



## REVIEW ARTICLE

## Autophagy and Transforming Growth Factor Beta1 in Renal Tubular Epithelia Cells

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### Abstract

Transforming growth factor beta1 (TGF- $\beta$ 1) is regarded as the important factor in many fibrotic kidney diseases. Autophagy is a vital mechanism which maintains intracellular homeostasis in eukaryotic cells and involves in various renal physiologic and pathological processes. Current studies indicate that autophagy in renal tubular epithelial cells serves as a renoprotective mechanism which modulates the course of diverse kidney disease. Thus, this review aims to show the possible linkage between TGF- $\beta$ 1 and autophagy in the renal tubular epithelial cells.

### Introduction

Kidneys are essential organs in human body and play a crucial role in modulating body fluids and blood pressure, excreting waste production, promoting erythropoiesis, etc. Kidney diseases have become a worldwide healthy issue with high morbidity and mortality. Tubular epithelial cells apoptosis, renal interstitial fibrosis and renal interstitial inflammatory response are common pathological processes in acute kidney injury and chronic kidney diseases. These pathologic processes always result in progressive loss of renal function or even complete loss.

Autophagy is a highly conserved homeostatic mechanism for cell survival under conditions of stress and catabolic process in which a double-membrane structure, named autophagosome, sequesters and delivers long-lived proteins, cellular macromolecules, and intracellular damaged organelles to the lysosome

for degradation. Free amino acids and fatty acids generated on degradation of cellular components are recycled to synthesize new proteins and bioenergetic supplies of the cell. Thus, under normal physiological conditions, basal autophagy plays a homeostatic role that maintains cellular homeostasis and quality control. Autophagy is induced in response to many stressors including cell starvation, growth factor deprivation, hypoxia, and oxidant injury. Under stress conditions, autophagy induction is regarded as an adaptive role to ensure cell survival. The formation of an autophagosome is initiated by several autophagic protein complexes, including the unc-51-like kinase 1 or 2 (ULK1 or ULK2) complex, the class III phosphatidylinositol 3-kinase complex, Atg12-Atg5-Atg16 conjugation, and lipidation of microtubule-associated protein 1 light chain 3 (LC3) with phosphatidylethanolamine to form LC3-II. The elongation and expansion steps in autophagosome formation involve two ubiquitin-like proteins, Atg12 and Atg8/LC3-II. The conjugation of Atg12 to Atg5 is catalyzed by Atg7 and Atg10 (E1- and E2-like enzymes, respectively) to form covalently linked Atg12-Atg5. Following formation of the Atg12-Atg5 conjugate, Atg16L non-covalently associates with this conjugate to produce the Atg12-Atg5-Atg16 multimeric complex. These conjugation systems are recruited to the phagophore membrane for phagophore expansion to complete the formation of the autophagosome. Once the autophagosome is formed, most of the Atg proteins are dissociated, which allows fusion with the lysosome

to form the autolysosome. LC3-II, a marker of autophagic flux, remains present in both the membranes of the autophagosome. The sequestered contents and the inner membrane of the autolysosome are degraded by the lysosomal hydrolases.

Recent studies suggested that autophagy is a protective mechanism that helps renal intrinsic cells to survive in response to various stressors, defends kidneys against elements of nephrotoxicity, pro-fibrotic factors and inflammatory response injuries under pathological condition and plays a renoprotective role in acute kidney injury and chronic kidney diseases.

### **TGF- $\beta$ 1 Plays a Crucial Role in the Renal Fibrosis**

Renal fibrosis is the main feature of chronic kidney disease (CKD) in despite of the initial causes. Transforming growth factor beta (TGF- $\beta$ ) has extensive biological functions in different cell types, and is a critical mediator in the course of renal fibrosis [1,2]. TGF- $\beta$ 1 can be secreted by all kinds of renal cells. Growing evidence has indicated a crucial role of TGF- $\beta$ 1 in renal fibrosis in both experimental and human kidney diseases. TGF- $\beta$ 1 is significantly up-regulated in the fibrotic kidney diseases [1,3]. Overexpression of mature TGF- $\beta$ 1 in rodent liver promoted the progression of fibrosis in kidneys, revealing a functional importance of TGF- $\beta$ 1 in CKDs [4,5]. Recent studies further confirmed that blockade of TGF- $\beta$ 1 with neutralizing TGF- $\beta$  antibodies or antisense oligonucleotides significantly ameliorates renal fibrosis in vivo and in vitro [6]. These findings strongly suggested a pro-fibrotic effect of TGF- $\beta$ 1 in the fibrotic kidney diseases.

### **TGF- $\beta$ 1 Induced Autophagy and Led to Epithelia Decomposition and Apoptosis in the Renal Tubular Epithelial Cells**

Yanfang Xu, et al. investigated the effects of exogenous TGF- $\beta$ 1 on cultured human renal proximal tubular epithelial cells (HRPTEpiCs) and found that TGF- $\beta$ 1 induced up-regulation of autophagy-related genes, Atg5, Atg7 and Beclin1 and accumulation of autophagosomes in a time- and dose-dependent manner. Furthermore, TGF- $\beta$ 1 activated autophagy by generating ROS and promoted apoptosis in tubular cells as indicated by elevated epithelia apoptosis rate, enhanced expression of the pro-apoptotic gene Bim and reduced expression of the anti-apoptotic gene Bcl-2 in HRPTEpiCs incubated with TGF- $\beta$ 1 [7].

