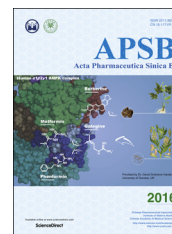




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REVIEW

5'-Monophosphate-activated protein kinase (AMPK) improves autophagic activity in diabetes and diabetic complications



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Abstract Diabetes mellitus (DM), an endocrine disorder, will be one of the leading causes of death world-wide in about two decades. Cellular injuries and disorders of energy metabolism are two key factors in the pathogenesis of diabetes, which also become the important causes for the process of diabetic complications. AMPK is a key enzyme in maintaining metabolic homeostasis and has been implicated in the activation of autophagy in distinct tissues. An increasing number of researchers have confirmed that autophagy is a potential factor to affect or induce diabetes and its complications nowadays, which could remove cytotoxic proteins and dysfunctional organelles. This review will summarize the regulation of autophagy and AMPK in diabetes and its complications, and explore how AMPK stimulates autophagy in different diabetic syndromes. A deeper understanding of the regulation and activity of AMPK in autophagy would enhance its development as a promising therapeutic target for diabetes treatment.

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Abbreviations: ACC, carboxylase; AdipoR, adiponectin receptors; ADP, adenosine diphosphate; AMP, adenosine monophosphate; AMPK, 5'-monophosphate-activated protein kinase; ATP, adenosine triphosphate; CaMKK, Ca²⁺ calmodulin-dependent protein kinase kinase; DEPTOR, DEP domain-containing mTOR-interacting protein; DM, Diabetes mellitus; DN, Diabetic nephropathy; ERK, extracellular signal-regulated kinase; FoxO, forkhead box class O; GFRs, glomerular filtration rates; IKK, IκB kinase; JLDG, Jinlida granule; JNK, janus kinase; LC3, light chain 3; LKB1, liver kinase B1; mTOR, mammalian target of rapamycin; mTORC1, mammalian target of rapamycin (mTOR) complex 1; PKC, protein kinase C; PRAS40, proline-rich Akt substrate 40 kDa; RAPTOR, regulator associated protein of mTOR; SOGA, suppressor of glucose form autophagy; SQSTM1, sequestosome 1; STZ, streptozotocin; TSC, tuberous sclerosis complex; ULK1, Unc-51-like kinase 1; VPS34, vacuolar protein-sorting 34

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1. AMP-activated protein kinases (AMPK) and autophagy

1.1. Introduction of AMPK

The heterotrimeric protein, AMPK, plays a pivotal regulatory role in cellular energy homeostasis and metabolism. This serine/threonine kinase is formed by a catalytic subunit α and two regulatory subunits β and γ ¹⁻³. Each subunit has its own isoforms and binds different ligands. As an example, for decreasing the activity of AMPK, adenosine triphosphate (ATP) could be bound with in an opposite manner, while adenosine diphosphate (ADP) could bind with the γ subunit for protecting AMPK *via* Thr-172 dephosphorylation^{1,4}. These AMPK subunit isoforms could combine or interact with each other, which might form about 12 heterotrimers in different tissues^{5,6}. Furthermore, activated AMPK, as an energy sensor, could block cellular proliferation and synthesis of cholesterol or fatty acid⁷, but stimulate glucose uptake and autophagy^{8,9}. Due to its significant sensor role in modulating pathways in catabolic processes, AMPK inhibition is correlated with endocrine metabolic conditions, including diabetes and its induced complications. Signaling pathways affected by inhibition AMPK have been studied extensively in diabetes and its complications, such as autophagy, mitochondrial biogenesis and energy regulation. In the present review, we will focus on the important role of AMPK and autophagy in diabetes and its complications.

1.2. Introduction of autophagy

Autophagy, a kind of intracellular recycling system, is the basic catabolism activated by lysosomes or autolysosomes, including degradation of dysfunctional or unnecessary cellular organelles and nutrients^{2,10}. It has been reported that autophagy plays an important role in the regulation of catabolic nutrients; when nutrients are abundant, insulin secretion would be stimulated. But when nutrients are lacking autophagy might be evoked at a cellular level¹¹. Autophagy could be induced by oxidative stress or any other cellular injuries and loss. There are three types of autophagy, involving macroautophagy, microautophagy and chaperone-mediated autophagy. The macroautophagy involving fusion with lysosomes is the common autophagy, which is also what we will review below.

