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Regulation of Autophagy by TGF- β : Emerging Role in Kidney Fibrosis

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Abstract

Autophagy is a highly conserved homeostatic mechanism for cell survival under conditions of stress, and is widely implicated as an important pathway in many biological processes and diseases. In progressive kidney diseases, fibrosis represents the common pathway to end-stage kidney failure. Transforming growth factor- β 1 (TGF- β 1) is a pleiotropic cytokine that has been established as a central mediator of kidney fibrosis. Recently emerging body of evidence from studies in renal cells in culture and experimental animal models suggests that TGF- β 1 regulates autophagy and that autophagy regulates many critical aspects of normal and disease conditions associated with kidney fibrosis, such as tubulointerstitial fibrosis, glomerulosclerosis, and diabetic nephropathy. Here, we review the recent advances exploring the process of autophagy, its regulation by TGF- β 1, and implication in the pathogenesis of progressive kidney fibrosis and injury responses. Understanding the cellular and molecular bases of this process is crucial for identifying potential new diagnostic and therapeutic targets of kidney fibrosis.

Keywords

Autophagy; transforming growth factor- β 1; fibrosis; chronic kidney disease

INTRODUCTION

Autophagy

Autophagy is a process by which cytoplasmic components including macromolecules such as proteins, glycogens, lipids and nucleotides, and organelles such as mitochondria, peroxisomes and endoplasmic reticulum (ER) are degraded by the lysosome. There are at least three different types of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy. Macroautophagy (hereafter referred to as autophagy) is the most extensively studied and its process involves delivery of cytosolic contents to the lysosome by autophagosomes. In contrast to autophagy, microautophagy involves inward invagination of the lysosomal membrane, and in chaperone-mediated autophagy, proteins are selectively recognized by the cytosolic chaperone and directly translocate across the lysosomal membrane.^{1–3}

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The autophagic pathway proceeds through several phases, including initiation, vesicle elongation, autophagosome maturation and cargo sequestration, and autophagosome-lysosome fusion. In the final stage, autophagosomal contents are degraded by lysosomal acid hydrolases and the contents of the autolysosome are released for metabolic recycling. Yeast genetic studies have identified a set of autophagy-related (*Atg*) genes that are required for autophagy and its related processes. These genes are highly conserved among eukaryotes. In initiation process, the Beclin 1-interacting complex consists of BECLIN 1, BCL-2 family proteins which inhibit autophagy, the class III phosphatidylinositol 3-kinase (PI3K) vacuolar protein sorting 34 (VPS34), and ATG14L.⁴ Autophagosomal elongation requires two ubiquitin-like conjugation systems: the ATG5–ATG12 conjugation system and the microtubule associated protein light chain 3 (LC3/ATG8) conjugation system. The conversion of a cytosolic truncated form of LC3 (LC3-I) to phosphatidylethanolamine-conjugated form (LC3-II) indicates autophagosome formation.³ Impaired autophagosome-lysosome fusion may result in increases in the number of autophagosomes, as observed in several diseases.³

TGF- β and kidney fibrosis

Renal fibrosis is a major hallmark of chronic kidney disease (CKD) regardless of the initial causes. Transforming growth factor (TGF)- β has a broad spectrum of biological functions in a variety of cell types, and is a well-known mediator in the pathogenesis of renal fibrosis.^{5,6} TGF- β 1, the most abundant isoform of TGF- β family members, can be secreted by all types of renal cells and infiltrating inflammatory cells as a precursor called latent TGF- β 1, which binds to latent TGF- β -binding protein (LTBP). TGF- β 1 is released from the latency-associated peptide and LTBP when exposed to many factors, such as reactive oxygen species (ROS), plasmin, and acid.^{7–10} The mature TGF- β 1 binds to its receptor, TGF- β type II receptor, which in turn activates TGF- β type I receptor kinase, and initiates the downstream signals, including both Smad-dependent and Smad-independent pathways.¹¹

Accumulating evidence has well established a central role for TGF- β 1 in renal fibrosis in both experimental and human kidney diseases. TGF- β 1 is significantly up-regulated in the fibrotic kidney regardless of the initial causes of kidney diseases.^{5,9} Overexpression of mature TGF- β 1 in rodent liver is capable of promoting the progression of fibrosis in kidneys, revealing a functional importance of TGF- β 1 in CKDs.^{12,13} The pro-fibrotic effect of TGF- β 1 is confirmed further by the findings that blockade of TGF- β 1 with neutralizing TGF- β antibodies or antisense oligonucleotides significantly ameliorates renal fibrosis in vivo and in vitro.¹⁴

