

# The activity and role of autophagy in the pathogenesis of diabetic nephropathy

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**Abstract. – OBJECTIVE:** Evidence suggested that deficiency of autophagy is involved in the pathogenesis of diabetic nephropathy (DN). However, some recent studies have also shown that autophagy is activated in renal cells under diabetic conditions. In this review, we discuss whether autophagy is inactivated in renal cells in DN as well as the therapeutic potential of autophagy for treating DN, in order to aid future investigation in this field.

**MATERIALS AND METHODS:** Relevant information, original research articles and reviews, were gathered primarily through a search in PubMed and Cochrane database. The activity and role of autophagy, as well as the relevant signaling pathways, were analyzed in different intrinsic renal cells, including podocyte, renal tubular epithelial cell, glomerular mesangial and endothelial cells.

**RESULTS:** The upstream of autophagic pathway, but not whole pathway, was predominately studied in these intrinsic renal cells, such as the induction of autophagy, an amount of autophagic vacuoles and so on. In most cases, autophagic inactivation occurred, which is an important mechanism underlying DN progression. Targeting the autophagic pathway to activate autophagy activity might have renoprotective effect. However, autophagic activation was also found in a few studies, in which there was a debate on the role of activated autophagy: mounting an adaptive response or leading to autophagic apoptosis.

**CONCLUSIONS:** The downstream of autophagic pathway, including the degradation of autophagic vacuoles, and lysosomal function, should be well studied to clarify the activity and role of autophagy in the progression of DN. Autophagy activation is likely a potential therapy for combatting DN. Diabetic nephropathy (DN) is a key chronic complication of diabetes mellitus and is the main cause of death and disability

in diabetic patients. Multiple therapeutic methods have failed to fully prevent the progression of DN. With the increasing prevalence of diabetes, the demand for a new therapeutic target to prevent DN has become increasingly urgent. Autophagy is a catabolic process that degrades damaged proteins and organelles and recycles macromolecules, thereby playing a critical role in the maintenance of cellular homeostasis. Autophagy has become a hot topic in the field of kidney disease and research as, until recently, the evidence suggested that deficiency of autophagy is involved in the pathogenesis of DN and that targeting the autophagic pathway to activate autophagy activity may have a renoprotective effect. Indeed, the majority of studies show that autophagic activity is suppressed under diabetic conditions. However, some recent studies have also shown that autophagy is activated in renal cells under diabetic conditions.

In this review, we discuss whether autophagy is inactivated in renal cells in DN as well as the therapeutic potential of autophagy for treating DN, in order to aid future investigation in this field.

*Key Words:*

Autophagy, Diabetic nephropathy, Podocyte, Renal tubular epithelial cell, Mesangial cell, Glomerular endothelial cells.

## Introduction

With the increasing incidence of diabetes mellitus (DM), the prevalence of diabetic nephropathy (DN) continues to rise worldwide. DN is a serious complication of DM and is a leading cause of endstage re-

nal disease (ESRD) on a global level<sup>1</sup>. The number of diabetes worldwide is up to 347 million<sup>2</sup>, and about 25% to 40% of diabetics will develop diabetic nephropathy (DN)<sup>3</sup>. Current therapies for DN include controlling blood pressure and blood glucose levels as well as inhibiting the renin angiotensin system (RAS) in order to reduce or abrogate proteinuria<sup>4-6</sup>. While these strategies have some effect with regard to managing DN, results are far from satisfactory and many patients experience a progressive decline in kidney function leading to ESRD. Thus, the identification of new and effective therapeutic targets is a top priority for the prevention and control of ESRD. Recent studies have focused on autophagy as it plays a role in the stress-responsive machinery, the disturbance of which is involved in the pathogenesis of age- and diabetes-related diseases<sup>7,8</sup>. Autophagy is involved in catabolic processes and plays a key role in the degradation of damaged intracellular proteins and organelles in order to maintain intracellular homeostasis and cell integrity<sup>9</sup> in both normal and diseased states; including immunity, inflammation, adaptation to stress<sup>10</sup>, development and aging, metabolic and neurodegenerative disorders<sup>11</sup>, and cancer<sup>12-14</sup>. Increasing evidence implies that autophagy activity is altered in certain organs under conditions of obesity<sup>15-18</sup> and the functional role of autophagy in the kidney has gradually begun to be identified. It has been reported that autophagy may play a renoprotective role in various animal models, including those used to study aging and acute kidney injury<sup>19-23</sup>. In recent years, autophagy has become a hot topic in the field of kidney disease, suggesting that it is likely to be a key target for the prevention and treatment of DN, although the activity of autophagy in the renal cells and changes in the autophagy-lysosomal system during DN is still under debate. Therefore, in this review we discuss the relationship between autophagy activity and DN.

