellular bulk degradation/recycling system that plays fundamental roles in cellular homeostasis. To date, at least three modes of autophagy have been identified: macroautophagy [1, 2], microautophagy [3] and chaperone-mediated autophagy [4].

In macroautophagy, when cells are deprived of nutrients or contain damaged organelles or protein aggregates, a small region of the cytoplasm is sequestered in a membrane sac (isolation membrane), which is derived from the endoplasmic reticulum (ER), mitochondria, plasma membrane or ER–mitochondria contact site [5]. Isolation membrane elongates at both ends and closes to form a double-membrane structure, the autophagosome. The autophagosome finally fuses with lysosomes, and thereafter the inner membrane and enclosed cytoplasmic materials are degraded by lysosomal enzymes [6]. In microautophagy, small regions of the cytoplasm are directly engulfed by inward invagination of the lysosomal or late endosomal membrane. The third type of autophagy, chaperone-mediated autophagy, does not involve membrane reorganization; instead, substrate proteins containing a Lys-Phe-Glu-Arg-Gln-like pentapeptide sequence are first recognized by cytosolic heat shock cognate 70 and co-chaperones. Then they are directly translocated into the lysosomal lumen after binding with the lysosomal receptor, LAMP-2A. After any type of autophagic process, the resulting degradation products can be used for different purposes such as new protein synthesis, energy production and gluconeogenesis. Of these, macroautophagy is believed to be the major type of autophagy and has been most extensively analyzed, probably because many of the specific molecules involved have been identified.

Although canonical macroautophagy (hereafter referred to as merely autophagy) is thought to be a bulk degradation process, special types of autophagy have been discovered [7]. Selective autophagy is directed at specific organelles, such as mitochondria (mitophagy) [8] and peroxisomes (pexophagy) [9], and to intracellular bacteria (xenophagy) (Figure 1) [10]. Xenophagy extends beyond the original concept of ‘auto’
phagy. Selective autophagy needs to distinguish between normal and ‘abnormal’ cell contents. The exact mechanism of such substrate recognition remains obscure. However, evidence for the involvement of ubiquitin and ubiquitin-binding receptors such as p62/SQSTM1 in this process is emerging (Figure 1) [11]. Before autophagic clearance, these receptors need to ‘tether’ the ubiquitinated substrate to the nascent autophagosome, which carries microtubule-associated protein light chain 3 (LC3) proteins on its surface. Thus, autophagy receptors binding to both ubiquitin and LC3 proteins are able to control protein degradation by selective autophagy.

**The core machinery of autophagy and its regulation**

Although autophagy is currently the subject of extensive investigations in numerous fields in biology, it is not a new idea. Acid phosphatase-positive structures containing mitochondria, ER membranes and ribosomes have been known since the 1960s. Autophagy research has long been stagnant because it has relied on laborious morphological approaches using electron microscopic analysis. The breakthrough in the elucidation of the molecular machinery involved in autophagy came from yeast genetic studies in the 1990s [12]. The discovery of autophagy in yeast and the genetic tractability of this organism have allowed us to identify genes that are responsible for this process, leading to the explosive growth in this research field that is seen today. To date, 36 genes/proteins have been identified and the nomenclature of autophagy related genes has been unified to Atg (AuTophaGy-related).

Analyses of Atg proteins have revealed dynamic and diverse mechanisms that underlie membrane formation during autophagy. After receiving input from environmental cues, some of these Atg proteins form a functional complex and orchestrate autophagy induction, autophagosomal membrane nucleation, elongation, closure and maturation in a defined manner [6, 13]. Autophagy is tightly regulated because too little or too much autophagy can be deleterious [14]. Autophagy induction is initiated by the activation of the Atg1 complex. The mammalian Atg1 complex is composed of the mammalian Atg1 homolog Unc-51-like kinases 1 or 2 (ULK1 or ULK2, respectively), mammalian Atg13, FIP200 and the others. The target of rapamycin (TOR)—specifically, TOR complex 1 (TORC1)—is an upstream negative regulator of this complex. Under nutrient-rich conditions, the active TORC1 associates with the ULK complex, phosphorylates ULK1 and hyperphosphorylates Atg13, which inhibits the kinase activity of ULK1 and thus blocks autophagy induction. Under starving conditions, TORC1 dissociates from the ULK complex, preventing phosphorylation of Atg13 and ULK1 by TORC1 and leads to autophagy induction. Vesicle nucleation is the initial step for autophagosome construction. Activation of a phosphatidylinositol 3-kinase (PtdIns3K) complex is essential for this process. The mammalian class III PtdIns3K complex is composed of beclin 1, Vps34 and Vps15. Proteins such as UVRAG, AMBRA1, Atg14L and Bif-1 positively regulate autophagy, whereas other class III PtdIns3K-interacting proteins such as rubicon, Bcl-2 and Bcl-xL are negative regulators. The next step is membrane

