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Autophagy in Acute Kidney Injury

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Abstract

Acute kidney injury is a major kidney disease associated with poor clinical outcomes. The pathogenesis of acute kidney injury is multifactorial and is characterized by tubular cell injury and death. Recent studies have demonstrated autophagy induction in proximal tubular cells during acute kidney injury. The regulatory mechanisms of tubular cell autophagy are poorly understood; however, some recent findings have set up a foundation for further investigation. Although autophagy may promote cell death under certain experimental conditions, pharmacological and autophagy-related gene knockout studies have established a renoprotective role for autophagy in acute kidney injury. The mechanisms by which autophagy protects cells from injury and how, possibly, its pro-survival role switches to pro-death under certain conditions are discussed. Further research is expected to help us understand the regulatory network of tubular cell autophagy, define its precise roles in specific context of acute kidney injury, and identify autophagy-targeting strategies for the prevention and treatment of acute kidney injury.

Keywords

acute kidney injury; autophagy; cell survival; cell death

Introduction

Acute kidney injury (AKI), mostly caused by renal ischemia-reperfusion, sepsis, and nephrotoxins, is characterized by a rapid loss of kidney function that leads to declined glomerular filtration, accumulation of nitrogenous wastes, and imbalance of water, electrolyte and acid-base. Despite advances in basic research and medical care during the past several decades, AKI is still a common and serious clinical problem in hospitalized patients and is associated with increasing incidence, high mortality, and few preventive and therapeutic options¹. Moreover, AKI is an important risk factor for the development of chronic kidney disease and the eventual progression to end-stage renal disease, which further imposes enormous medical and economic burdens on the society^{2, 3}.

The pathogenesis of AKI is very complex and multifactorial, with emerging tubular, microvascular and inflammatory mechanisms that interplay with and amplify each other.

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Tubular cell injury and death is the key pathological feature in AKI, which results in the generation of inflammatory and vasoactive mediators. These chemical mediators induce vasoconstriction and tissue inflammation, further exacerbating tubular damage and renal dysfunction⁴⁻⁶. In experimental models of AKI, the proximal tubules, especially the S3 segment located within the outer medulla of the kidney, suffer the most severe injury^{7, 8}.

Autophagy is a “self-eating” response to stress and plays important roles in the pathogenesis of a variety of diseases⁹. The research in the last few years has demonstrated the induction of autophagy in proximal tubular cells and kidneys during AKI. This review summarizes our current insights on the role of autophagy and its regulation in experimental models of AKI. The therapeutic potential of targeting autophagy for AKI prevention and treatment is also discussed.

Autophagy induction in proximal tubular cells during AKI

In a rat model of renal ischemia-reperfusion, Lai and colleagues showed an increased expression of autophagy-related (ATG) proteins (BECLIN-1 and LC3) in renal tubules^{10, 11}. Suzuki et al. further showed increased numbers of LC3- and LAMP2-positive vacuoles in a human kidney proximal tubular cell line (HK-2) following hypoxia incubation, and in mouse kidneys during ischemia-reperfusion. Under these conditions, LC3-positive vacuoles colocalize with LAMP2-positive vacuoles, suggesting the fusion of autophagosomes with lysosomes for degradation¹². The formation of autophagic vacuoles was further shown in tubular cells in transplanted human kidneys by electron microscopy (EM)¹². Autophagy is also induced by hypoxia in a rat proximal tubular cell line (RPTC), as indicated by the formation of fluorescent puncta in GFP-LC3-transfected cells and the accumulation of LC3-II, a lipidated form of LC3 that localizes on autophagosomes. Importantly, induction of autophagy is an early response to hypoxic stress, prior to tubular cell apoptosis¹³. In anoxia-reoxygenation, an in vitro model of ischemia-reperfusion, anoxia alone induces LC3-II accumulation but not formation of GFP-LC3 puncta. Nonetheless, autophagy is induced undoubtedly during reoxygenation as indicated by both GFP-LC3 punctuated cells and LC3-II accumulation¹³. These observations are consistent with an autophagic response during in vitro ischemia-reperfusion of a cardiac cell line¹⁴. While the evidence of autophagy from these in vitro and in vivo studies is circumstantial, autophagy was recently demonstrated clearly during renal ischemia-reperfusion in C57BL/6 mice by biochemical and morphological analyses. Although not obvious during the ischemic period, LC3-II accumulates in renal tissues following reperfusion (Figure 1A). The appearance of autophagic vacuoles in proximal tubular cells is further revealed by EM (Figure 1B)¹³. Consistently, later studies using GFP-LC3 transgenic mice showed an increased formation of GFP-LC3 dots in proximal tubules during renal ischemia-reperfusion^{15, 16}. It is noteworthy that autophagic flux was also determined by comparisons of LC3-II level and autophagosome number in the presence and absence of lysosomal inhibitors. Lysosomal inhibitors increase LC3-II accumulation in RPTC cells during hypoxia or anoxia incubation, and in kidney tissues during renal ischemia-reperfusion¹³. Similarly, Suzuki et al. showed that the number of LC3-positive vacuoles is significantly increased by lysosomal inhibitors during hypoxic treatment of HK-2 cells¹². Together, these studies indicate that autophagy is indeed induced in tubular cells during renal ischemia-reperfusion.

