Autophagy: A new treatment strategy for MSC-based therapy in acute kidney injury (Review)

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Abstract. Acute kidney injury (AKI) is a common and serious medical condition associated with poor health outcomes. Autophagy is a conserved multistep pathway that serves a major role in many biological processes and diseases. Recent studies have demonstrated that autophagy is induced in proximal tubular cells during AKI. Autophagy serves a pro-survival or pro-death role under certain conditions. Furthermore, mesenchymal stem cells (MSCs) have therapeutic potential in the repair of renal injury. This review summarizes the recent progress on the role of autophagy in AKI and MSCs-based therapy for AKI. Further research is expected to prevent and treat acute kidney injury.

Contents

1. Introduction

Autophagy is imperative in normal and pathological conditions, including inflammation, adaptation to stress, aging, immunity, metabolic and neurodegenerative disorders, and cancer (1-7). Both *in vivo* animal models and *in vitro* cell culture studies suggest that autophagy serves a role in the maintenance of renal function under pathological conditions including nephrotoxic drugs treatment or ischemia/reperfusion (I/R) injury (8,9).

Acute kidney injury (AKI) is characterized by tubular cell injury and death that leads to a rapid and progressive loss of renal function, including declined glomerular filtration, accumulation of nitrogenous wastes and imbalance of water, electrolytes and acid-base reactions. AKI is a common disease and constitutes a risk factor for chronic kidney disease (10). The incidence of AKI during an ICU stay ranges from 22 to 67% (11). Although there is certain progress in basic research and clinical application, AKI with an increasing morbidity and mortality rate and few prevention and treatment approaches, remains a common and serious clinical condition in hospitalized patients (11).

2. Autophagy in disease

Autophagy is classified into three subtypes: macroautophagy, chaperone-mediated autophagy, and microautophagy (12). Macroautophagy, generally referred to as autophagy, is well-studied and is the focus of this review. Autophagy is an 'auto-eating' process in a cell where intracellular organelles, proteins, and other macromolecules are sequestered into autophagic vesicles (known as autophagosomes) and then degraded by the hydrolases of lysosomes (13). The formation of autophagosomes involves the following steps: Induction, nucleation and elongation that form a complete isolation membrane. Subsequently, double-membrane autophagosomes with sequestered materials inside fuses with the lysosome and form autolysosomes (Fig. 1). Cytoplasmic constituents are degraded by the lysosomal hydrolases for cyclic utilization (14). The initiation step of autophagosome formation involved the ULK1/2-Atg13-FIP200 complex, which is required for the phagophore membranes. The phagophore membrane PI3K/Vps34 complex containing Vps34, Vps15, Beclin-1 and Atg14 L is recruited for nucleation. Autophagy-related gene 9 (Atg 9) and vacuole membrane protein 1 (VMP1) are necessary for membrane expansion. Atg1-Atg13-Atg17 complex promote Atg9 cycling between the phagophore assembly site (PAS) and non-PAS compartments and aided in localization of Atg9 to the PAS (15,16). Indeed, Atg12-Atg5-Atg16 complex and Atg8/light chain 3-II are two ubiquitin-like conjugation systems that are involved in the elongation and expansion steps during autophagosome formation (17). Mi *et al* (18) demonstrated that actin

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served a role in autophagosome formation. They demonstrated that polymerized actin puncta were co-localized with DFCP1 [a PtdIns(3)P-binding ER protein that marks omegasomes] and LC3-positive puncta. PtdIns(3)P promoted actin network formation inside membranes, thereby branched actin networks are essential for autophagosomal membrane shaping.

Recent studies have documented the key role of autophagy in normal physiological conditions as well as in pathogenesis conditions. In physiological processes, a low level of basal autophagy occurs to maintain cellular homeostasis. In pathological conditions, cellular stress including hypoxia, cell starvation, oxidant injury, genotoxic agents, and other damaging factors contribute to the induction of autophagy (19,20).

Autophagy has both pro-survival and pro-death functions in gastric cancer cells (21). Autophagy is upregulated in cancer cells to provide nutritive material for cell survival (22). Pla *et al* (23) demonstrated that autophagy decreased ethanol toxicity in mouse neurons. Autophagy and autophagic flux decreased in aging hearts, stimulation of autophagy alleviated aging-associated pathology in the heart (24). Furthermore, autophagy is considered to play a renoprotective role in kidney injury (25).