**FIGURE 1:** Multiple roles of non-selective and selective autophagy. Upon nutrient deprivation, activated autophagy catabolizes cytosolic materials non-selectively and breaks down to their basic components such as amino acids. A defect in this process causes amino acid insufficiency, which impairs production of new component and energy for cell survival. Protein aggregates and damaged mitochondria, which are produced in various pathological settings, are degraded by autophagy. In many cases, The LC3-binding protein p62 mediates sequestration of ubiquitinated substrates into autophagosomes. Autophagy deficiency can cause neurodegenerative diseases including Parkinson’s disease and contribute to aging stress. Invading bacteria are ubiquitinated, and autophagic receptors including p62 mediate autophagic sequestration of the microbes to restrict their growth. Failure of this xenophagy leads to chronic inflammation.
elongation and closure. Of note, autophagosomal elongation requires two ubiquitin-like conjugation systems: the Atg5–Atg12 conjugation system and LC3 conjugation system. During elongation, a cytosolic truncated form of LC3 (LC3-I) is converted to its autophagosomal membrane-associated, phosphatidylethanolamine-conjugated form (LC3-II) [15]. Thus, autophagosome formation can be seen as LC3-positive puncta with immunofluorescence. Among these Atg proteins, Atg5 and Atg7 are thought to be essential for autophagy and knockout or knockdown of these genes is widely used to create autophagy deficiency. However, autophagy was recently shown to occur through an Atg5/Atg7-independent alternative pathway without lipidation of LC3 to form LC3-II [16].

**Assessment of autophagic activity**

As many new scientists have begun to study autophagy, relevant new technologies have also emerged [17]. For example, transgenic mice expressing green fluorescent protein (GFP)-LC3 are widely used. In these mice, the transgene GFP-LC3 is under the control of the constitutively active CAG promoter, which produces nearly equivalent amounts of endogenous LC3. Hence, in these mice, GFP-positive dots represent autophagosomes. On the other hand, some confusion remains regarding acceptable methods to measure autophagic flux [17, 18]. We should emphasize that the numbers or volume of autophagosomes do not always reflect the autophagic flux, because theoretically and in many cases, autophagosomes accumulate due to a block in trafficking to lysosomes without a concomitant increase in autophagosome biogenesis. Therefore, to demonstrate the activation of autophagy, researchers should block autophagy and then observe autophagosome accumulation (LC3-II accumulation) or degradation of autophagy substrates such as p62. However, elucidating autophagic flux in vivo, especially in human samples, is difficult.

**Physiological and pathological roles of autophagy**

In addition to the basic cellular mechanism of autophagy, during the past decade since the discovery the Atg series of genes in yeast, tremendous development have been made in understanding its roles in human health and disease. In particular, analyses of autophagy-defective organisms have revealed numerous physiological and pathological roles of autophagy at both the cellular and whole-organism levels. These include renovation during differentiation and development such as early embryogenesis, adaptive immune responses such as antigen presentation and lymphocyte development, initiation and progression of cancer, tissue homeostasis in liver, brain, skeletal muscle, heart and pancreas [1, 2].