In nephrotoxic models of AKI, cisplatin was shown to induce autophagy in different renal proximal tubular cell lines including RPTC (rat)¹⁷, LLC-PK1 (porcine)¹⁸, and NRK-52E (rat)¹⁹. In all three cell lines, cisplatin induces autophagy in a treatment time-dependent manner, as shown by autophagosome formation and LC3-II accumulation. In addition, EM of cisplatin-treated RPTC cells identifies various characteristic structures that may represent the maturation of autophagic vesicle from phagophore through autolysosome¹⁷. Notably,

autophagy is activated by cisplatin within hours, earlier than apoptosis^{17–19}. Using GFP-LC3 transgenic mice, Inoue et al. further monitored autophagy in kidney tissues during cisplatin nephrotoxicity, unveiling autophagy mainly in proximal tubules (Figure 1C)¹⁹. These observations are consistent with an examination by EM, which demonstrated a time-dependent increase of autophagic vacuoles in proximal tubular cells following cisplatin treatment of C57BL/6 mice¹⁷. Autophagy is also induced in proximal tubular cells during cyclosporine nephrotoxicity and aristolochic acid nephropathy^{20, 21}. In addition, some environmental toxins, such as cadmium and arsenic, were also shown to induce autophagy in proximal tubular cells and kidneys^{22, 23}.

In addition to ischemic and nephrotoxic AKI, autophagy is induced in other settings of AKI as well. In a rat model that simulates human peritonitis by cecal ligation and puncture, autophagy increases transiently in kidneys at the early stage of sepsis, as indicated by LC3-positive aggregates primarily in proximal tubules and LC3-II accumulation. Interestingly, autophagy goes back to the basal level at the late stage, accompanied by renal dysfunction and tubular injury²⁴. Autophagy induction during septic AKI is also shown in the kidneys of rats treated with lipopolysaccharide²⁵. Moreover, LC3-II accumulates in kidney tissues following glycerol injection to rats, suggesting autophagy induction in the model of myoglobinuric AKI²⁶.

Regulation of tubular cell autophagy during AKI

The regulation of autophagy has been extensively studied in the past few years, and tremendous progress has recently been made in understanding the molecular mechanism and signaling pathways of autophagy from yeast to mammals^{27–30}. The ATG proteins constitute the core molecular machinery of autophagy and function at several successive steps of the autophagy cascade to orchestrate the process. Upstream of the core machinery, autophagy is regulated by a complex signaling network of multiple stimulatory and inhibitory inputs. Diverse signaling pathways regulate autophagy in response to various stresses. Moreover, these signaling pathways may interact with each other to determine the specificity and magnitude of autophagy. Despite these significant advances, regulation mechanisms that contribute to tubular cell autophagy in AKI remain unclear. Several recent studies have just started the journey of exploration.