3. Prevention and conventional treatment of AKI

AKI is mainly caused by I/R injury and nephrotoxic drugs which impair renal function Adequate hydration control, maintenance of arterial pressure, pre-emptive use of antioxidants may prevent AKI; few treatments could change the development of AKI (26). Previous studies have demonstrated autophagy serves a role in AKI (27-33).

Autophagy in ischemia reperfusion injury. I/R injury is a leading cause of AKI, which is frequently associated with many clinical conditions but lacks effective therapies (27). Recent studies have reported that autophagy is activated in renal I/R injury *in vitro* and *in vivo* models irrespective of the genetic or pharmacological impairment of autophagy (27-35).

In vivo renal I/R injury patterns, pretreated with 3-methyladenine (3-MA, an autophagy inhibitor) or pretreated with rapamycin (an autophagy activator), demonstrated that autophagy served a renoprotective effect by inhibiting apoptosis (27,28) or by decreasing pro-inflammatory cytokines (29). Similar results were reported by Jiang *et al* (30), indicated that inhibition of autophagy by chloroquine aggravated AKI, whereas the upregulation of autophagy by rapamycin improved lost renal function, further indicating the protective role of autophagy in AKI. In C57BL/6 mice, renal I/R injury is aggravated by the suppression of autophagy using chloroquine and 3-MA (31). Endoplasmic reticulum stress inducer tunicamycin, caused significant Grp78 expression and ameliorating renal I/R injury *in vivo* by inducing autophagy (32). It was also found that autophagy protects kidneys from I/R injury *in vivo*, but the exact mechanisms remain to be elucidated.

Pharmacological inhibitors or inducers of autophagy have non-specific effects and therefore previous studies demonstrated the effect of autophagy during renal I/R by using conditional kidney proximal tubule-specific Atg5- or Atg7-knockout mice (31,33). Kimura *et al* (33) demonstrated that I/R injury increases proximal tubule cell apoptosis in kidney proximal tubule-specific Atg5 mice compared with the wild-type mice. It is also demonstrated that autophagy serves a critical role in maintaining tubular cell integrity during stress conditions (34).

Jiang *et al* (30) revealed that autophagy was induced earlier than apoptosis in response to hypoxia, in GFP-LC3-transfected tubular cells. Blockade of autophagy by 3-methyladenine or small-interfering RNA knockdown of *Beclin-1* and *Atg5* (two essential autophagic genes) lead to hypoxia-induced apoptosis *in vitro* (31). Transfection of small interfering RNAs for heat shock protein1 have been shown to inhibit cell autophagy and increased apoptosis in GFP-LC3-transfected NRK-52e cells during H_2O_2 treatment (34,35). Besides rat tubular cells, Wang *et al* (36) demonstrated that adenosine monophosphate-activated kinase (AMPK) protected pig renal tubular cells (LLC-PK1) from I/R injury by regulating mTOR pathway *in vitro*.

On the other hand, excessive stimulation of autophagy has been described to exacerbate I/R injury in the kidney (37). Isaka *et al* (38) used LC3-GFP transgenic mice and demonstrated that I/R injury promoted the formation of LC3-GFP dots. By contrast, increased B-cell lymphoma (Bcl)-2 protein protected tubular epithelial cells from I/R injury by suppressing autophagy and inhibiting apoptosis (38). Furthermore, overexpression of Bcl-xL and Bcl-2 eliminate both apoptosis and autophagy (39). Suzuki *et al* (40) used HK-2 cells subjected to hypoxia or activation of reactive oxygen species and demonstrated that compared to normoxic conditions LC3-labeled autophagic vacuoles slightly increased and lysosome-associated membrane protein2-labeled lysosomes markedly increased following 48 h of hypoxia. Using lysosomal protease inhibitors autophagosomes increased significantly under hypoxia, suggesting that hypoxia highly induces autophagic generation and degradation. Moreover, HK2 cells with Atg7 deletion significantly inhibit H_2O_2-induced cell death (40). These results indicate that autophagy may contribute to cell death during renal ischemia-reperfusion injury. In another study it was demonstrated that kidneys of GFP-LC3 transgenic mice that were subjected to 48 h of cold ischemia in the presence of lysosomal inhibitor bafilomycin A1, the number of apoptotic cells were significantly reduced (41) suggesting that autophagy may serve a role in I/R injury.