Autophagy could be regulated by several factors and protein sensors, including amino acids or insulin. Autophagy induced by another condition of nutrient starvation like glucose deprivation is less common¹², but they are all associated with signaling pathways. Generally, besides the positive effects supported by few researches, negative effect, inhibited by mammalian target of rapamycin (mTOR) complex 1 (mTORC1) seems to be more related to glucose deprivation of autophagy¹³. It should be clarified that mTORC1 is a signaling complex, which involves mTOR, RAPTOR (regulator associated protein of mTOR), PRAS40 (proline-rich Akt substrate 40 kDa), DEPTOR (DEP domain-containing mTOR-interacting protein) and LST8 homolog (mTOR associated protein)¹⁴. The upstream regulatory factors respond to ATP depletion (AMPK, P53), reactive oxygen species [janus kinase (JNK), extracellular signal-regulated kinase (ERK)] and directly induction of glucose deprivation [forkhead box class O (FoxO), I κ B kinase (IKK)]. Furthermore, the direct up-regulatory kinase could be serine/threonine protein kinase Unc-51-like kinase 1 (ULK1) and Beclin1-BCL2 complex. For instance, ULK1 could be phosphorylated at Ser317 and Ser777 to induce autophagy. And

Beclin1 could also be phosphorylated to block the forming of the Beclin1-BCL2 complex, which would inhibit autophagy. Likewise, microtubule-associated protein 1 A/1B-light chain 3 (LC3) is an autophagosomal marker and one of the important measurement indicators, which could reflect the status of autophagic activity induced by starvation⁵. Thus, AMPK and autophagy will be summarized with regard to the mechanism of upstream and downstream modulation in classic pathways.

1.3. The mechanism of AMPK-regulated autophagy

AMPK is a major activator of autophagy in the catabolic process of oxidative stress and energy starvation. Their modulation has been concluded in a few classic pathways. In previous studies autophagy was just thought to play a dual role in either avoiding cell death in some conditions or inducing autophagic death¹⁵. After a few years, it has been linked with protein kinase-AMPK activation directly and indirectly. AMPK could activate autophagy by directly activating ULK1. Specifically, AMPK and mTORC1 are both initiators of autophagy *via* ULK1 activation⁹. AMPK could active ULK1 by phosphorylation not only at Ser317, Ser777, but also at Ser467, Ser555, Ser637 and Thr574⁸, while ULK1 is inhibited by mTORC1 phosphorylation at Ser317 and Ser777. To some extent, AMPK activity could be suppressed by mTORC1 *via* increased phosphorylation of ULK1 at Ser757^{9,14}. AMPK could activate autophagy indirectly by tuberous sclerosis complex 2 (TSC2) phosphorylation and subsequent mTORC1 inhibition¹⁶ (Fig. 1). Moreover, activation of AMPK also could stimulate JNK1 following dissociation of Beclin-1 from BCL-2 to form vacuolar protein-sorting 34 (VPS34) complex¹⁷ and turn on autophagy. Other studies also reported that AMPK modulates FoxO transcription factor expression, which leads to expression of autophagy-associated genes¹⁴. Thus, AMPK is a key junction as both an upstream energy sensor and a downstream autophagy activator, especially in endocrine disordered diseases such as diabetes.