**Autophagy and aging**

The notion that autophagy is a highly conserved mechanism that plays an essential role in maintaining cellular homeostasis leads us to speculate that an age-related decline in autophagic activity will contribute to different aspects of the aging phenotype [19]. Does aging lead to a decline in autophagic activity? The age-related changes in the regulation of autophagic proteolysis were studied in vitro with isolated rat hepatocytes. The maximum rate of proteolysis occurred in the cells from 6-month-old rats and declined thereafter in the cells from older rats [20]. In contrast, Wohlgemuth et al. [21] have reported that the presence of autophagic vacuoles is not affected by age in liver and heart. Another study reported the unexpected finding that old hematopoietic stem cells have higher basal autophagic flux than young hematopoietic stem cells, and retain their ability to induce autophagy upon metabolic stress [22]. Therefore, at present, we cannot determine whether activity of autophagy declines with aging.

Another challenging problem is that a (relative) decline in autophagy contributes to aging. Evidence from lower organisms such as *Drosophila melanogaster* and *Caenorhabditis elegans* has confirmed the tight connection between autophagy and aging. Overexpression of *sir2* or mutations in different genes such as *daf-2* that suppress the insulin-like signaling pathway can extend lifespan in *C. elegans*. Activation of autophagy is a common feature observed in these long-lived mutant worms. Deletion or depletion of essential autophagy genes in such mutants drastically abrogates longevity, indicating that functional autophagy is required to attain the maximal lifespan extension mediated by these genetic manipulations. With regard to mammals, Harrison et al. [23] recently reported that rapamycin, an inhibitor of the mTOR pathway (and an inducer of autophagy), extends the lifespan of both male and female mice when fed beginning at 600 or 270 days of age. It remains to be determined whether rapamycin prolongs longevity by autophagy, as it is possible that rapamycin-fed mice display lifespan extensions through anti-inflammatory functions. Since systemic knockout of autophagy genes leads to neonatal death, probably due to nutrient deprivation, conditional knockout mice in each organ should be investigated to determine whether autophagy deficiency contributes to aging. Most autophagy-deficient mice progressively accumulate biological ‘garbage’ such as damaged mitochondria and ubiquitinated protein aggregates, and mimics several signs of aging including sarcopenia, cardiac dysfunction, dysfunction of hematopoietic stem cells and tumorigenesis, suggesting that autophagy counteracts aging stress [24]. However, it remains elusive whether a (relative) decline in autophagic activity is a primary cause of natural aging process or not.

**Autophagy and the immune system**

Autophagy can also play crucial roles in shaping cellular immunity [25]. Autophagy contributes to the regulation and function of innate immune responses. Several pathogens including bacteria, viruses and parasites are degraded by autophagy, which play a protective role against these pathogens [10]. Autophagy controls inflammation through regulatory interactions with innate immune signaling pathways and by removing inflammasome activators such as damaged mitochondria. In addition, autophagy contributes to adaptive immune responses such as antigen presentation [26] and lymphocyte development [27]. Extracellular antigens captured by antigen presenting cells are delivered to autophagosomes, which generates immunogenic peptides and loads them onto major histocompatibility complex (MHC) class II molecules for presentation to CD4+ T cells. Autophagy-mediated MHC class II presentation has a role in the selection of naïve T cell
repetitiveness in the thymus. High constitutive expression of autophagy in thymic epithelial cells delivers endogenous proteins to MHC class II molecules and contributes to the selection of appropriate T cell receptor repertoires and for the elimination of autoreactive CD4+ T cells [27].

**AUTOPHAGY IN THE KIDNEY**

The kidney demands high blood flow relative to its weight, and in particular, the proximal tubules consume a large amount of energy during the process of electrolyte reabsorption [28]. These tubules contain large quantities of mitochondria, which provide the energy for this reabsorption [29]. The lysosomal machinery plays an essential role in the reabsorption and degradation of albumin and low molecular weight plasma proteins from the glomerular filtrate. Moreover, kidney proximal tubules are susceptible to many kinds of insults such as ischemia–reperfusion (I/R) injury and nephrototoxic substrates. Therefore, an important role of autophagy in proximal tubules is possible. Indeed, autophagy in the kidney was described as early as 1969, when newly formed cytosegresomes (autophagosomes) containing unaltered organelles including mitochondria were observed in renal proximal convoluted tubules in mice treated with nephrotoxic antibiotics [30].