### **Autophagy**

It is well known that cells initiate “self-eating” in order to degrade damaged proteins and organelles, as well as to recycle intracellular energy resources for the maintenance of cellular homeostasis under stressful conditions (such as nutrient starvation and hypoxia). The most important mechanism in this process is autophagy and three major types have been identified: macroautophagy, microautophagy, and chaperone-mediated autophagy. In this review, macroautophagy is referred to as autophagy as it occurs widely across the cellular degradation/recycling system. As illustrated by Klionsky et al<sup>24</sup>, autophagy is a dynamic process with multiple links.

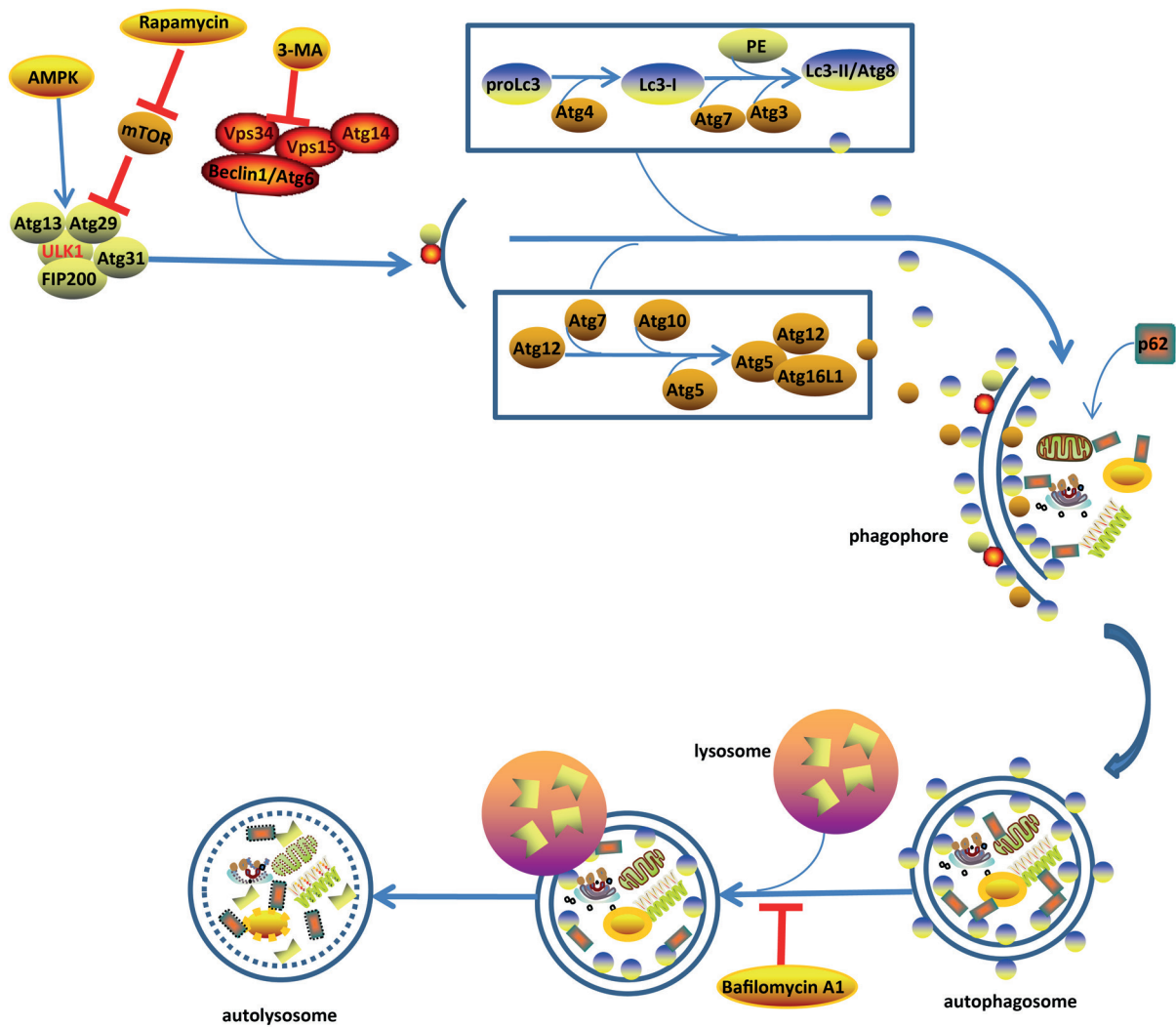
Autophagy is initiated by the unc-51-like kinase (Ulk) 1 complex (the mammalian ortholog of yeast Atg1), which comprises the Ulk1 Ser/Thr protein kinase, Atg13, and FIP200 (mammalian homolog of yeast Atg17). Ulk1-derived phosphorylation of Atg13 and FIP200 is essential for triggering autophagy. Phagophore nucleation is dependent on beclin 1 (Atg6 in yeast), an hVps34 or class III phosphatidylinositol 3-kinase complex consisting of hVps34, hVps15, beclin 1, and Atg14<sup>25-29</sup>. The formation of the phagophore, also known as the isolation membrane, occurs around cytoplasmic components to be sequestered by double-membrane autophagosomes forming at the endoplasmic reticulum (ER)-mitochondria contact site in mammalian cells<sup>30</sup>. LC3-II formation is recognized as a marker for autophagosomes in both cellular and animal experiments<sup>24,31,32</sup>. After formation, autophagosomes fuse with lysosomes to form autolysosomes. The protein p62, also known as sequestosome 1, localizes to autophagosomes via LC3 interaction and is constantly degraded by the autophagy-lysosome system. Indeed, accumulation of p62 is observed in autophagy deficient cells<sup>33,34</sup>. All of these processes form an integrated autophagy-lysosome pathway and each link may affect autophagy flux and activity should anything go awry, given the complexity of the process (Figure 1). Indeed, complications in the execution of this process have been linked to numerous pathological conditions, including neurodegeneration, aging, and cancer<sup>7,35-37</sup>.