Non-coding RNAs, including lncRNA and microRNAs, exhibit an effect on regulating autophagy during I/R injury (42-44). MicroRNAs serve a significant effect in autophagy during kidney I/R injury both *in vivo* and *in vitro* (43,44). Liu *et al* (43) demonstrated that miR-34a reduced the autophagic activity and caused injury in I/R tubular epithelial cells via a direct binding to the Atg4B 3'-untranslated region. In addition, miR-21 mimics directly targeted Rab11a 3'-UTR reducing beclin-1, LC3-II expression and increased p62 expression in the I/R model. Overexpression of Rab11a weakened the effect of miR-21 mimics and I/R on cell apoptosis (44). In a rat I/R model, pre-treatment with miR-21 inhibitor injection ameliorated the renal injury, and increased the expression of LC3-II and beclin-1 (44). The role of autophagy in I/R damage depends on the injury situation such as the duration of hypoxia, ischemia and reperfusion, and the effect of autophagy in different studies is shown in Table I.

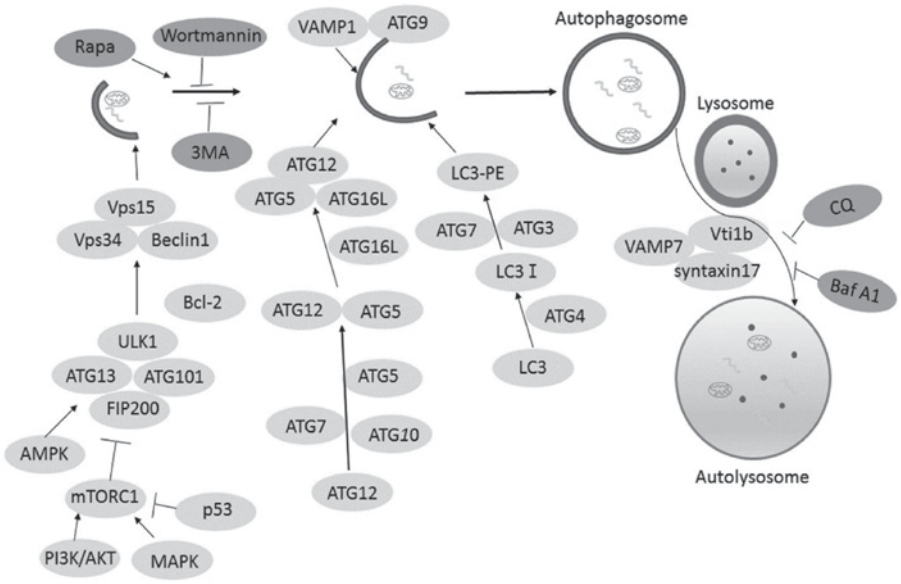

Figure 1. Overview of the autophagy process and its molecular machinery. The formation of autolysosome involves several processes and a number of autophagy-associated genes. PI3K/AKT and MAPK inhibit autophagy by regulating mTOR signaling pathway, p53 serves the opposite effect. AMPK upregulates autophagy by activating ULK1 complex. However, Bcl-2 inhibits autophagy by interacting with Beclin1. Rapamycin promotes the nucleation step of autophagosome, but wortmannin and 3MA inhibit this step. In addition, CQ and Baf A1 impair the autophagic flux by inhibiting the fusion autophagosome and lysosome. PI3K/AKT, phosphatidylinositol 4,5-bisphosphate/RAC-alpha serine/threonine-protein kinase; MAPK, mitogen-activated protein kinase; mTOR, serine/threonine-protein kinase mTOR; AMPK, 5' AMP-activated protein kinase; ULK1, Serine/threonine-protein kinase ULK1; Bcl-2, apoptosis regulator Bcl-2; CQ, chloroquine; BAF, bafilomycin A1; 3MA, 3-methyladenine.

Autophagy in drug-induced acute kidney injury. Cisplatin is a widely used chemotherapeutic drug, with major side effects in kidneys, leading to cell death and tissue damage (45,46). Several studies demonstrated that cisplatin is capable of activating autophagy both *in vitro* and *in vivo* AKI models (47-51).