Unlike all other cell types, podocytes have an extremely limited capacity for replacement, because they are terminally differentiated postmitotic cells [31]. Since damaged components in the cytosol that result from various stress conditions cannot be diluted by division in podocytes, it is easy to infer that autophagy plays an important role in the podocytes. Indeed, autophagosomes are abundant in conditionally immortalized mouse podocyte clone [32] and in human renal biopsies [33]. In addition, the GFP-LC3 transgenic mouse has revealed that glomerular podocytes contain abundant autophagosomes even under basal conditions [34, 35]. However, whether podocytes have high autophagic flux activity or simply accumulate autophagosomes, and have low autophagic flux has not been determined, probably due to the technical limitations of assessing autophagic flux in vivo.

**Physiological roles of autophagy in the kidney**

We and other groups have recently generated kidney tubule (proximal or collecting duct)-specific [36–38] and podocyte-specific [35] autophagy-deficient mice, which allow us to examine the role of autophagy in the kidney in both physiological and pathological settings.

To determine the basal function of autophagy in adult mouse proximal tubules of kidneys, we generated kidney an- drogen-regulated protein (KAP)-Cre/floxed Atg5 mice (prox-KO mice), in which autophagy is deficient in the outer stripe and the medullary ray of the kidney [36]. Kidney function is comparable in prox-KO mice and littermate controls up to 9 months of age; however, kidney function significantly deteriorate in prox-KO mice compared with littermate controls at 2 years of age concomitant with marked fibrosis (Y. Takabatake, T. Kimura, A. Takahashi and Y. Isaka, unpublished data). Kidneys from prox-KO mice show slight hypertrophy of the tubular cells without interstitial nephritis or fibrosis at 8 weeks, but they show atrophy at 2 years of age. Accumulation of cytosolic amorphous substrates in the proximal tubular cells becomes apparent after the age of 6 months. In addition, electron microscopic analysis of 9-month-old prox-KO mice shows accumulation of deformed mitochondria. Massive accumulation of p62- and ubiquitin-positive inclusions is observed in 9-month-old prox-KO mice, whereas, they were rarely seen in younger prox-KO mice.

Huber’s group [35] has established podocyte-specific deletion of Atg5 in mice. They showed that (i) Atg5-dependent autophagy is dispensable for glomerular development, and (ii) podocyte-specific Atg5-deficient mice develop mild albuminuria, vacuolar degeneration and large cystic structures seen only with electron microscopy 8–12 months after birth that lead to podocyte loss, late-onset glomerulosclerosis and accumulation of oxidized and ubiquitinated protein aggregates, ER stress and proteinuria 20–24 months after birth. Accumulating evidence suggests that defective proteins identified by quality control systems are degraded by either the autophagy–lysosome system or the ubiquitin–proteasome system [7]. Both protein degradation systems are closely linked and complement each other. Therefore, compensatory mechanisms can be activated in response to the autophagic failure in these podocyte-specific Atg5-deficient mice. In support of this possibility, acute induction of podocyte-specific Atg5 knock-out by doxycycline produces a more rapid onset of albuminuria, suggesting that loss of Atg5 is compensated for by other pathways such as upregulation of proteasome activity [35].

These data clearly highlight the importance of basal constitutive autophagy as a key homeostatic mechanism to maintain proximal tubules/podocyte integrity. Distal tubule- and collecting duct-specific autophagy-deficient mice did not show any histological changes ([38] and our unpublished data), suggesting that proximal tubule cells depend on basal autophagic activity more than cells in other tubule segments.