### **Autophagy Activity in Kidney Cells**

Given the prevalence of DN, the relationship between this condition and autophagy has received much attention in recent years. Investigators initially conducted preliminary research to study changes to the autophagy pathway during DN and found that p62/Sequestosome 1 (SQSTM1), a substrate of the autophagy-lysosomal degradation pathway, was significantly increased in the renal tissue of animals with DN<sup>38</sup>. Furthermore, Atg5, Atg8, beclin 1 and LC3 mRNA level were found to be dramatically reduced in the renal tissue of DN patients in a study with a small sample size<sup>39</sup>, suggesting that autophagy activity declines in certain renal inherent cells and that DN plays a role in inhibiting autophagy induction. However, identification of the type of cells involved in this decline of autophagy activity remains controversial.

### **Podocytes**

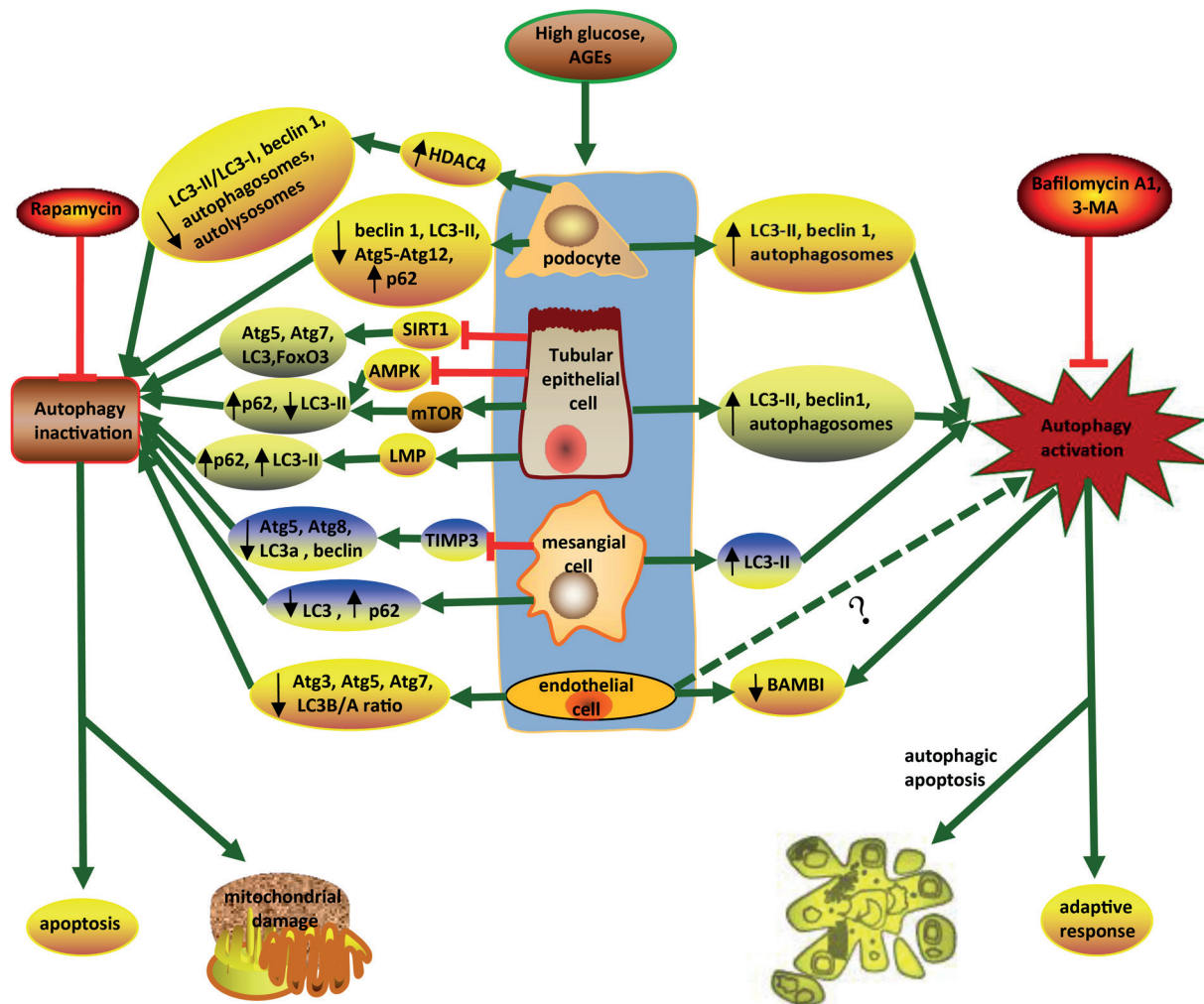
Under normal physiological conditions, podocytes exhibit high basal levels of autophagy