Depending on the experimental conditions, autophagy could act as a mechanism of cytoprotection (47,48). Cisplatin induced an increase of LC3-II expression that is further enhanced by emodin treatment in NRK-52E cells (49). Autophagy activity is increased after 6 h of cisplatin treatment and began a gradual decrease from 12 h in proximal tubular cells (RPTCs), which was transiently transfected with GFP-LC3, indicating that autophagy is induced before apoptosis in RPTCs during cisplatin treatment (50). Consistently, using mRFP-LC3-transfected NRK-52E cells it was demonstrated that emodin increased the formation of mRFP-LC3 dots and decreased apoptosis during cisplatin treatment (51). Inhibition of autophagy with either 3-MA or bafilomycin A1 elevated renal proximal tubular cell apoptosis and abolished the protective effects of emodin during cisplatin treatment (50,51), suggesting a renoprotective role of autophagy in cisplatin-induced AKI. HEK cells with Beclin-1 knockdown prevent the formation of GFP-LC3 dots during cisplatin treatment and sensitize cells to cisplatin-induced apoptosis (50). By contrast, overexpressed beclin-1 and Atg5 in LLC-PK1 cells inhibit caspase activation and protecte tubular cells from cisplatin toxicity (51). This further supports that autophagy serves a protective role against cisplatin injury to proximal tubular epithelial cells.

Furthermore, further *in vivo* studies have affirmed that autophagy serves the same cytoprotective role as in the *in vitro* findings. An *in vivo* model autophagy was induced in proximal kidney tubules during cisplatin treatment in C57BL/6 mice (50). Autophagic vesicles were increased in C57BL/6 mice after cisplatin treatment, as detected by electron microscopy (50). Activation of autophagy by rapamycin ameliorated cisplatin-induced AKI, whereas the inhibition of autophagy by chloroquine aggravated cisplatin-induced AKI in C57BL/6 mice (31,51). In addition, proximal tubule-specific Atg7-deficient mice, which were more sensitive to cisplatin-induced injury compared with the wild-type mice (31).

The activity of autophagy is crucial to protect kidney against chemotherapeutics injury (49). The underlying mechanism of how autophagy protects the kidney from the damage induced by cisplatin is not clearly understood (31). P53 is reported to participate in cisplatin-induced renal cell apoptosis in *in vitro* and *in vivo* models (52-58). Feng *et al* (59) suggested that p53 regulates autophagy. Periyasamy-Thandavan *et al* (50) demonstrated that pifithrin-α, a pharmacological inhibitor of p53, inhibited autophagosome formation during cisplatin treatment in RPTCs. Increased p53 and JNK activation aggravate cisplatin-induced proximal tubular cells apoptosis in PT-Atg7-KO mice (31).

mTORC1 is a critical serine-threonine kinase in the autophagy regulation pathway that negatively regulates autophagy activity (49). However, AMPK inhibited the activity of mTORC1, a AMPK inhibitor compound C significantly suppressed emodin-induced AMPK phosphorylation and LC3 conversion, consequently inhibited the autophagic activity and increased the cisplatin-induced proximal tubular cells apoptosis (49). Cells transfected with AMPK small interference (si)RNA were sensitive to cisplatin-induced AKI (60). NAD(P)H: quinone oxidoreductase 1 (NQO1) knockout enhances autophagy in ACHN cells and mice during cisplatin treatment by the AMPK/mTOR signaling pathway (61).

Caspase inhibitor zVAD-fmk prevented the degradation of Atg5, Atg12, and beclin-1 thereby increasing GFP-LC3-II dots in

Table I. Overview of different studies of autophagy in renal I/R injury.