Oxidative stress

Bolisetty et al. examined the regulation of autophagy by heme oxygenase 1 (HMOX1) during cisplatin-induced AKI. They found that cisplatin induces HMOX1, oxidative stress and autophagy in cultured renal tubular cells and mouse kidneys. In *Hmox1*-null models, oxidative stress is elevated, resulting in an increased sensitivity to autophagy and tubular cell death. Restoring HMOX1 in tubular cells reverses the autophagic response. Furthermore, overexpression of HMOX1 reduces oxidative stress, delays autophagy induction and protects against cisplatin-induced cell death³¹. These results suggest that oxidative stress may lead to autophagy in renal tubular cells. Lately, Parajuli et al. generated a mouse model with manganese superoxide dismutase (*Mnsod*) specifically deleted from distal nephrons. As a result of *Mnsod* knockout, mitochondrial oxidant production is increased, leading to autophagy activation³². Moreover, N-acetyl-L-cysteine, an antioxidant, inhibits both autophagosome formation and LC3-II accumulation in RPTC cells subjected to anoxia-reoxygenation, further suggesting the regulation of tubular cell autophagy by oxidative stress during AKI (Livingston M and Dong Z, unpublished data).

ER stress

In mammals, endoplasmic reticulum (ER) stress through unfolded protein response (UPR) and intracellular calcium has been implicated in autophagy regulation³³. In cyclosporine-treated human renal tubular cells, ER stress is induced along with autophagy activation. Salubrinal, an ER stress inhibitor, suppresses cyclosporine-induced autophagy, suggesting the involvement of ER stress in tubular cell autophagy during cyclosporine nephrotoxicity²⁰. A recent study examined ER stress response in cisplatin-treated NRK-52E cells and found that cisplatin at different doses elicits different UPR. 10 μ M cisplatin induces autophagy and upregulates two ER chaperones, glucose-regulated proteins 78 (GRP78) and 94 (GRP94). The expression of GRP78 and GRP94 is significantly reduced by 50 μ M cisplatin, leading to autophagy inhibition and apoptosis induction. Under this condition, pre-conditioning with taurine, an antioxidant, restores the expression level of both ER chaperones and switches the cellular response from apoptosis to autophagy³⁴. The signaling pathways by which ER stress induces tubular cell autophagy were then studied in the models of tunicamycin-induced kidney injury. Gozuacik et al. showed that death-associated protein kinase (DAPK) is activated by ER stress through protein phosphatase 2A dephosphorylation. Importantly, ER stress-induced autophagy is inhibited in *Dapk*-null cells, suggesting that DAPK may be a positive mediator of autophagy³⁵. Using inhibitors of the mitogen-activated protein kinases (MAPKs), Kawakami et al. demonstrated that activation of extracellular signal-regulated kinase (ERK), but not c-Jun N-terminal kinase (JNK) or p38, is necessary for the induction of tubular cell autophagy by ER stress³⁶. Further investigations should test how DAPK and ERK regulate autophagy in tubular cells. Given that ER stress is activated by a variety of insults that cause AKI³⁷, it would also be important to examine if ER stress is a common modulator of tubular cell autophagy.

HIF1 α -BNIP3

Regulation of autophagy by hypoxia-inducible factor 1 (HIF1) and its transcriptional target BCL-2 nineteen-kilodalton interacting protein 3 (BNIP3) was first demonstrated in the *Hif1 α* knockout MEF cells exposed to hypoxia³⁸. The involvement of HIF1 α -BNIP3 in tubular cell autophagy during AKI was then studied. Preliminary work showed that HIF1 α is induced in RPTC cells within hours of hypoxia, paralleled with autophagy induction. BNIP3 is also induced in a HIF1 α -dependent manner. Importantly, hypoxia-induced autophagy in RPTC cells is abolished by genetic knockdown of HIF1 α (Livingston M and Dong Z, unpublished data). These observations were further extended by a recent study using a different proximal tubular cell line. BNIP3 is induced in rat proximal tubules after renal ischemia-reperfusion and in NRK-52E cells in response to hypoxia or cobalt chloride, a chemical inducer of HIF1. While overexpression of BNIP3 in NRK-52E cells induces formation of autophagosomes that mainly colocalize with mitochondria, knockdown of BNIP3 significantly suppresses hypoxia-induced autophagy and mitophagy³⁹. These results suggest that HIF1 α -BNIP3 may contribute to the regulation of autophagy in renal tubular cells.