**Autophagy and kidney aging**

Age-associated tissue damage is typically seen in kidneys, and the increased incidence of chronic kidney disease in the elderly is a health problem worldwide [39]. Age-related structural kidney changes are characterized by a loss of podocytes, glomerulosclerosis, vascular changes such as arteriolosclerosis and intimal and medial hypertrophy and tubule-interstitial changes such as fibrosis and tubular atrophy [39, 40]. Although the aging process is complicated, and several mechanisms of aging have been identified, the time-dependent accumulation of cellular damage is widely considered to be the general cause of aging. In particular, damaged mitochondria produce increased amounts of reactive oxygen species (ROS), leading to further accumulation of cellular damage. Autophagy-deficient podocytes and proximal tubules, as discussed above, perfectly phenocopy age-related cellular alterations such as the accumulation of lipofuscin, the formation of ubiquitinated protein aggregates, the occurrence of damaged mitochondria and the increase in the oxidized protein load, indicating that autophagy in these cells/segments slows kidney aging [35, 36].
In clinical practice, devising a way to activate autophagy to protect the aged kidney is important. Kume et al. [41] addressed this question by showing that (i) decline of Sirt1 activity in the normal aging process fails to promote cell adaptation to hypoxia via autophagy, leading to mitochondrial damage, and that (ii) adult-onset and long-term calorie restriction (CR) in mice promotes increased expression of Sirt1 in aged kidney and attenuates hypoxia-associated mitochondrial and renal damage by restoring autophagic activity [41]. In addition, an in vitro study revealed that Sirt1-mediated deacetylation of forkhead box O3, which is stimulated by CR, is essential for autophagy. Thus, they provide a rationale of CR-mediated antiaging effect, and pave the way for new therapies for age- and hypoxia-related kidney damage.

Role of autophagy in acute kidney injury

Acute kidney injury (AKI) is not only a determinant of renal outcome but an independent risk factor for mortality [42]. Ischemia and nephrotoxins are the major causative factors that contribute to the pathophysiology of AKI [43]. Upregulation of autophagy in the kidney proximal tubules has been observed in several experimental AKI models including I/R injury [44, 45], cisplatin nephropathy [46, 47] and cyclosporine (CsA) nephropathy [48]. However, the findings of most of these studies are often contradictory or unconvincing regarding the role of autophagy in the kidney. One study has suggested a cytoprotective role of autophagy during cisplatin treatment of proximal tubular cells by demonstrating that blockade of autophagy using pharmacologic inhibitors (3-methyladenine or bafilomycin) or short hairpin RNA for beclin increased tubular cell apoptosis during cisplatin treatment [47]. By contrast, another study has reported that autophagy is positively involved in cell death in renal tubules in the same experimental model [46]. The conflicting data may be due to the use of ‘autophagy inhibitors’, which are not entirely specific and could induce potential adverse effects [18]. Experiments with specific inhibition of autophagy are needed to more strictly assess the role of autophagy in the kidney.

In recent years, several studies have revealed that autophagy is induced by hypoxia or I/R injury in various organs including the heart [49], liver [50] and brain [51]. Multiple signals such as reduced cellular adenosine triphosphate (ATP) [52], BCL2/adenosivirus E1B 19 kDa interacting protein 3 (BNIP3) [53], ROS [54] and mitochondrial permeability transition pore opening [55] are likely to activate autophagy during I/R. The kidney is no exception; under I/R stress, autophagic vesicles have been observed in cultured proximal tubular cells (HK-2) [44], in proximal tubular cells of GFP-LC3 mice [36] and in transplanted human kidney [44].

We analyzed the role of autophagy in I/R injury using prox-KO mice [36]. I/R injury increases the number of autophagic vesicles, predominantly in proximal tubules, as assessed by massive accumulation of LC3 dots in GFP-LC3 transgenic mice. Severely injured tubules with massive tubular sediments and vacuolation are observed in the kidney cortex of prox-KO mice compared with control littermates after I/R injury. Bilateral I/R injury causes significant deterioration of kidney function in prox-KO mice compared with I/R-injured control littermates. Massive accumulation of ubiquitin-positive and p62-positive large dots is observed in the tubular cells of the kidney in prox-KO mice after I/R injury.

Other groups have also demonstrated that autophagy is renoprotective following ischemic AKI [37, 38]. One study employed drug-induced kidney tubule-specific Atg5 KO mice to exclude the possibility of confounding developmental or compensatory phenotypes [38]. They demonstrated that autophagosomes and autolysosomes are partially filled with mitochondria in proximal tubular cells of control mice after I/R injury, but that Atg5-null tubular cells exhibit severe tubular injury with concentric membranes surrounding damaged mitochondria, suggesting that autophagy is essential for scavenging damaged mitochondria. Together with our observations, autophagy plays a protective role during I/R injury.