Cause of AKI	Model	Autophagy modulation method	Role of autophagy	(Refs.)
Ischemia 45 min Reperfusion 24 h	Male Wistar rats	3MA Rapa	Protective	(27)
Hypoxia 24 h Reoxygenation 2-24 h	TCMK-1 cells	3MA Rapa	Protective	(28)
Ischemia 30 min reperfusion 0 h-7 d	Male BalB/c mice			
Ischemia 30 min Reperfusion 3, 12, 24 h	Male SD rats	3MA Rapa	Protective	(29)
Hypoxic 24 h	RPTC cells	GFP-LC3 3MA ATG5 Beclin shRNA	Protective	(30)
Ischemia 30 min Reperfusion 0, 6, 24, 48 h	C57BL/6 mice	3MA CQ		
Ischemia 25 min reperfusion 24 h	C57BL/6 mice	CQ Rapa ATG7 KO	Protective	(31)
H_2O_2 200 Mm 4 h	LLC-PK1 cells	Beclin ATG5 siRNA GFP-LC3 3MA CQ wortmannin tunicamycin	Protective	(32)
Ischemia 35 min Reperfusion 3, 6, 12, 24, 36, 48 h	C57BL/6 mice	ATG5 KO GFP-LC3 transgenic	Protective	(33)
H_2O_2 200, 400, 600 mM 4 h	NRK-52E cells	HSPB1 siRNA LC3 siRNA Rapa BAF	Protective	(35)
Ischemia 30 min Reperfusion 0-72 h	Male SD rats	-		
Antimycin A and 2-deoxyglucose 1.5 h reperfusion 24 h	NRK-52E cells LLC-PK1 cells	GFP-LC3 shRNA AMPK 3MA Rapa	Protective	(36)
Ischemia 45 min Reperfusion 96 h	Mice	LC3-GFP transgenic Bcl-2/LC3-GFP transgenic	Detrimental	(38)
Hypoxia 6-24 h H_2O_2 500 μM 8-12 h	HK-2	3MA E64d ATG7 siRNA pepstatin A	Protective	(40)
Ischemia 30 min Reperfusion 0-48 h	Male C57BL/6 mice	-		
Cold ischemia 48 h	Mice	GFP-LC3 transgenic	Detrimental	(41)
Ischemia 35 min Reperfusion 1, 3, 7 d	Male C57BL/6 mice	-	Protective	(43)
Hypoxia 24 h Reoxygenation 24h	NRK-52E cells	Rab11a siRNA pcDNA3.1-Rab11a	Protective	(44)

RPTC, renal proximal tubular cell; Rapa, rapamycin; CQ, chloroquine; BAF, bafilomycin A1; 3MA, 3-methyladenine.

LLC-PK1 during cisplatin treatment. However, autophagosome formation and p62 expression were not significantly increased in the presence of zVAD-fmk and chloroquine, demonstrating that zVAD-fmk impaired autophagic flux by blocking the autophagosome clearance (62). zVAD-fmk suppressed lysosomal function and impaired autophagic flux by inhibiting lysosomal cathepsins (63) and calpains (64,65). A similar result is obtained zVAD-fmk prevented beclin-1 cleavage to impair autophagic flux and increase cisplatin-induced cellular injury in a mouse model (62). There is a connection between apoptosis and autophagy, where a signaling pathway that regulates autophagy can also regulate apoptosis (17). Therfore, 10 μM cisplatin induced autophagy to maintain cell homeostasis, whereas 50 μM cisplatin induced cell apoptosis in NRK-52E cells. However, Rovetta *et al* (66) demonstrated that ER signaling pathway regulated the balance between autophagy and apoptosis induced by cisplatin. Autophagy mostly served a renoprotective action in cisplatin-induced AKI (Table II).

Table II. Overview of different studies of autophagy in renal drugs injury.

Cause of AKI	Model	Autophagy modulation method	Role of autophagy	(Refs.)
Cisplatin 10-50 μM 24 h	NRK-52E cells	Rapa BAF compound C	Protective	(49)
Cisplatin 20 μM 0-16 h	RPTC	Beclin shRNA Bcl-2 transfection	Protective	(50)
30 mg/kg 0-3 d	C57BL/6 mice	-		
Cisplatin 10 μg/ml 48 h	Primary kidney cells	Compound C	Protective	(59)
25 mg/kg 48 h	C57BL/6 mice	AMPK siRNA		
Cisplatin 16 mg/kg 3 d	Male Wistar rats	CQ Rapa	Protective	(60)
Cisplatin 20 μM 24 h	ACHN cells	3MA CQ Rapa	Protective	(61)
18 mg/kg	C57BL/6 mice	Compound C NQO1-KO		
Cyclosporine A 20 μM	HK-2	BAF	Protective	(69)
	TMBIM6 KO mice	-		

RPTC, renal proximal tubular cell; Rapa, rapamycin; CQ, chloroquine; BAF, bafilomycin A1; 3MA, 3-methyladenine; NQO1, quinone oxidoreductase 1.