Koesters, et al. found that tubular overexpression of TGF- $\beta$ 1 induced autophagy and resulted into the development of tubulointerstitial fibrosis, without evidence of epithelial-mesenchymal transition (EMT) [8].

Huang C, et al. found that TGF- $\beta$ 1 increased the formation of autophagic vacuoles, LC3 expression in the HK-2 cell [9].

These findings indicate that TGF- $\beta$ 1 induces autophagy in renal tubular epithelial cells and mediate renal fibrosis.

### **Activation of Autophagy Attenuates Renal Tubulointerstitial Fibrosis**

In many studies, the unilateral ureteral obstruction (UUO) model is a classical model of progressive renal interstitial fibrosis with increased autophagy, apoptosis and necrosis in the tubules [10-15]. Kim WY, et al. found that autophagy was induced through Akt-mammalian target of rapamycin (Akt-mTOR) signaling pathway and inhibition of autophagy by 3MA enhanced tubular cells apoptosis and deteriorated tubulointerstitial fibrosis in the obstructed kidney after UUO [12]. In the study of Wu MJ and Wang S, Rapamycin, an effective inducer of autophagy in many kinds of cells, significantly mitigated the progress of renal interstitial fibrosis, included reducing scores for tubular dilatation, interstitial collagen deposition and alpha-smooth muscle actin in renal interstitial [16,17]. Yan Ding, et al. found that autophagy regulated TGF- $\beta$  expression and suppressed kidney fibrosis induced by ureteral obstruction via using different kinds of transgenic mice. Their study shows that autophagy was induced in renal tubular epithelial cells of obstructed kidneys in the GFP-LC3 transgenic mice after UUO. Furthermore, both deletion of LC3B (LC3<sup>-/-</sup> mice) and beclin1 heterozygous (beclin1<sup>+/-</sup> mice) led to an increase in the collagen deposition and mature pro-fibrotic factor TGF- $\beta$  levels in the obstructed kidneys after UUO [18]. These studies indicate that autophagy plays a vital renoprotective role in alleviating tubular damage and kidney fibrosis.

### **Autophagy in Renal Tubular Epithelial Cells Attenuates Kidney Fibrosis through Degrading TGF- $\beta$ 1**

Yan Ding, et al. found that autophagy regulated the TGF- $\beta$  expression and suppressed kidney fibrosis induced by ureteral obstruction. Their study shows that mature TGF- $\beta$  but not pro-TGF- $\beta$  levels were significantly increased in the obstructed kidneys of LC3<sup>-/-</sup> mice compared with wild-type LC3<sup>+/+</sup> mice after UUO. Furthermore, LC3 deficiency resulted in an increased expression of mature TGF- $\beta$  in primary RTECs. Using bafilomycin A1 to inhibit the degradation of autolysosomal protein elevated the mature TGF- $\beta$  protein levels without alterations in TGF- $\beta$ 1 mRNA [18]. What's more, recent studies implicated that dysfunctional autophagy in disorders characterized by fibrosis in various tissues, including cardiac fibrosis, liver fibrosis, and idiopathic pulmonary fibrosis (IPF) [19]. These findings suggest that autophagy may play a novel protective role in renal fibrosis by negatively regulating the production of mature TGF- $\beta$  proteins in the renal epithelial cells, and in turn reducing the TGF- $\beta$  secretion and delaying the progress of interstitial fibrosis in kidney injury.

These studies strongly indicated a bidirectional regulated mechanism in which TGF- $\beta$  is capable of inducing autophagy in renal tubular epithelial cells and the activated autophagy, in turn, modulates the generation and secretion of TGF- $\beta$  in epithelia. Thus, the negative regulation of autophagy on the generation and secretion of TGF- $\beta$  may provide a novel strategy in curing or delaying kidney fibrosis.

## Conclusions and Perspectives

Autophagy, a highly conservative and important intracellular degradation pathway, plays a vital role in maintaining intracellular homeostasis in glomerulus and tubule cells and is closely associated with kidney damage, age and diseases. Autophagy is an essential adaptive mechanism in acute and chronic kidney injury. In present review, autophagy was induced by exogenous and endogenous TGF- $\beta$ , on the other hand, autophagy modulates the generation and secretion of TGF- $\beta$  in renal tubule epithelial cells and alleviates renal tubulointerstitium fibrosis. These findings indicated that autophagy and the TGF- $\beta$  could affect each other in the renal tubular epithelial cells under different pathogenic conditions. However, the renal tubular epithelial cells are not only a victim but also an abuser, because renal tubular epithelial cells can generate various cytokines, some are benefited and some are harmful, in acute and chronic kidney diseases. The linkage between autophagy and cytokines, including IL-1, IL-6, IL-8, IL-10, IFN and TNF, could influence the prognosis of the renal tubular epithelial cells in the pathogenesis of nephropathy. Understanding these linkages may provide new therapeutic strategy in the treatment of acute and chronic kidney diseases.

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