Cisplatin also induces autophagy in vitro and in vivo [47]. Using proximal tubule-specific autophagy-deficient mice, we and another group have shown that autophagy is protective against cisplatin-induced AKI [37, 56]. Prox-KO mice exhibit more severe cisplatin-induced AKI than control mice, as assessed by kidney function and morphologic findings [56]. In addition, cisplatin induces more severe DNA damage and p53 activation, and a massive accumulation of protein aggregates in autophagy-deficient proximal tubules. Cisplatin treatment significantly increases damaged mitochondria that produce ROS in immortalized autophagy-deficient proximal tubular cells compared with autophagy-competent control cells. Thus, autophagy guards kidney proximal tubules against cisplatin-induced AKI, possibly by alleviating DNA damage and ROS production and by eliminating toxic protein aggregates.

Cancer cells use autophagy as a source of energy in the unfavorable metastatic environment of chaotic vasculature. Thus, a number of clinical trials are now revealing a promising role of chloroquine, an autophagy inhibitor, as a new adjuvant in anticancer therapy. As we showed a renoprotective role of autophagy against cisplatin-induced kidney injury, we need to issue a warning about the use of chloroquine in combination with anticancer drugs such as cisplatin [57]. Indeed, Jiang et al. [37] directly demonstrated that inhibition of autophagy by chloroquine worsens cisplatin-induced AKI in mice.

As described above, autophagy protects cells from cell death in AKI by multiple mechanisms that include recycling of misfolded and aggregate-prone proteins and removal of damaged mitochondria. Recently, mutual exclusion between autophagy and apoptosis has emerged [58]. Autophagy eliminates potential sources of proapoptotic stimuli such as mitochondrial membrane permeabilization, thereby setting a higher threshold against apoptosis induction. By contrast, the apoptosis-associated activation of proteases such as calpain and caspase-3 may destroy autophagy-specific factors (Atg4D, Beclin 1 or Atg5), thereby suppressing autophagy. The observation that the number of apoptotic cells is increased in the kidney of Prox-KO mice after I/R injury [36] or cisplatin treatment [56] supports this concept.

Role of autophagy in metabolic disorders of the kidney

Autophagy is a major contributor to cellular metabolism. When external nutrients are unavailable, it provides internal...
Diabetic nephropathy is characterized by cellular hypertrophy, exaggerated proliferative responses, increased apoptosis and excessive deposition of extracellular matrix that eventually lead to glomerulosclerosis, tubulointerstitial degeneration and fibrosis associated with precipitous decline of glomerular filtration rate [59]. In experimental diabetic kidney, there is a significant activation of mTOR-dependent pathways [60] and inactivation of AMP-activated protein kinase (AMPK)-dependent pathways [61], both of which are believed to downregulate autophagy. Indeed the streptozotocin (STZ)-induced diabetic rodent models demonstrate the early inhibition of autophagy in both proximal and distal tubular segments [62]. Since diabetic kidney is exposed to cellular stresses including elevated ROS and ER stress [59], the need for cytoprotective autophagy is significantly increased. Therefore, it is possible that at early phases of nephropathy, a reduction of basal autophagy may lead to the pathological changes of the diabetic nephropathy. The actual involvement of impaired autophagy in diabetic nephropathy requires further research.

Recently, Yamahara et al. [63] elucidated the role of autophagy in obesity-mediated exacerbation of proteinuria-induced proximal tubular cell damage. They observed that more severe proteinuria-induced cellular damage occurred in obese than in non-obese conditions, which was associated with autophagy insufficiency. Prox-KO mice, subjected to intraperitoneal free fatty acid–albumin overload, developed severe proteinuria-induced tubular damage, suggesting that proteinuria-induced autophagy is renoprotective. They further found that inappropriate hyperactivation of the mTOR pathway was associated with obesity-mediated insufficiency of autophagy.