Table III. Overview of different studies of autophagy in renal sepsis injury.

Cause of AKI	Model	Autophagy modulation method	Role of autophagy	(Refs.)
LPS 1 mg/ml 24 h	RTEC	Bafilomycin A1	Protective	(72)
LPS 15 mg/kg 48 h	Male C57BL/6 mice	ATG7 KO		
TNF-α 2 ng/μl 24 h	NRK-52E cells	siRNAs Atg7 Rapa	Protective	(73)
CLP 3, 6, 9, 18 h	male SD rats	-		
LPS 10 mg/kg 24 h	Male C57BL/6 mice	CQ PT-Atg7- KO RFP-EGFP-LC3	Protective	(74)
LPS 1.5 mg/kg 48 h	Male C57BL/6 mice	mTOR inhibitor activator of AMPK siRNA VPS34	Protective	(75)

RTEC, renal tubular epithelial cell; Rapa, rapamycin; CQ, chloroquine.

Cyclosporine A (CsA) is an immune-suppressor used in renal allograft transplantation (67). Previous studies demonstrated that CsA induced the accumulation of autophagosomes and inhibited autophagic clearance (68). Yadav *et al* (69) demonstrated that expression of LC3-II was increased and SQSTM1 accumulation was decreased following CsA treatment in transmembrane BAX inhibitor motif containing 6 (TMBIM6)-expressing HK-2 cells compared to NC cells, suggesting that the autophagic flux was standard in TMBIM6 cells. A similar result was obtained *in vivo*, in CsA-treated TMBIM6-/- mice where autophagosome formation was increased and the formation and activity of lysosome were decreased (69). The mechanism of TMBIM6-induced autophagy was that it activated PRKAA and suppressed mTORC1 in CsA-treated HK-2 cells. This further demonstrated that TMBIM6 reversed the impaired autophagic flux by stimulating lysosome biogenesis through TFEB activation.

Autophagy in sepsis renal injury. Sepsis-related AKI is an important clinical issue occurring during severe infection including cecal ligation and puncture (CLP) and LPS (70,71). The effect of autophagy in septic AKI remains unclear, compared with the genetic evidence in I/R and cisplatin models (17). Lipopolysaccharide (LPS)-induced accumulation of LC3-II in HPT1 cells derived from human proximal tubular epithelial cells (PTEC) (72). Preincubation of rapamycin decreased tumor necrosis factor (TNF) and induces NRK-52E cell death, whereas the knockdown of Atg7 exaggerated TNF-induced DNA fragmentation (73). This demonstrated that autophagy serves a cytoprotective role in the sepsis-induced AKI. Similar results were found *in vivo*, LPS induces the accumulation of LC3-positive puncta in GFP-LC3 transgenic mice (74). CLP-induced autophagy was time-dependent in rat, LC3-II was elevated transiently at 3 h but declined at 9 h until 18 h after CLP, renal dysfunction occurred at 9 and 18 h after CLP (73), indicating that autophagy occurred prior to apoptosis and the decline of autophagy aggravated TNF-α-induced cell death. Chloroquine, an inhibitor of lysosomes also aggravates LPS-induced AKI in C57BL/6 mice (74). Tubular injury was more severe in ATG7KO mice than in controls and the