**Figure 1.** Schematic diagram of autophagy pathway.

gy (much higher than that of other renal cells), indicating a key role for autophagy in the maintenance of podocyte homeostasis under non-stressful conditions<sup>40</sup>, leading to additional studies on podocytes autophagy. Certain reports have found that autophagic activity in podocytes under streptozotocin (STZ)-induced type 1 diabetic or high glucose conditions decreases with reduced levels of autophagy-related protein expression, including beclin-1, LC3-II and the Atg5–Atg12 complex<sup>15,41–45</sup>, and that defective autophagy may be restored by HDAC4 knockdown<sup>43</sup>, rapamycin<sup>44,45</sup>, and taurine-conjugated derivatives (TUDCA)<sup>41</sup>. In addition, Li and Siragy<sup>46</sup> showed deficient autophagy in podocytes with LC3II decline and accumula-

tion of p62. These results suggest that hyperglycaemia reduces autophagic activity in podocytes. However, increased expression of the autophagy-related protein LC3-II, beclin-1, and autophagosomes in podocytes during high glucose treatment has also been reported<sup>47,48</sup>, which is indicative of high glucose-promoted autophagy in podocytes (Figure 2). Similar results on primary podocytes and podocyte cell lines have been shown by the Lenoir et al<sup>49</sup> and Dong et al<sup>50</sup>. Wei et al<sup>48</sup> further showed that high glucose promotes autophagy in podocytes with accumulation of autophagosomes and that this effect is further enhanced by bafilomycin A1, a specific inhibitor of vacuolar-type H (1)-ATPase that inhibits acidification and protein degradation



**Figure 2.** Overview of autophagic activity and role in renal inherent cells under diabetic conditions. Autophagy inactivation was reported in most of the studies by assessing autophagic induction and autophagy substrate. However, autophagy activation was also implicated in a few investigations, as evidenced by an up-regulation of beclin 1 and an accumulation of autophagosomes under diabetic condition.

in the lysosomes of cultured cells. However, the effect may be inhibited by 3-methyadenine, proving that enhanced autophagy results from augmentation of autophagic flux and not from prohibition of autophagosome-lysosome fusion.  
*Renal Tubular Epithelial Cells*

In contrast to that observed in podocytes, tubular epithelial cells (TECs) under basal conditions show a low level of autophagy. The increased p62 in the proximal and distal tubular cells of both types 1 and 2 diabetic animals has previously been shown in several assays<sup>38,51,52</sup>. Also, accumulation of p62 protein and hyperactivation of mTORC1 have been observed in proximal tubular cells under diabetic conditions and from patients with type 2 DM, indicating