TGF- β signaling

The Smad signaling pathway is widely known as a canonical pathway induced by TGF- β 1.¹⁵ The heteromeric signaling complex, resulting from binding of TGF- β 1 to type II receptor and in turn type I receptor, transduces signals through receptor-regulated Smads (Smad2/3) and common-partner Smad (Smad4), leading to transcriptional regulation of target genes. A number of non-canonical TGF- β signaling pathways have also been identified, including the rho-like guanosine triphosphatases,^{16,17} PI3K/Akt,^{18–21} and the mitogen-activated protein kinases (MAPKs), namely extracellular signal-regulated kinase 1/2,^{22,23} c-Jun N-terminal kinase (JNK),^{24–26} and p38 MAPK.^{27–30} We and others have shown that TGF- β -activated kinase 1 (TAK1) is a major upstream signaling molecule in TGF- β 1-induced type I collagen and fibronectin expression through activation of the MAPK kinase (MKK)3-p38 and MKK4-JNK signaling cascades, respectively.^{31–33} The role of TGF- β signaling via TAK1 pathway in kidney fibrosis has been discussed in a previous review.³⁴

Mechanisms of kidney fibrosis

The pathogenesis of renal fibrosis in chronic kidney disease is characterized by excessive accumulation of extracellular matrix (ECM). This underlying mechanisms by which TGF- β 1 mediates fibrogenesis involve in broad strokes the following: (i) TGF- β 1 induces matrix production such as type I collagen and fibronectin through Smad3-dependent or non-canonical mechanisms; (ii) TGF- β 1 inhibits ECM degradation by suppressing matrix metalloproteinases (MMPs) and inducing the natural inhibitor of MMPs [TIMPs (tissue inhibitor of metalloproteinases)].¹⁴ Moreover, TGF- β 1 has been shown to induce tubular epithelial–mesenchymal transition (EMT) in renal epithelial cells, and thought to directly contribute to the myofibroblast pool in renal injury, the cell type most responsible for interstitial matrix accumulation.³⁵ This notion was based largely on *in vitro* cell culture studies. However, the recent application of genetic fate mapping techniques in mouse fibrosis models argues against EMT as a direct contributor to the kidney myofibroblast population.³⁶ Therefore, this function of TGF- β 1 and the origin(s) of interstitial myofibroblasts contributing to the genesis of renal fibrosis have been recently challenged and the subject of debate. Cellular interactions that lead to TGF- β -mediated tubulointerstitial fibrosis are not well understood. Various forms of injury (e.g. mechanical stretch, hypoxia) target the renal tubular epithelium, leading to production of inflammatory cytokines such as monocyte chemoattractant protein-1, which recruits macrophages. The infiltrating macrophages are potent sources of TGF- β that can signal on neighboring epithelial cells or renal fibroblasts. TGF- β from either macrophages or injured tubular epithelium stimulates fibroblasts to produce matrix components such as collagen I and fibronectin. The increased TGF- β production by injured epithelium can signal in an autocrine fashion, leading to further TGF- β production, dedifferentiation, and possibly increased collagen IV production. Tubular injury also may increase integrin α v β 6 expression and activation of latent TGF- β .³⁷

TGF- β REGULATES AUTOPHAGY IN THE KIDNEY

TGF- β 1 activating autophagy is a recently recognized biological function of TGF- β 1 that is just beginning to be elucidated. Few studies have previously reported that TGF- β 1 induces autophagy in bovine mammary gland epithelial cells and neonatal piglet gut epithelium in the context that autophagy represents type II programmed cell death, which is complementary to apoptosis type of cell death induced by TGF- β 1 treatment.^{38,39} Recently, TGF- β has been demonstrated to activate autophagy in certain hepatocellular carcinoma and breast cancer cell lines, which undergo cell cycle arrest and apoptosis in response to TGF- β . In those cancer cells, TGF- β stimulation increases the expression of mRNA transcripts of several autophagy-related genes, such as *Beclin 1*, *Atg5*, *Atg7*, and *Dapk* (death-associated protein kinase), and induces accumulation of autophagosomes and activation of autophagic flux.⁴⁰ Moreover, induction of autophagy by TGF- β is suppressed by knockdown of Smad2/3 or Smad4 suggesting that TGF- β induces autophagy, at least in part, via the Smad pathway.⁴⁰ In addition, knockdown of DAPK or inhibition of JNK also suppresses TGF- β -induced autophagy, indicating the involvement of both Smad-dependent and Smad-independent pathways. Involvement of other pathways for the transcriptional activation of autophagy-related genes and regulation of autophagy, including the PI3K/Akt/FoxO3, E2F1, and p53 and its homologue p73 has also been reported.⁴¹ Interestingly, TGF- β can also activate the mammalian target of rapamycin (mTOR) pathway via PI3K/Akt, and therefore, TGF- β may exert both stimulatory and inhibitory effects on autophagy. The dual functions of TGF- β 1 capable of opposing effects, for instance, to suppress or promote tumorigenesis, or to inhibit or stimulate cell growth and cell death, are well known, and may depend on the specific cell type and context.