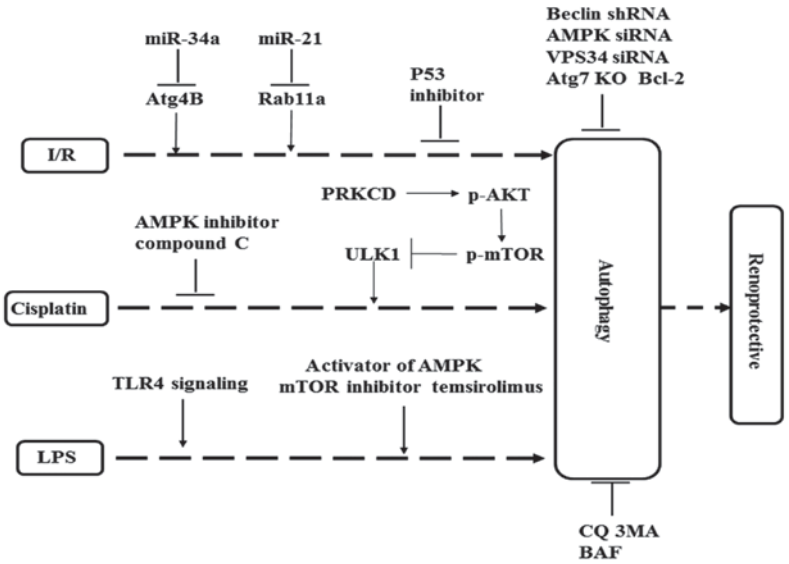

Figure 2. Overview of the protective mechanism of autophagy in AKI. In I/R induced AKI models, miR-34a and miR-21 activate autophagy by direct binding to Atg4B and Rab11a respectively. In cisplatin-induced AKI models, AMPK and p53 inhibitor decrease the autophagy. In addition, the PRKCD also inhibits autophagy by regulating AKT/mTOR signaling pathway. In LPS induced AKI models, TLR4 signaling pathway promotes autophagy and activator of AMPK or inhibitor of mTOR has the same effect on autophagy. Knock down of certain autophagy-associated genes or administration of pharmacological inhibitors of autophagy also inhibits the protective effect of autophagy in AKI. I/R, ischemia/reperfusion; AKI, acute kidney injury; miR, microRNA; Atg4B, cysteine protease ATG4B; Rab11a, Ras-related protein Rab-11A; AMPK, 5' AMP-activated protein kinase; PRKCD, protein kinase C δ type; AKT, RAC-alpha serine/threonine-protein kinase; mTOR, serine/threonine-protein kinase mTOR; TLR4, toll-like receptor 4.

expression of IL-6 was significantly increased in ATG7KO kidneys compared with controls following LPS treatment (73), indicating that autophagy protected kidneys from CLP and LPS-induced sepsis injury. Leventhal *et al* (72) reported that LPS-induced autophagy in RTEC was TLR4-dependent, there were no differences in LC3-II accumulation in C57Bl\10ScN mice, which was no functional TLR4 compared with saline-injected mice. Autophagy was decreased in adult mice during LPS-induced AKI, administration of the mTOR inhibitor temsirolimus increased autophagy and improved renal function in adult mice during LPS-induced AKI (75). In young mice (8 weeks of age), the inhibitor of VPS34 damaged renal function after LPS treatment (75). These results suggest that autophagy serves a renoprotective role irrespective of age (young or aged mice) during LPS treatment (Table III).

4. MSCs and extracellular vesicles therapy in AKI

Mesenchymal stem cells (MSCs) are multipotent stem cells isolated from different tissues including bone marrow, umbilical cord, adipose tissue or muscle (76). These cells have self-renewal and multiple differentiation potential including adipocytes, chondrocytes and osteocytes (77). A large number of studies have documented the potential therapeutic effects of MSC, including cardiopathy (78), hepatic diseases (79), and renal injury (80). The therapeutic potential of MSCs includes the anti-inflammatory, antioxidant, anti-fibrotic, anti-apoptotic, pro-angiogenic, stimulation of endogenous progenitor cells, and promotion of cellular reprogramming (78-80). Exosomes derived from MSC have beneficial effects in distinct models of injury. Our previous studies suggested that exosomes derived from human umbilical cord MSCs alleviated liver fibrosis (81), cutaneous wound healing (82), and acute myocardial ischemic injury (83).

MSCs are recruited to injured tissues and release certain cytokines and growth factors, such as insulin-like, hepatocyte and vascular endothelial growth factors, which could activate endogenous cellular repair programs contributing to the growth and survival of endothelial and epithelial tubular cells thus promoting renal angiogenesis and regeneration (84,85). Bruno *et al* (86) demonstrated that MSC-derived microvesicles express MSC markers and transferr cellular materials to neighboring cells, including RNA and proteins, consequently promoting cell proliferation and inhibited apoptosis.