p53

In cisplatin-treated renal tubular cells, autophagy is partially suppressed by chemical inhibition of p53, suggesting that p53 may positively regulate autophagy in this experimental condition¹⁷. The pro-autophagy role of p53 depends on its nuclear localization and is associated with an increasing number of transcriptional targets⁴⁰. It has been shown that p53 transactivates 5'-AMP activated protein kinase (AMPK) to inhibit mammalian target of rapamycin complex 1 (mTORC1), resulting in autophagy⁴¹. Alternatively, p53 can induce autophagy by activating damage-regulated autophagy modulator (DRAM), a lysosomal membrane protein that stimulates autophagosome-lysosome fusion⁴². In response

to DNA damage, p53 upregulates ULK1 for sustained autophagy and subsequent cell death⁴³. Novel p53 transcriptional targets that can activate autophagy are being discovered, including BNIP3 and Sestrin2^{44, 45}. A latest study has further identified a plethora of p53-bound genes that encode proteins involved in almost all steps of the autophagy pathway⁴⁶. While promoting autophagy in these studies, p53 may induce TP53-induced glycolysis and apoptosis regulator (TIGAR) to suppress oxidative stress and inhibit autophagy via an mTOR-independent pathway⁴⁷. Whether these mechanisms are responsible for autophagy regulation by p53 in tubular cells during AKI remains to be determined.

BCL-2 family proteins

Inhibitory effects of BCL-2 or BCL-2L1/Bcl-xl on tubular cell autophagy during AKI have been suggested by several studies. In rats, intrarenal arterial delivery of adenoviral *Bcl-xl* gene reduces autophagy in tubular cells following ischemia-reperfusion¹⁰. Suppression of autophagy by BCL-2 was also shown in Bcl-2/GFP-LC3 double transgenic mice under this condition⁴⁸. Moreover, cisplatin-induced autophagy is almost completely blocked in a BCL-2 stable expression tubular cell line¹⁷. A well-recognized mechanism by which BCL-2 downregulates autophagy is that BCL-2 binds Beclin-1 through a BCL-2 homology 3 (BH3) domain to prevent Beclin-1 from assembling the class III PtdIns3K complex that is indispensable for autophagosome formation^{49, 50}. Notably, only ER-targeted BCL-2 inhibits autophagy⁴⁹. However, in BCL-2 overexpressing renal tubular cells BCL-2- Beclin-1 interaction is not detected by co-immunoprecipitation assay¹⁷. Recently, Chang et al. showed that nutrient-deprivation autophagy factor 1 (NAF1) binds BCL-2 at the ER and is required for the interaction of BCL-2 with Beclin-1, and BCL-2 inhibition of autophagy⁵¹. In addition to the association with Beclin-1, ER-localized BCL-2 may also suppress autophagy by diminishing stress-induced Ca²⁺ release and consequent activation of Ca²⁺/calmodulin-dependent protein kinase-AMPK signaling pathway⁵². These interesting possibilities need to be investigated in experimental models of AKI.

ULK1 and its regulation

ULK1 is a mammalian homolog of yeast Atg1, which functions as an initiating kinase in the autophagy core machinery⁵³. ULK1 is activated in tubular cells during severe hypoxia or anoxia and following reoxygenation, and in kidney tissues during renal ischemia-reperfusion. Importantly, tubular cell autophagy induced under these conditions is significantly suppressed by ULK1 knockdown, suggesting a role for ULK1 on autophagy regulation in ischemic AKI. Mechanistically, ULK1 activation is coordinately mediated by multiple upstream signals including mTORC1, AMPK and JNK (Livingston M and Dong Z. unpublished data). Despite these findings, the mechanisms by which ULK1 regulates tubular cell autophagy during AKI remain unknown. Under starvation conditions, ULK1 initiates autophagy through the recruitment of other ATG proteins to the pre-autophagosomal structure (PAS) and phosphorylation of downstream substrates⁵³. The search for ULK1 interacting proteins and substrates has gained impressive progress. ULK1 can phosphorylate both AMBRA1 and Beclin-1 to activate the class III PtdIns3K complex and thereby induce autophagy^{54, 55}. Gammoh et al. further identified an interaction between FIP200 and ATG16L1, essential components of the ULK1 and ATG5 complexes, respectively⁵⁶. ULK1 also participates in the feedback regulation of mTORC1 and AMPK to fine-tune autophagy⁵⁷. These findings will certainly inspire research into the regulation mechanisms of ULK1 and its role in tubular cell autophagy during AKI.