Chronic use of CsA, a frequently used immunosuppressant, is often limited due to its nephrotoxicity. The mechanisms of chronic CsA nephrotoxicity have been studied extensively [64]. These include hemodynamic changes as well as toxic effects on tubular cells such as ROS and transforming growth factor-β production, increased apoptosis and ER stress. A few prior papers have shown that CsA imposes metabolic stress by affecting mitochondrial respiration [65]. In addition, it has been reported that CsA activates autophagy [48]. Therefore, we speculated that autophagy plays a protective role against CsA-induced metabolic stress in kidney proximal tubule epithelial cells. A metabolomics approach showed that CsA imposes substantial metabolic stress on autophagy-competent proximal tubular cells as characterized by decreased levels of amino acids, increased tricarboxylic acid ratio (the levels of intermediates of the latter part of the tricarboxylic acid cycle to levels of intermediates in the earlier part), reduced NAD+/NADH ratio, and decreased products of oxidative phosphorylation (ATP) [66]. In autophagy-deficient cells, CsA induces significant deterioration of all these metabolic parameters, suggesting that cells employ autophagy as an adaptation to CsA-induced metabolic stress. In support of this idea, compensatory upregulation of the glycolytic pathway is observed in autophagy-deficient cells cultured with CsA. These data provide a new perspective regarding the possible role of autophagy, which alleviates metabolic stress in other kidney diseases.

### Role of autophagy in glomerular diseases

Autophagy seems to act as a quality control mechanism in podocytes, and it is also involved in glomerular disease [35]. Autophagy is substantially activated in glomeruli from mice with induced proteinuria (by bovine serum albumin overload) and in glomeruli from patients with acquired proteinuric diseases. Moreover, mice lacking Atg5 in podocytes exhibit strongly increased susceptibility to models of glomerular disease as suggested by a significant increase of albuminuria and subsequent development of glomerulosclerosis after injection of puromycin aminonucleoside or adriamycin, both of which have almost no effect on wild-type mice on the C57BL/6 background.

Fabry disease is a progressive, X-linked inherited disorder of glycosphingolipid metabolism due to deficient or absent lysosomal α-galactosidase A activity [67]. This disease is characterized by severe pathology in major organs including neurological (pain), cutaneous (angiokeratoma), renal (proteinuria, kidney failure), cardiovascular (cardiomyopathy, arrhythmia) and cochleo-vestibular and cerebrovascular (strokes) manifestations. Recently, disruptions in autophagic processes have been reported in Fabry disease [68]. Renal biopsies obtained from five adult male Fabry disease patients before and after 3 years of enzyme replacement therapy with α-galactosidase indicate that the vacuole accumulation that was seen in renal biopsies from all patients prior to therapy was drastically decreased after 3 years of enzyme replacement therapy. These effects were primarily seen in renal endothelial cells and mesangial cells but not in podocytes. Furthermore, cultured cells from Fabry disease patients compared with non-Fabry disease individuals revealed impaired autophagic flux in Fabry disease. These data suggest that impairment in the normal autophagic processes may contribute to renal pathology in Fabry disease.

### Selective autophagy in the kidney

Mitophagy is a specific type of autophagy in which specifically targeted mitochondria is degraded by autophagic process, and it plays an important role in the quality control of mitochondria [69]. Observations from mitophagy in yeast have shown that canonical (non-selective) autophagy and mitophagy share similar core autophagosome/mitophagosome-formation machinery, but that distinct steps and molecules are involved in each autophagic process. As described above, autophagic degradation of mitochondria has been observed in the kidney, particularly in proximal tubules, both in the physiological [70] and pathological settings including I/R injury [36–38] and nephropathic cystinosis [71]; however, in a strict sense, we cannot exclude the possibility that mitochondria is ‘predominantly’ degraded by canonical autophagy. More precise molecular mechanism by which damaged mitochondria is recognized and is targeted to autophagosomes in proximal tubular cells should be elucidated in the future.
Pathogenic invasion or the uptake of mineral crystals such as silica and monosodium urate, can threaten cells due to lysosomal rupture, which can lead to oxidative stress, inflammation and cell death [72]. Recently, we and another group have shown that autophagic renovation of damaged lysosome (called lysophagy) is indispensable for cellular and tissue homeostasis [73, 74]. Moreover, we demonstrated that with lysosomal damage, loss of autophagy inhibits lysosomal biogenesis and leads to deterioration of kidney injury in the acute hyperuricemic nephropathy model. Kidney function in uric acid (UA)- and oxonic acid (OA, an uricase inhibitor)-treated prox-KO mice deteriorates significantly compared with that of UA- and OA-treated control mice, and pathology is increased. Localization and morphology of LAMP2-positive lysosomes are different in UA- and OA-treated prox-KO mice. A subpopulation of LAMP1-positive puncta beneath the brush border colocalizes with ubiquitin following UA- and OA-treatment, and this phenomenon is more prominent in prox-KO mice than in control mice, indicating that lysosomal rupture occurs in hyperuricemia. Electron microscopic observations have shown that the abnormal lysosome-like structures are indeed engulfed by the double-membranous isolation membranes in UA- and OA-treated control mice but not in UA- and OA-treated prox-KO mice. Collectively, lysophagy, is a surveillance mechanism that alleviates cells from the adverse effects of lysosomal damage.