TGF- β -induced autophagy in glomerular mesangial cells

Glomerular mesangial cells are generally considered as specialized contractile pericytes, unique to the kidney, and located within the mesangium, providing structural support as well as forming a functional unit for the glomerular tuft, together with adjacent glomerular capillary endothelial cells and podocytes, to regulate glomerular filtration. Mesangial cells are major contributors to the ECM that constitutes the mesangium, and are important in the maintenance of mesangial matrix homeostasis. They are also major targets of a number of glomerular diseases such as IgA nephropathy and diabetic nephropathy. In response to injury and progressive kidney disease, mesangial cells proliferate and produce excessive ECM, leading to the development of glomerulosclerosis and kidney fibrosis.

To date, there have been few studies examining the role of autophagy in mesangial cells. Our studies have shown that TGF- β 1 induced autophagy in mesangial cells.^{21,42} Moreover, our recent investigations unveiled a novel role of autophagy in negatively regulating matrix production in mesangial cells by promoting the degradation of intracellular type I collagen induced by TGF- β 1⁴² (Fig. 1A). The induction of autophagy in mesangial cells by treatment with trifluoperazine (TFP), an inducer of autophagy, or low-dose carbon monoxide (CO), which we had previously shown to exert anti-fibrotic effects in a model of kidney fibrosis induced by unilateral ureteral obstruction (UUO),⁴³ also resulted in decreased type I collagen protein levels induced by TGF- β 1, without alterations in collagen (Col-1 α 1) mRNA⁴² (Fig. 1A). These studies demonstrated that CO induced autophagy in the kidneys of mice exposed to low-dose CO and in mesangial cells treated with CO-releasing molecule 2 (CORM-2). Treatment with CORM-2 in wild-type mesangial cells also reduced type I collagen protein stimulated by TGF- β 1, whereas these CORM-2 effects were abrogated in autophagy-deficient *beclin 1*^{+/-} mesangial cells, suggesting that CO suppresses accumulation of collagen protein induced by TGF- β 1, at least in part, through induction of autophagy (Fig. 1A).

We previously demonstrated the role of TAK1-MKK3-p38 signaling pathway in TGF- β 1-induced collagen production in mesangial cells,^{29,32} and interestingly, the MKK3 pathway also mediated TGF- β 1 induction of autophagy, indicating the vital role of this signaling cascade in controlling the level of type I collagen (Fig. 1A). Thus, mesangial cells produce and regulate turnover of its ECM, and the balance between synthesis and degradation of collagen is crucial for the maintenance of tissue homeostasis. TGF- β 1 is known as a prototypic multifunctional cytokine, and the duality of TGF- β 1 functions, as both an inducer of collagen synthesis and an inducer of autophagy and collagen degradation, underscores the multifunctionality of TGF- β 1.

We have recently reported that under the condition of serum deprivation, TGF- β 1 induced autophagy in mesangial cells, and autophagy enhanced cell survival by inhibiting mesangial cells from undergoing apoptosis.²¹ Deficiency of autophagy in mesangial cells, through either knockdown of LC3 by small interfering RNA (siRNA) or *LC3* gene deletion in cells obtained from LC3 null (*LC3*^{-/-}) mice, resulted in failure of TGF- β 1 to rescue mesangial cells from serum deprivation-induced apoptosis. TGF- β 1 also enhanced cell cycle progression under serum deprivation condition in mesangial cells through up-regulation of cyclin D1 and E, while down-regulating cyclin-dependent kinase inhibitor p27, to promote cell survival. We also showed that the induction of autophagy by TGF- β 1 in mesangial cells was mediated via TAK1 and PI3K-Akt dependent pathways.²¹ Collectively, these findings provide support for the cytoprotective role of autophagy in mesangial cells.