Pathological role of tubular cell autophagy in AKI: pro-survival or pro-death?

Clearly, the observations that autophagy occurs prior to apoptosis in renal tubular cells during AKI suggest that autophagy is an early response of the cells to stress and not a result of apoptosis. However, what role autophagy plays under this condition is still controversial.

In cisplatin-treated RPTC cells, inhibition of autophagy by pharmacological inhibitors (3-methyladenine or bafilomycin A₁) or genetic knockdown of Beclin-1 or ATG5 increases apoptosis, suggesting a protective role for autophagy in cisplatin-induced tubular cell injury¹⁷. Similarly, Yang et al. demonstrated that cisplatin-induced autophagy in LLC-PK1 cells acts as a prosurvival mechanism against cell apoptosis¹⁸. Moreover, Rovetta et al. showed two “sensitivity-thresholds” to cisplatin in NRK-52E cells. Cisplatin at 10 μM activates autophagy to preserve cell viability and prevent cell death. In contrast, autophagy is blocked in the cells treated with 50 μM cisplatin and as a result, apoptosis is induced. These results also suggest autophagy plays a protective role³⁴. Later in vivo work further confirmed and extended the in vitro findings. In a mouse model of cisplatin nephrotoxicity, pharmacological blockade of autophagic flux by chloroquine significantly enhances cisplatin-induced kidney injury whereas activation of autophagy by rapamycin protects proximal tubules from injury⁵⁸. A cytoprotective role of autophagy was also shown in primary culture of human proximal tubular cells during cyclosporine nephrotoxicity²⁰. Zeng et al. further suggested that autophagy induced in NRK-52E cells via ERK pathway is a protective mechanism for cell survival during aristolochic acid nephropathy²¹. Using both in vitro and in vivo experimental models, a recent study examined the role of autophagy in renal ischemia-reperfusion. In vitro, pharmacological or genetic suppression of autophagy sensitizes tubular cells to apoptosis induced by hypoxia incubation or anoxia-reoxygenation. Inhibition of autophagy in vivo by chloroquine or 3-methyladenine worsens ischemia-reperfusion renal injury, as indicated by renal function, histology, and tubular apoptosis. Together, these results suggest that autophagy is a renoprotective mechanism for cell survival in ischemic AKI¹³. A latest study demonstrated that autophagy may play a major role in mediating the renoprotective effects of caloric restriction preconditioning against renal ischemia-reperfusion injury in rats, suggesting a connection between these two renoprotective mechanisms⁵⁹. The renoprotective role of autophagy was also shown in experimental models of septic AKI. In rats subjected to cecal ligation and puncture operation, the decline of an early increased autophagic response is associated with the development of kidney injury at the late stage of sepsis. Knockdown of ATG7 exaggerates, whereas preincubation of rapamycin diminishes tumor necrosis factor α-induced cell death in NRK-52E cells²⁴.

In contrast, there are several studies suggesting that tubular cell autophagy may contribute to cell death during AKI. In a rat model of renal ischemia-reperfusion, Lai and colleagues showed increased Beclin-1 and LC3 expression as well as apoptosis in injured renal tubules. Both autophagy and apoptosis are suppressed by BCL-2 overexpression or ischemic preconditioning, accompanied by the amelioration of kidney injury^{10, 11}. Similarly, Suzuki et al. found that autophagy occurs in renal tubules with disrupted morphology in GFP-LC3 transgenic mice after renal ischemia-reperfusion. While autophagy is reduced in BCL-2/GFP-LC3 double transgenic mice, tubular damage is also attenuated. Along with the in vitro observation that autophagy inhibitors protect HK2 cells from H₂O₂-induced cell death, it was concluded that autophagy might be detrimental during renal ischemia-reperfusion¹². In tunicamycin-treated mice, Gozuacik et al. showed that ER stress induces apoptosis and autophagy concomitantly in the same damaged tubular cells. Interestingly, inhibition of autophagy by itself does not change cell death or survival; however, in combination with