Human MSCs repair HK2 cell after ischemia injury by stimulating normal reactive oxygen species handling, anti-apoptotic activity, energy production, protein synthesis, cytoskeleton organization and cell proliferation (87). In I/R rats, MSCs repair kidney by anti-inflammatory, anti-apoptotic and by enhancing the repair of peritubular capillaries and tubular epithelial cells (88). Furthermore, extracellular vesicles derived from MSCs alleviate kidney injury through anti-oxidation by strengthening Nrf2/ARE activation in I/R rats (89). BM-MSCs ameliorate cisplatin-induced AKI by increasing $Foxp^{3+}$ T-regulatory cell infiltration in a monkey model (90). Human adipose-derived mesenchymal stem cells protect against cisplatin-induced AKI via anti-apoptotic pathways (91). In a previous study, it demonstrated that human umbilical cord mesenchymal stem cells-derived exosomes decrease cisplatin-induced renal oxidative stress and apoptosis in NRK-52E cells and in rats (92), indicating that MSCs and microvesicles released by MSCs may improve renal injury in AKI.

5. The role of autophagy in MSCs cell-based therapies

Autophagy plays a critical role in MSCs-based therapy of tissues injury. Hypoxia and heat shock pretreatment enhance

survival in BM-MSCs and increase the therapeutic potential of the stem cell by increasing autophagy, providing a novel strategy to improve MSC-based therapies (93,94).

Bone marrow (BM)-MSCs alleviate chronic high glucose-induced injury in INS-1 cells (95) and reduce the severity of lung I/R injury in human pulmonary microvascular endothelial cells (HPMVECs) (96) by enhanced autophagic activity. Similar results are presented *in vivo*, where BM-MSCs promote the recovery of pancreatic damage in T2D rats (95) and reduce the severity of lung I/R injury in C57BL/6J mice (96). Shin *et al* (97) demonstrated that MSCs significantly enhance autophagy and the clearance of amyloid-β in Alzheimer disease models to increase neuronal survival against Aβ toxicity. In a Parkinsonian model, BM-MSCs increase the cell viability and reduce α-synuclein by upregulating autophagolysosome formation (98). Tonsil-derived MSCs ameliorate liver fibrosis via the downregulation of TGF-β and autophagy activation (99). These findings support the protective effect of autophagy in MSCs, thereby repairing the pancreas, lung, nervous diseases. In our previous studies, it was demonstrated that human umbilical cord mesenchymal stem cell-derived exosomes prevent cisplatin-induced AKI by autophagy (100) which providing a novel strategy of MSC-based therapy for AKI.

6. Conclusions

In conclusion, autophagy is induced in kidneys in response to AKI, autophagy in AKI is multifaceted and complex, and it can protect against kidney injury or promote cell death depending on experimental conditions (17). The activity of autophagy is time-dependent. In the early stages of renal injury, autophagy contributes to cell survival, whereas in later stages, autophagy may activate apoptosis signaling pathways sequentially promoting cell death and renal function injury, but the mechanism remains unknown. Autophagy and apoptosis share similar regulators and are mutually inhibitory (101). Many factors that activate autophagy also activate cell apoptosis, which usually precedes cell apoptosis (17). Several critical signaling pathways positively regulate autophagy and apoptosis, such as the tumor suppressor p53, bcl-2 family, death-associated protein kinases and c-Jun N terminal kinases (101). In the model of cisplatin-induced nephrotoxicity, PRKCD suppressed autophagy and promoted renal cell death by AKT/mTOR signal pathway (102). The possible protective mechanism of autophagy in AKI is summarized in Fig. 2. Autophagic flux is frequently defined as a measure of autophagic degradation activity. Continuous observation autophagy is imperative, and to assess the efficiency of the autophagic flux is necessarily in measuring autophagic degradation activity. However, the molecules participating in the autophagosome formation and the regulating mechanism are not known. The signaling pathways, which have been reported in AKI models, such as AMPK and mTOR, are critical in inducing and regulating autophagy but these are also poorly understood.

Pharmacological inhibitors (3-MA or bafilomycin A or wortmannin) or inducers (Rapa) of autophagy are non-specific for autophagy (17). It is necessary to find autophagy inducers in proximal tubules and examine their effects in models of AKI. A comprehensive understanding of autophagy may improve renal function and prevent AKI progression.

MSC-based therapies have been extensively studied as a potential treatment for several diseases (78-80). However, our understanding of the regulatory mechanisms of MSCs or MSC-derived vesicles in kidney disease, remain to be fully elucidated. Autophagy serves a key role in MSCs-based therapy, but effects of autophagy in AKI, which are repaired by MSCs are not very clear. Therefore, elucidating its role may provide a novel approach towards the therapy of AKI.

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