TGF- β -induced autophagy in tubular epithelial cells

Renal tubular epithelial cells are key targets in acute kidney injury (AKI) and in chronic kidney diseases, and histological studies have indicated that renal function correlates better with tubular and interstitial changes than glomerular changes. Proximal tubular epithelial cells are particularly susceptible to ischemia-reperfusion- and nephrotoxin-induced kidney injury, and the induction of autophagy has been reported under these settings. For instance, in a nephrotoxic model of AKI, cisplatin was shown to induce autophagy in renal proximal tubular epithelial cells.⁴⁴ Recent studies in cultured human renal proximal tubular epithelial cells demonstrated that TGF- β 1 induced up-regulation of autophagy-related genes, *Atg5*, *Atg7* and *Beclin 1* and accumulation of autophagosomes in a time- and dose-dependent manner.⁴⁵ Furthermore, TGF- β 1 activated autophagy through the generation of ROS, and promoted apoptosis in tubular cells⁴⁵ (Fig. 2A). It is generally believed that ROS induces autophagy, and autophagy serves as a mechanism to eliminate oxidized proteins and dysfunctional organelles, such as damaged mitochondria, to mitigate oxidative stress and restore cellular ROS balance.

In studies by Koesters et al. the role of TGF- β 1 in the induction of autophagy was examined using a transgenic mouse model with tetracycline-controlled overexpression of TGF- β 1 in renal tubular epithelial cells.⁴⁶ Their studies show that tubular overexpression of TGF- β 1 induced dedifferentiation and decomposition of tubular cells by autophagy, and led to the development of tubulointerstitial fibrosis, without evidence of EMT. Renal tubulointerstitial fibrosis, accompanied by tubular degeneration and atrophy, is the hallmark of progressive CKD and these findings indicate that TGF- β 1 induces autophagy in renal tubular epithelial cells and mediate tubular decomposition and pathogenesis of tubulointerstitial fibrosis⁴⁶ (Fig. 2A).

AUTOPHAGY REGULATES KIDNEY FIBROSIS

Activation of autophagy may represent a cellular defense against various stress stimuli, and a growing body of evidence supports a link between autophagy and the pathogenesis of fibrotic diseases. Dysregulated autophagy has been implicated in disorders characterized by fibrosis in various tissues, including idiopathic pulmonary fibrosis, liver fibrosis, cardiac fibrosis, and renal fibrosis.^{47–49} Here, we highlight the current insights regarding the process of autophagy and its role in the pathogenesis of renal fibrosis with particular focus on tubulointerstitial fibrosis, glomerulosclerosis, and diabetic nephropathy, which are disorders in which central role of TGF- β 1 has been well documented. TGF- β 1 is arguably the most potent pro-fibrotic cytokine known and a key mediator of renal fibrosis. Multifunctional actions of TGF- β 1 are well-known, and similarly, dysregulation of autophagy and its role in fibrotic disease pathogenesis may be multifunctional and complex.

Autophagy deficiency in the kidney

Evidence from autophagy deficient mice suggests that autophagy is important in maintenance of matrix protein homeostasis. In the kidneys of mice deficient in autophagic protein Beclin 1, through heterozygous deletion of *beclin 1* (*beclin 1*^{+/-}), increased collagen deposition was noted compared to littermate controls.⁴² Mesangial cells isolated from *beclin 1*^{+/-} mice or transfected with Beclin 1 siRNA expressed higher basal level of type I collagen, and treatment with autophagy inhibitor Bafilomycin A1, but not proteasome inhibitor MG132, increased type I collagen protein levels which colocalized with LC3 as well as lysosomal marker LAMP1 (lysosomal-associated membrane protein 1).⁴² Studies in primary mouse mesangial cells confirmed that collagen and aggregated, insoluble procollagen undergo intracellular degradation through autophagy.⁴² Taken together, these findings suggest a critical role of autophagy as a cytoprotective mechanism to negatively

regulate and limit excess collagen accumulation in the kidney, and suggest that autophagy may be a new therapeutic target to prevent or mitigate pathogenesis of kidney fibrosis.