2. The regulation of autophagy by AMPK in diabetes and its complications

2.1. AMPK and autophagy in pancreatic β cells

The pancreatic β cell in pancreatic islets is a significant glucose regulatory cell regard to insulin secretion. Pancreatic islets are also correlated with lipid metabolism. When disordered pancreatic islet or dysfunctional pancreatic β cells occur, it is always related to abnormal glucose and lipid levels¹⁸. Additionally, Dong and Czaja or Singh et al.^{19,20} have reported that intracellular lipid droplets might be one substrate of autophagy. In the process of lipid overloading, autophagy could metabolize lipid by moving the unnecessary part. Because activated autophagy might not only alleviate endoplasmic reticulum stress, but also restart the process of lipophagy for pancreatic islets protection²¹. Cellular level studies in pancreatic β cells also show that autophagy plays a pivotal role. The relative pathway might contain LC3, PKC (protein kinase C), JNK as well as others²²⁻²⁴. Moreover, in normal conditions, metformin could suppress Min6 β cell proliferation and promote cell apoptosis through an AMPK independent and autophagic mechanism. However, as an effective anti-diabetic drug, metformin could protect pancreatic β cells in particular from the apoptosis induced by palmitic acid²². Another

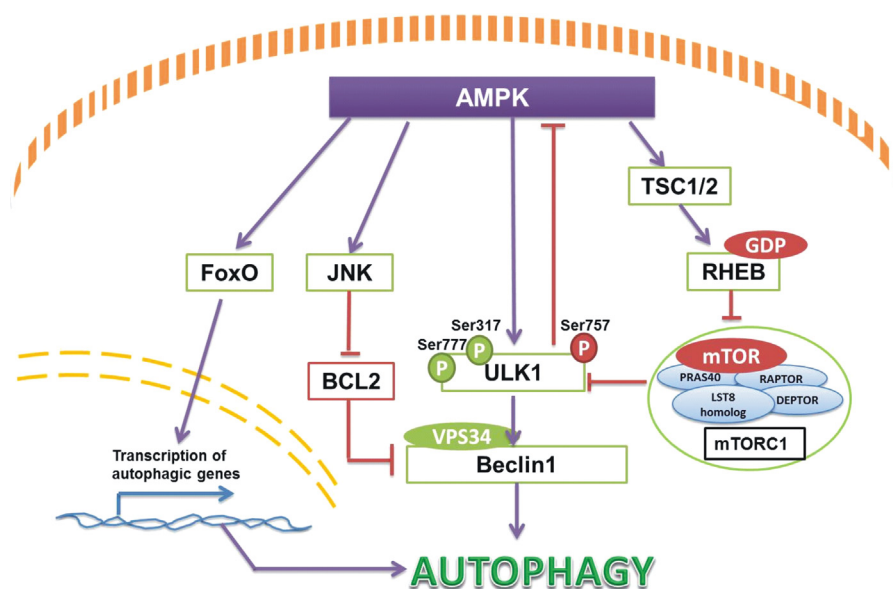


Figure 1 Autophagy stimulated by AMPK activation contains four normal pathways found till now. When energy is insufficient, AMPK could phosphorylate JNK, ULK1, TSC1/2 and FoxO transcription factor to activate autophagy in many tissues generally. And the upstream pathways in autophagy activity regulation also contain BCL2 inhibition, Beclin1 stimulation and mTORC1 blocking.

Chinese traditional medicine Jinlida granule (JLDG) has also been shown to reduce lipid accumulation in pancreatic β cells and lead to autophagy *via* protection of AMPK activation. The study demonstrated that JLDG might not only inhibit lipogenesis by downregulation of acetyl coenzyme A carboxylase (ACC) and so on, but also reduce the expression of mTOR and stimulate expression of tuberous sclerosis complex 1 (TSC1) and LC3 to induce the autophagy¹⁸. Except the protective mechanism, Chen et al.²³ have argued that as an adaptive response, β cell autophagy could inhibit increased insulin resistance. Take the diabetic fatty mouse model as an example: it has been found that autophagy was necessary to maintain the normal architecture of islets and intracellular insulin metabolism by increasing the insulin degradation rate in β cells of the *Rab3A*^{-/-} null mouse. Altered autophagy might also induce loss of pancreatic β cell mass in diabetes^{23,25}. Nonetheless, decreasing beclin1 expression could protect Min6 cells to some extent. It has been reported that a certain degree of autophagy is essential for Min6 cell survival²⁶. So the mechanism and regulation is between autophagy and autophagic cell death. But whether autophagy could boost cell death or regulate cell survival of pancreatic β cells and diabetes therapy need to be elucidated. The evidence nonetheless indicates that autophagy could play an essential protective role in glucose starvation *via* AMPK stimulation.