a deficiency in autophagy<sup>53</sup>. Furthermore, LC3-II expression is significantly reduced in tubules under STZ-induced type 1 diabetic conditions or high glucose treatment. Indeed, not just reduced autophagy but increased mitochondrial fragmentation under high glucose conditions has been observed in tubular cells in both *in vitro* and *in vivo* studies<sup>54</sup>, suggesting that impairment of the autophagy system induces mitochondrial damage. Similar to the results observed in the afore-mentioned studies, our recent investigation showed that both LC3-II and p62 were significantly enhanced after exposure of HK-2 cells to advanced glycation end products due to lysosomal membrane permeabilization (LMP) and lysosomal dysfunction<sup>55</sup>, suggesting that the

accumulation of autophagosomes resulted from the decreased lysosomal degradation in renal tubules<sup>56</sup>. Both hyperactivation of the mTOR pathway and inhibition of the AMPK pathway play an important role in autophagy deficiency in DN<sup>57</sup>. Sirt1, an important autophagy-activating regulator via directly deacetylating Atg5, Atg7, Atg8 and FoxO3, is also down-regulated in DN<sup>58-60</sup>. In contrast, levels of LC3-II, autophagosomes and beclin1 in HK-2 cells as well as the kidneys of diabetic rats have been shown to be significantly up-regulated, leading the author of that particular study to conclude that high glucose conditions may activate the autophagy pathway<sup>61</sup> (Figure 2). However, it is well known that an accumulation of autophagosomes does not definitively indicate autophagy induction and may represent inhibited maturation of autolysosomes. The downstream of autophagic pathway should therefore be studied in order to validate these results.

#### *Glomerular Mesangial Cells*

Relatively speaking, autophagy activity in mesangial cells is the most controversial of all the intrinsic renal cell types. Several studies have shown that high glucose conditions inhibit rat mesangial cell I autophagy by upregulating p62/SQSTM1 and downregulating LC3 expression at 24 h<sup>62</sup> and 72 h<sup>63,64</sup>, and that insufficient autophagy can be attenuated with the use of ursolic acid<sup>62</sup> and rapamycin<sup>64</sup>. It has been reported that TIMP3 (a type of tissue inhibitor) expression is reduced in the renal cells of both STZ-induced diabetic mice and patients with DN, and that TIMP3 knockdown mesangial cells showed decreased expression of the autophagy genes Atg5, Atg8, Lc3a and beclin as well as Foxo1 and FoxO3 expression, suggesting that TIMP3 deficiency under diabetic conditions could suppress autophagy<sup>39</sup>. These results indicate that DN inhibits the mesangial cells autophagy pathway. However, other studies have reported that the expression of LC3-II is significantly increased in sections of diabetic kidneys or mesangial cells treated with high glucose and that mesangial cell autophagy is in fact activated under diabetic conditions<sup>65</sup> (Figure 2).

**Glomerular Endothelial Cells** While glomerular endothelial dysfunction is an important characteristic of DN, few studies have previously investigated the role of autophagy in glomerular endothelial cells under diabetic conditions. It has been reported that high glucose decreases the expression of Atg5, Atg7, Atg3 and consequently the LC3B/A ratio, thus inhibiting autophagy induction<sup>66</sup>. The

most commonly reported component of glomerular endothelial cell autophagy is bone morphogenetic protein and activin receptor membrane-bound inhibitor (BAMBI), the expression of which is suppressed in both human and murine models of DN<sup>67</sup>. As BAMBI is degraded by autophagosomal and autolysosomal processes<sup>68</sup>, an excessive degradation of BAMBI may result from autophagy activation in DN (Figure 2).

## Conclusions

When considering the afore-mentioned studies, it is clear that in most cases, autophagy is inactivated in DN. The body of evidence shows that impaired autophagy is involved in the pathogenesis of DN, suggesting that autophagy activation could be a potential therapy for combatting DN. However, activation of autophagy has also been reported. The root cause of this dispute must be clarified. While the underlying causes may vary, observing only the changes in Atg, LC3-II and beclin 1 is not sufficient for the evaluation of autophagy activity, as accumulation of autophagosomes and induction of autophagy does not fully represent activation of the autophagy pathway. As we know, both increased induction and inhibited degradation of autophagy could lead to an increase in autophagic vacuoles. Therefore, in order to better evaluate autophagic activity, improved assessment of the degradation process is required; including determining the efficiency of autophagosome fusion to lysosomes, the activity of lysosomal enzymes, and whether or not autophagic vacuoles can be digested and the protein effectively degraded. As the blocked autophagy pathway caused by lysosome dysfunction plays an important role in the pathogenesis of various diseases, including those related to the nervous system, the relationship between the autophagy-lysosome pathway and DN requires further exploration. In addition, whether autophagy activation is a safe therapy for DN, and the specific role it plays, must be defined.

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#### Conflict of Interest

The Authors declare that they have no conflict of interest.

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