Investigations also suggest that induction of autophagy may promote fibrogenesis. A recent study that focused on examining the role of autophagy in the pathogenesis of liver fibrosis reported that induction of liver injury in mice with carbon tetrachloride (CCl₄) or thioacetamide (TAA) activated hepatic stellate cells to undergo autophagy and promoted loss of lipids in hepatic stellate cells through intracellular degradation of lipids in lysosomes and liver fibrosis.⁴⁸ However, in transgenic mice with targeted deletion of *Atg7* in stellate cells displayed reduced stellate cell activation and fibrosis following liver injury.⁴⁸ Furthermore, inhibition of autophagy in cultured stellate cells using siRNAs against *Atg5* or *Atg7* and chemical inhibitor 3-methyladenine (3-MA) reduced expression of matrix proteins. Similarly, *Atg7* siRNA or 3-MA treatment in a mouse mesangial cell line decreased expression of collagen, α -smooth muscle actin, and β -platelet-derived growth factor receptor⁴⁸ (Fig. 1B). These findings are in contrast to the previously discussed studies,⁴² but it is important to point out several notable differences in the two studies, including the different strategies employed to block autophagy, such as heterozygous deletion of *Beclin 1* (*Beclin 1*^{+/-}), *Beclin 1* siRNA, and Bafilomycin by Kim et al.⁴² vs. *Atg7* siRNA, and 3-MA by Hernández-Gea et al.,⁴⁸ which brings to focus the matter of distinct roles played by various autophagic proteins. There may also be differences in the cells used in the two studies: primary cultured mouse mesangial cells⁴² vs. mouse mesangial cell line.⁴⁸ Importantly, Kim et al. examined autophagy in the context of TGF- β 1 stimulation, whereas Hernández-Gea et al. examined basal autophagy in mesangial cells. Basal autophagy is present in all cell types but can be rapidly up-regulated as an adaptive response under conditions of cellular stress as a means to generate intracellular nutrients and energy.² The dual, seemingly paradoxical, functions of TGF- β is well-documented, and it seems that both up-and down-regulation of autophagy have been hypothesized to be involved in fibrogenesis. Certainly further research is needed to further clarify and deepen our understanding of the role(s) of autophagy, and to determine whether autophagy may be a new therapeutic target to prevent or mitigate pathogenesis of kidney fibrosis.

Autophagy in tubulointerstitial fibrosis

Renal tubulointerstitial fibrosis is the hallmark of most progressive CKD and end-stage kidney diseases. The UUO model is a well-established model of progressive renal interstitial fibrosis in which increased autophagy, apoptosis, and necrosis in the tubules have been demonstrated in several studies.^{50–55} Kidney injury by UUO activates autophagy mediated via Akt-mTOR signaling pathway, and the induction of autophagy preceded tubular apoptosis and interstitial fibrosis and peaked after 3 days of UUO in the obstructed kidney of rats, and inhibition of autophagy with 3-MA enhanced tubular cell apoptosis and interstitial fibrosis⁵² (Fig. 2B). These studies indicate that autophagy has a cytoprotective role in alleviating tubular damage and kidney fibrosis.

UUO-induced kidney injury also exacerbates oxidative stress and inflammation to induce autophagy, apoptosis, and pyroptosis. Nuclear factor erythroid-2-related factor 2 (Nrf2) is a redox-sensitive basic leucine zipper family transcription factor shown to transactivate cytoprotective pathways and provide cardioprotection, anti-inflammation, and renoprotection. Nrf2 activation, induced by antioxidant sulforaphane, ameliorated UUO-induced kidney damage through preserved mitochondrial function and suppressed UUO-induced renal oxidative stress, inflammation, fibrosis, autophagy, apoptosis and pyroptosis⁵⁵ (Fig. 2B). Sulforaphane also reduced TGF- β expression and hydroxyproline level resulting in decreasing UUO-induced fibrosis and mitigated renal injury.⁵⁵

In other models of tubular injury, the induction of autophagy by a number of nephrotoxins has been reported recently. Autophagy is induced in proximal tubular cells during cyclosporine-induced nephrotoxicity.⁵⁶ Cyclosporine is a potent immunosuppressive drug widely used in preventing transplant rejection and treating autoimmune diseases, but its long-term use leads to the development of a chronic nephrotoxicity characterized by tubular atrophy, interstitial fibrosis, glomerulosclerosis, and impaired renal function. Cyclosporine induces ER stress, which is a potent stimulus for autophagy activation. Autophagy inhibition during cyclosporine treatment with beclin1 siRNA significantly increases tubular cell death.⁵⁶ Thus, induction of autophagy is protective against cyclosporine-induced tubular cell death⁵⁶ (Fig. 2C). Autophagy may serve as an adaptive mechanism to remove the damaged proteins generated from ER stress.