Exploring autophagy as a therapeutic strategy for kidney disease

Given these observations, pharmacological approaches to modulate autophagy are currently receiving considerable attention [75, 76]. For example, inducing or augmenting basal autophagic activity in certain cell types may be therapeutically beneficial in several pathological states including kidney diseases, whereas, autophagy inhibition is being investigated as a strategy for treating some cancers. One class of candidate drugs that upregulates autophagy is the mTOR inhibitors. Rapamycin and its derivatives (temsirolimus, everolimus and ridaforolimus) are first-generation mTOR inhibitors which can induce autophagy in yeast and mammalian cell lines. ATP-competitive small molecule mTOR inhibitors, such as PP242 and Torin1, and catalytic inhibitors that selectively block the mTOR kinase, such as AZD8055 have been identified and have proven effective in greater inhibition of mTORC1 and mTORC2 activity than that produced by rapamycin. Despite its potential advantages in experimental models, rapamycin has been shown in several clinical trials to cause de novo or worsening proteinuria [77]. In line with this issue, Quaggin’s group [78] has developed a mouse model with podocyte-selective deletion of the Mtor gene (Mtor pod-KO). These mice develop proteinuria at 3 weeks of age and end stage renal failure by 5 weeks of age. Unexpectedly, podocytes from these mice exhibit disruption of the autophagic pathway, suggesting that it may play a role in the pathogenesis of proteinuria in patients treated with mTOR inhibitors. They speculate that inhibition of Mtor activity stimulates autophagy but mTOR reactivation is required for reformation of autophagolysosomes and lysosomes and for the cycle of autophagy to complete itself. Therefore, we should keep in mind that mTOR inhibitors could have unforeseen autophagy-blocking effects. In addition to the compounds that inhibit the mTOR pathway, numerous other compounds have been described that induce autophagy, including AMPK activators, inhibitors of the phosphatidylinositol signaling pathway and others [75, 76].

To avoid pleiotropic effects other than autophagy, one group has developed an autophagy-inducing peptide [79]. During human immunodeficiency virus (HIV)-1 infection, the Nef protein of HIV-1 inhibits autophagy by interacting
with beclin-1. The authors also found that GAPR-1, a cellular protein that associates with the Golgi, binds the same domain of beclin-1 to inhibit autophagy. They developed a peptide derived from the domain of beclin-1 which interacts with Nef or GAPR-1. This peptide decreases the accumulation of polyglutamine expansion protein aggregates and the replication of several pathogens (including HIV-1) in vitro, and reduces mortality in mice infected with chikungunya or West Nile virus.

Further work is needed to identify compounds that target unique molecular effectors/regulators of autophagy to selectively modulate the various stages of autophagy in different tissues and to design therapeutic interventions applicable to a broad variety of kidney diseases.

**CONCLUSION**

Basal autophagy potently coordinates cellular homeostasis in the kidney, particularly in podocytes and proximal tubular cells, thereby allowing cell-specific function and slowing kidney aging (Figure 2). In addition, autophagy is a surveillance sensor for kidney cells, and is upregulated by stress stimuli such as ischemia and nephrotoxins. Autophagy thereby counteracts kidney disease by eliminating damaged organelles or supplying energy. Although the precise mechanism of the renoprotective role of autophagy remains to be elucidated, strategies aimed at modulating autophagy hold promise for treating kidney disease.

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**CONFLICT OF INTEREST STATEMENT**

None declared.

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