caspase blockade, autophagy inhibition increases cell viability. Furthermore, *Dapk* knockout mice are resistant to ER stress-induced kidney injury as tubular cell autophagy is suppressed in those mice. Based on these results, it was suggested that autophagy may serve as a second cell killing mechanism that acts in concert with apoptosis to trigger kidney injury during ER stress³⁵. A cell death-promoting role of autophagy was also suggested by Inoue et al. showing that pharmacological or genetic inhibition of autophagy suppresses cisplatin-induced caspase activation and apoptosis in NRK-52E cells¹⁹.

The cause of the obvious discrepancy among the aforementioned studies is unclear, although it is generally believed that, depending on experimental conditions, autophagy can be either protective or detrimental. It is important to note that the occurrence of autophagy and apoptosis in the same cells or cell population does not indicate that autophagy contributes to apoptosis in these cells; rather it suggests the presence of cellular stress leading to both apoptosis and autophagy. In the same line of thinking, simultaneous inhibition of apoptosis and autophagy (e.g. by BCL-2 or preconditioning^{10, 11}) does not necessarily imply a role of autophagy in apoptosis. In addition, the non-specific effects of pharmacological inhibitors of autophagy have to be considered. In this regard, in vivo tests using kidney tissue-specific *Atg* gene knockout models have obvious advantages. To this end, Kimura et al. and Liu et al. established tubule-specific *Atg5* knockout mouse models, which show the accumulation of deformed mitochondria, aberrant concentric membranous structures, and cytoplasmic inclusions including SQSTM1/P62- and ubiquitin-positive protein aggregates in renal tubules, leading to cellular degeneration. During renal ischemia-reperfusion, tubular cell autophagy is inhibited in the *Atg5* conditional knockout mice and, importantly, more severe kidney injury is induced in these mice compared with wild type animals^{15, 16}. Moreover, a mouse model of *Atg7* knockout in proximal tubules (PT-*Atg7* KO) was established recently. Knockout of *Atg7* leads to impairment of the autophagy-conjugation systems, resulting in inhibition of autophagy and accumulation of autophagy-selective substrates such as SQSTM1/P62 during cisplatin treatment (Figure 2A, 2B). Compared with their wild-type littermates, PT-*Atg7* KO mice show accelerated loss of renal function and aggravated kidney tissue damage and tubular apoptosis (Figure 2C, 2D). Primary proximal tubular cells isolated from PT-*Atg7* KO mice are more sensitive to cisplatin-induced caspase activation and apoptosis than the cells from wild-type mice. PT-*Atg7* KO mice are also more sensitive to renal ischemia-reperfusion injury than their wild-type littermates⁵⁸. Further, Takahashi et al. demonstrated that *Atg5* knockout in proximal tubules results in a more severe cisplatin-induced kidney injury, accompanied by accelerated DNA damage and P53 activation⁶⁰. Together, these conditional knockout studies have demonstrated definitive evidence for a renoprotective role of tubular cell autophagy during AKI.

It is not yet understood how autophagy protects tubular cells from injury or apoptosis. Upon metabolic stress in which the availability of oxygen and nutrient is poor, this catabolic pathway can generate amino acids and lipids that can be reused for protein synthesis and ATP production, which are essential for the adaptation to bioenergetic catastrophe⁹. As a cellular housekeeping process, autophagy can clear misfolded proteins and damaged organelles to maintain cellular homeostasis and thereby set a higher threshold against apoptosis induction^{9, 61}. Indeed, when autophagy is impaired in proximal tubules by *Atg5* knockout, damaged mitochondria and abnormal protein aggregates accumulate in the cells. As a result, these autophagy-deficient mice are more sensitive to kidney injury than wild-type mice that have intact autophagy^{15, 16, 60}. Similar findings are shown in a study when autophagy is inhibited by chloroquine or ATG7 deficiency⁵⁸. In addition, it is plausible that the signaling activated during autophagy can interfere with or compromise cell death pathways. This possibility has been implicated in the studies showing that, on one hand, liberation of BCL-2 and FLIP from activated autophagy protein complexes may block the intrinsic and extrinsic pathways of apoptosis^{49, 62}; on the other hand, autophagic degradation