Autophagy in glomerulosclerosis

Podocyte injury leading to podocyte depletion is a major pathomechanism in the development of proteinuria and glomerulosclerosis.⁵⁷ Autophagy is a fundamental homeostatic process that cells use to degrade and recycle cellular proteins and remove damaged organelles. This self-repair mechanism is especially important in terminally differentiated podocytes that have a very limited capacity for cell division and replacement, and podocytes exhibit a high basal level of autophagy.⁵⁸ Moreover, the proteasome pathway can serve as a compensatory mechanism, such that enhanced proteasome activity, compensating for autophagy deficiency, prevents significant accumulation of poly-ubiquitinated proteins.⁵⁸ However, reduction of proteasome activity with bortezomib, or with aging, caused increased ER stress, and accumulation of oxidized proteins and ubiquitinated protein aggregates, damaged mitochondria, and lipofuscin accumulation, and led to more severe proteinuria and glomerulosclerosis⁵⁸ (Fig. 3A). These studies underscore the importance of basal autophagy and compensatory proteasome pathway in the maintenance of glomerular homeostasis and function. In mice with podocyte-specific deletion of the *Atg5* gene, exposure to inducers of proteinuric glomerular injury [puromycin aminonucleoside (PAN) or adriamycin (ADR), low dose bovine serum albumin (BSA), or lipopolysaccharide (LPS)] resulted in more severe albuminuria, foot process effacement, loss of podocytes, and glomerulosclerosis, compared with littermate control mice⁵⁸ (Fig. 3A). These studies suggest that autophagy is a stress adaptive mechanism that is cytoprotective and that autophagy deficiency in podocytes enhances susceptibility to development of glomerular diseases.

Evidence suggests that the process of autophagy represents a key mechanism in maintaining the integrity of podocytes. Class III PI3K Vps34 is generally thought to play an important role in the initiation step of autophagosome formation. Mice with podocyte-specific knockout of Vps34 developed significant proteinuria by 3 weeks of age, developed severe kidney lesions by 5–6 weeks of age, and died before 9 weeks of age.⁵⁹ Kidneys from these knockout mice displayed enlarged vacuoles and increased autophagosomes in the podocytes, foot process effacement, proteinaceous casts, with glomerulosclerosis and interstitial fibrosis. Increased markers for lysosomes (LAMP1 and LAMP2) and autophagosomes (LC3-II/I) were observed in the isolated glomerular lysates, which is mediated via the mTOR pathway⁵⁹ (Fig. 3B). These results suggest that Vps34 participates in maintaining autophagic flux in podocytes, and protect the normal cellular metabolism, structure, and function of podocytes.

Autophagy in human proteinuric glomerular diseases

Immunofluorescence studies in kidney biopsy samples from patients with membranous glomerulonephritis confirmed increased LC3-positive autophagosomes in podocytes, compared with controls obtained from pretransplant allograft biopsies.⁵⁸ Accordingly, *Atg3*

mRNA expression was significantly higher in microdissected glomeruli obtained from kidney biopsy samples from patients with focal segmental glomerulosclerosis and membranous glomerulonephritis than in normal controls⁵⁸ (Fig. 3C). These findings demonstrate up-regulation of autophagy in podocytes in human proteinuric glomerular diseases. Correlative studies in GFP-LC3 transgenic mice, the administration of albumin (BSA)-overload resulted in a 3-fold increase in GFP-LC3 positive autophagosomes.⁵⁸ These data strongly support involvement of autophagy in podocyte injury response in proteinuric glomerular diseases.

Autophagy in diabetic nephropathy

Impaired autophagic activity is implicated in the pathogenesis of diabetic kidney disease and the therapeutic potential of targeting autophagic pathway in diabetic nephropathy is intriguing.⁶⁰ Reports in the 1990s revealed evidence of inhibition of cellular autophagy in streptozotocin (STZ)-induced diabetes in rats by electron microscopy analysis.^{61,62} The volume and number of autophagic vacuoles were significantly lower in proximal⁶¹ and distal tubules⁶² of diabetic rats. The inhibition of autophagy was partially reversed by insulin treatment or islet transplantation in the diabetic rats.^{61,62} Recent studies showed that the levels of p62/SQSTM1 were significantly elevated, indicating impaired autophagy in the kidneys of mice with STZ-induced diabetes⁶³ and Wistar fatty (fa/fa) rats,⁶⁴ which are models of type 1 and type 2 diabetes, respectively.