of active caspase 8 is responsible for the inhibition of apoptotic cell death⁶³. Moreover, certain stress stimuli can activate other cytoprotective responses to cooperate with autophagy to achieve optimal cellular repair and adaptation³⁰. Finally, data obtained from studying programmed cell death in embryo development indicate that autophagy is required for the maintenance of high ATP levels that may in turn facilitate the elimination of apoptotic cells. This function of autophagy could prevent a detrimental inflammatory response both during normal development and after exposure to pathological stimuli⁶¹. Further research is needed to gain insight into the mechanisms underlying the renoprotective effect of tubular cell autophagy in AKI.

It is generally acknowledged that autophagy is induced to serve primarily as an adaptive and defensive mechanism for cell survival, because *Atg* gene knockdown or knockout accelerates rather than delays cell death; however, in certain settings⁶⁴, uncontrolled massive autophagy may lead to cell death. How this prosurvival attempt fails and then switches to an alternative cell death pathway is unclear. It possibly is a result of an irreversible collapse of cell viability due to non-specific destruction of large proportions of cytoplasmic contents or a result of selective degradation of cytoprotective elements⁶¹. Some molecules, such as Draper, JNK and DAPK, have been shown to direct autophagy from a survival to a death pathway, although the exact mechanisms are unknown⁶⁵. Notably, many examples of *Atg* gene-dependent cell death occur in cells whose apoptotic machinery is compromised; however, a caveat should be considered since knockout or overexpression of a single *Atg* gene could have unknown indirect effects beyond autophagy⁶⁶. In addition to triggering cell death on its own, autophagy may also join apoptosis to coordinately determine a cell's fate. The functional relationship between autophagy and apoptosis is complex and generally presents as three scenarios^{61, 64, 67}. First, the two pathways share common regulatory signals and each can regulate and modify the activity of the other. Second, autophagy acts either upstream of apoptosis to enable apoptotic signaling or during the final stage of apoptosis to participate in certain morphological changes by providing ATP. Third, autophagy and apoptosis develop in a mutually exclusive manner under certain conditions, probably as a result of distinct thresholds for each process or mutual inhibition between the two processes. The intricate interplay and the cross-regulation between autophagy and apoptosis pathways further complicate the conundrum of how autophagy contributes to the life and death decisions of a stressed cell.

Autophagy as a therapeutic target for AKI?

Pharmacological approaches to activate or inhibit autophagy are currently receiving considerable attentions for therapeutic purposes of some diseases including neurodegenerative disease, infectious disease and cancer. Various screens have identified drugs and compounds that modulate autophagy. Although many of the compounds are tool compounds and may not be suitable for clinical use, a few of them are in clinical trials for the treatment of certain cancers and Huntington's disease⁶⁸. Given the evidence that autophagy is induced in AKI and manipulation of autophagy can affect the development and severity of AKI in many experimental settings, targeting autophagy could be a novel and potential strategy for the treatment of this disorder. There are, however, challenges that must be addressed before this strategy can be considered tenable and feasible.

The first challenge is the question of what precise role autophagy plays in AKI. Based on current evidence, particularly the evidence from kidney tubule-specific *Atg* gene knockout animals, autophagy is most likely renoprotective during AKI. Nonetheless, whether or not upregulating autophagy is "good" is not simplistic and may depend on magnitude, timing, and probably, other factors. It would be very important to determine an optimal condition and therapeutic window in which induction of autophagy will yield protective effects. To

accomplish this, monitoring autophagy, especially autophagic flux, in the correct tissue at the correct time will be a major challenge in the kidney.

Second, the drugs that activate autophagy via inhibiting mTOR include rapamycin and its analogues⁶⁸. A study in rats has suggested that the mTOR pathway is involved in tubular repair after AKI and rapamycin compromises recovery from AKI by inducing apoptosis and inhibiting proliferation of tubular cells⁶⁹. Therefore, specific and selective inhibitors of the autophagic machinery would be more attractive for clinical use. There have been thus far no reports of deleterious effects associated with specific autophagy upregulation in vivo. From a therapeutic view, however, constitutive autophagy induction may not be necessary; instead, intermittent upregulation of autophagy may be more effective with fewer side effects⁶⁸.

Third, thus far all studies suggesting that autophagy is associated with renoprotection were conducted in healthy adult animals. Given the fact that AKI is often a serious problem in hospitalized patients with other diseases, particularly in ICU patients with critical conditions, it will be necessary to determine: (1) whether induction of autophagy can still be renoprotective in those patients; (2) whether upregulation of autophagy for AKI treatment has side effects on or interferes with the treatment of other diseases. Eventually, the question of whether autophagy represents a useful target in AKI treatment will need to be addressed by conducting clinical trials in patients.

Conclusions and Perspectives

Despite some controversies, pharmacological and genetic knockdown or knockout studies have suggested a renoprotective role of autophagy in renal tubular cells in AKI. The mechanism by which autophagy protects tubular cells is currently unclear. In addition, whether and how autophagy changes its role from a pro-survival mechanism to a pro-death factor are currently unknown. The key signaling pathways that induce and regulate autophagy in AKI are also poorly understood. Further research should focus on these areas to elucidate the mechanism of autophagy induction in tubular cells in AKI, delineate the underlying signaling pathways, and define the precise roles played by autophagy in tubular regulation in this disease. A comprehensive understanding of the regulatory network of tubular cell autophagy will facilitate the discovery of genetic and pharmacological modulators for the prevention and treatment of kidney diseases including AKI.

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Figure 1. Autophagy in acute kidney injury

(A) Immunoblot analysis of LC3-II accumulation in kidney tissues after 30 minutes of bilateral renal ischemia, followed by 0–48 hours of reperfusion. The figure is adopted from Jiang et al.¹³, with permission from Elsevier. **(B)** Formation of autophagosomes and autolysosomes in tubular cells during renal ischemia-reperfusion. Kidney tissues were fixed for EM examination of autophagosomes (left panel, arrows: double or multiple membrane structures containing cytoplasm; middle panel, arrow: undigested organelles such as mitochondria) and autolysosomes (right panel, arrowheads: single membrane structures with remnants of cytoplasmic components). This figure is adopted from Jiang et al.¹³, with permission from Elsevier. **(C)** Cisplatin-induced autophagy in proximal tubular cells in kidney tissues. GFP-LC3 transgenic mice were treated with 20mg/kg cisplatin for 24 hours to harvest kidney tissues for immunostaining of aquaporin 1 (AQP1; marker of proximal tubules). Co-localization of GFP-LC3 dots with AQP1 suggests autophagy induction in proximal tubular cells. This figure is adopted from Inoue et al.¹⁹, with permission from Springer.

Figure 2. Autophagy inhibition in PT-Atg7 KO mice worsens cisplatin-induced acute kidney injury

Wild-type and PT-Atg7 KO mice were injected with 25mg/kg cisplatin or saline as control. (A) Kidneys were harvested to collect cortical tissues for immunoblot analysis of Atg7, LC3, Atg5 (Atg12 conjugated), p62, and β-actin. (B) Kidneys of cisplatin-treated mice were collected for immunofluorescence staining of LC3, fluorescein isothiocyanate (FITC)-labeled phaseolus vulgaris agglutinin (PHA; marker of proximal tubules), and Hoechst33342. Arrows indicate formation of LC3 dots and inset shows LC3 dots at high magnification. (C) Blood samples were collected for measurement of serum creatinine. Data are expressed as mean ± SD. * $P < 0.05$, significantly different from the control groups; # $P < 0.05$, significantly different from the relevant wild-type group. (D) Histology of kidney

cortex (hematoxylin-eosin staining). This figure is adopted from Jiang et al.⁵⁸, with permission from Nature Publishing Group.