Impairment of chaperone-mediated autophagy has also been implicated to play a role in diabetic kidney disease. The abundance of proteins containing the chaperone-mediated autophagy KFERQ signal motif and the abundance of substrates carrying the KFERQ motif were increased in the renal cortex of STZ-induced diabetic rats vs. control non-diabetic rats.⁶⁵ The levels of M2 isoform of pyruvate kinase (M2PK) and GAPDH, two representative proteins degraded by chaperone-mediated autophagy, were elevated in the diabetic rats. In addition, the lysosomal enrichment of molecular chaperone heat shock cognate protein of 73 kD (hsc-73) and lysosomal-associated membrane protein 2a (LAMP2a) were decreased in renal cortical lysosomes of diabetic rats.⁶⁵ Thus, there was a decrease in proteins which regulate chaperone-mediated autophagy, and an increase in target proteins degraded by this pathway, indicating that this form of autophagy was impaired in diabetes and may contribute to the accumulation of certain proteins in diabetic-induced renal hypertrophy.

Silent information regulator T1 (SIRT1) is an NAD⁺-dependent deacetylase that is known to function as an intracellular energy sensor, to detect the concentration of NAD⁺ and controls in vivo metabolic changes under caloric restriction and starvation. Like the prototypic energy sensor AMP-activated protein kinase (AMPK), SIRT1 is activated under low-energy conditions, and negatively regulates mTOR, thereby inducing autophagy and renoprotection.⁶⁶ In diabetes, decreased activities of AMPK and SIRT1, noted in the glomeruli of diabetic patients and in the kidneys from experimental type 1 and type 2 diabetic animals, are thought to contribute to diabetic kidney injury, such as renal hypertrophy, lipid accumulation, and albumin excretion.⁶⁶ Restoration of AMPK and SIRT1 activities by AMPK activators, such as resveratrol, and caloric restriction results in induction of autophagy and ameliorates diabetic kidney injury.^{66,67} Thus, together with strategies to AMPK and SIRT1 may be an attractive therapeutic target aimed against diabetic kidney disease.

Autophagy in endothelial cell-induced fibrosis

Although the glomerular endothelial cells are important components of the glomerular filtration unit, together with the podocytes and mesangial cells, and in addition to their

contribution to glomerular barrier function, the renal microvasculature plays key roles in physiological processes such as the regulation of vasomotor tone, vascular permeability, leukocyte recruitment, and antithrombogenic responses. Renal endothelial dysfunction, therefore, leads to kidney dysfunction and may contribute to progression of CKD and renal fibrosis. In this regard, the renal endothelium has garnered much interest, however, its role(s) in the initiation and the progression of renal fibrosis remains poorly understood. Akin to EMT in tubular epithelial cells, the concept that endothelial cells may also acquire functional and structural characteristics of mesenchymal cells, called Endothelial-Mesenchymal Transition (EndoMT), following tissue injury.⁶⁸

Recent studies in glomerular endothelial cells have demonstrated that the process of autophagy in regulating certain protein degradation may influence endothelial function. BAMBI (BMP and Activin Membrane Bound Inhibitor) is a decoy TGF- β receptor, and its transduction significantly inhibits TGF- β /Smad signaling.⁶⁹ It was noted that BAMBI localizes to endothelial cells in the kidney and BAMBI protein is regulated by lysosomal and autolysosomal degradation, resulting in relatively quick turnover of BAMBI.⁷⁰ Serum starvation as an inducer of autophagy caused marked BAMBI degradation, which could be totally prevented by inhibition of lysosomal and autolysosomal degradation with Bafilomycin, and partially by inhibition of autophagy with 3-MA, but not by proteasomal inhibitors. Rapamycin activates autophagy by inhibiting mTOR, and resulted in BAMBI protein degradation. Both serum starvation and rapamycin increased the conversion of the autophagy marker LC3 from LC3-I to LC3-II and also enhanced co-staining for BAMBI and LC3 in autolysosomal vesicles⁷⁰ (Fig. 4). Thus, autophagy regulates turnover of BAMBI protein, and owing to its endothelial localization in the kidney, BAMBI may function as a regulator of TGF- β /Smad signaling in endothelial cells, and hence modulate TGF- β actions, and thereby fibrosis.

CONCLUSION

It is becoming increasingly evident that autophagy is important in many biological processes, and dysregulation of autophagy leads to the pathogenesis of kidney diseases. Induction of autophagy may constitute an adaptive mechanism to kidney injury. Many studies demonstrate a link between dysregulated autophagy and fibrotic disease, and support the paradigm that autophagy is a cytoprotective mechanism. Autophagy is indispensable for stress adaptive responses in kidney injuries, and by removal of protein aggregates and damaged organelles, promotes cell survival and anti-fibrotic actions by negatively regulating and preventing excess matrix protein accumulation in the kidney. Nonetheless there are also studies that suggest autophagy being involved in promoting fibrosis. The role of autophagy in disease pathogenesis is likely multifaceted and complex. TGF- β 1 is a prototypic multifunctional cytokine capable of dual functions, as both an inducer of collagen synthesis and an inducer of autophagy and collagen degradation. Future investigations are necessary to further advance our understanding of the functions of autophagy, and to determine whether autophagy may be a new therapeutic target to prevent or mitigate pathogenesis of kidney fibrosis.

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Fig. 1.

Autophagy in mesangial cells. (A) TGF- β 1 signals via the MKK3/p38 to enhance type I collagen mRNA and protein (Col-I α 1 and Col-I, respectively) in mesangial cells. TGF- β 1-induced autophagy negatively regulates matrix production in mesangial cells by promoting degradation of intracellular type I collagen (Col-I). Treatment with inducers of autophagy, trifluoperazine (TFP) and low-dose carbon monoxide (CO), also results in decreased type I collagen protein levels induced by TGF- β 1, without alterations in collagen (Col-I α 1) mRNA. (B) Treatment with autophagy inhibitor 3-MA and genetic knockdown of *Atg7* decrease Col-I, α -SMA and -PDGFR protein expression in mouse mesangial cells.

Fig. 2.

Autophagy and tubular interstitial fibrosis. (A) TGF-1 overexpression in tubular epithelial cells induces autophagy in vivo and in vitro, through ROS, resulting in tubulointerstitial fibrosis, tubular apoptosis and decomposition. (B) Autophagy is activated in a model of renal fibrosis induced by unilateral ureteral obstruction (UUO), and is associated with inhibition of tubular apoptosis and interstitial fibrosis in the obstructed kidney, and reduces cell proliferation in the contralateral kidney. Antioxidant sulforaphane preserves mitochondrial function and suppresses UUO-induced renal oxidative stress, inflammation, fibrosis, autophagy, apoptosis, and pyroptosis. (C) Long-term use of Cyclosporine causes tubular atrophy, interstitial fibrosis and glomerulosclerosis and impairs renal function. Autophagy is activated via induction of ER stress by cyclosporine and protects tubular cells from cell death.

Fig. 3.

Autophagy and glomerulosclerosis. (A) In podocyte-specific *Atg5* knockout mice (*Atg5^{podocyte}*), compensatory proteasome activity decreases with aging, leading to severe proteinuria, loss of podocytes, and glomerulosclerosis. Knockdown of ATG5 in podocyte increases susceptibility to drug puromycin (PAN)-, adriamycin (ADR)-, albumin (BSA)-, and lipopolysaccharide (LPS)-induced glomerular injury. (B) Podocyte-specific *Vps34* knockout mice (*Vps34^{podocyte}*) develop significant glomerulosclerosis, interstitial fibrosis, severe albuminuria, and podocyte loss through inhibiting the formation of phagophore and the mTOR pathway. (C) Autophagy is activated in podocytes in both human proteinuric glomerular diseases and in experimental model of albumin (BSA)-overload-induced glomerular injury.

Fig. 4.

Autophagy and endothelial cell-induced fibrosis. BAMBI localizes to the endothelial cells in the kidney, and autophagy regulates turnover of BAMBI protein. Both serum starvation and rapamycin induce BAMBI protein degradation through activation of autophagy. BAMBI may function as a regulator of TGF- β /Smad signaling in endothelial cells, and hence modulate TGF- β actions, and thereby fibrosis.