2.2. AMPK and autophagy in muscle insulin sensitivity

Skeletal muscle insulin sensitivity can be improved by physical exercise, which reduces the risk of diabetes and cardiovascular disease²⁷. Intensity and duration of exercise might determine the status of AMPK activation, which has a strong relationship with the increasing status of insulin sensitivity²⁴. Likewise, it has been found that autophagy could modulate glucose homeostasis in skeletal muscle and increase insulin sensitivity. So there is a link between AMPK and autophagy in exercise and insulin sensitivity in skeletal muscle. Firstly, one study has shown that mutation of AMPK sucrose non-fermenting 1 (*SNF1*) gene might induced

defective energy metabolism in the yeast and ATG1 and ATG13, which are autophagy related protein kinases, are also involved in this regulation^{18,27}. Liu et al.¹⁰ have also reported that muscle-specific *AMPK α 2*-deficient mice, which lost *AMPK α 2*, could impair activation of autophagy in muscle exercise, suggesting that stimulation of AMPK is essential for muscle autophagy induced by physical exercise. The possible pathway might include the Beclin1-BCL2 complex. Furthermore, *BCL2* mutant mice research has proved that autophagy is a critical regulator in metabolism related exercise, and this is correlated with insulin resistance. For a detailed pathway, AMPK phosphorylated FoxO3 to induce the expression of LC3, beclin1 in skeletal muscle, which is associated with autophagy²⁸. AMPK could interact with ULK1 directly to stimulate autophagy as described above¹⁰. Additionally, dihydromyricetin, which is a natural flavonoid, has been shown to improved skeletal muscle insulin resistance by stimulation of autophagy through AMPK-beclin1 or LC3 pathways. And the AMPK inhibitor compound C has been used to prove that the autophagy was induced by AMPK, because the blocking of AMPK suppressed the activation of autophagy and the improvement in skeletal muscle insulin resistance²⁹. Therefore, several study results have demonstrated that AMPK activates autophagy to increase insulin sensitivity in skeletal muscle, and AMPK is a necessary sensor in these modulation processes.

2.3. AMPK and autophagy in liver gluconeogenesis

Liver, another glucose metabolic organ, can play a role in suppressing hyperglycemia. There are two mechanisms to decrease glucose production in liver to influence metabolism and diabetes. One is to activate glycolysis or increase the synthesis of glycogen, protein and lipid, and another is to inhibit glycogenolysis, gluconeogenesis, proteolysis or lipolysis³⁰. AMPK and autophagy can participate in glucose regulation of liver. On one hand, AMPK could suppress ACC to be a rate-limiting step for lipogenesis by liver *via* accelerating oxidation of long chain fat acids and inhibiting insulin-mediated lipid synthesis. On the other hand,

activation of AMPK could lead to autophagy improvement, which would be decreased in liver hyperglycemia^{30,31}. So autophagy might be related to lipid synthesis in liver. There are two possible ways to affect lipid synthesis with regard to AMPK, including liver kinase B1 (LKB1) and Ca²⁺ calmodulin-dependent protein kinase kinase (CaMKK). To be specific, LKB1 might stimulate AMPK to translocate to cytosol. In mouse hepatocytes, adaptor protein containing pleckstrin homology domain, phosphotyrosine, interaction, pH domain and leucine zipper motif 1 (APPL1) could combine with adiponectin receptors 1 and 2 (AdipoR1 and AdipoR2) at N-terminal domains, which induced LKB1 cytosolic localization for AMPK activation^{32,33}. Moreover, AdipoR1 and AdipoR2 binding could promote APPL1 homodimerization³⁴. For the CaMKK pathway, it binds the similar significant sequence and shares structural homology with LKB1 to be the upregulator for AMPK, which could be activated by adiponectin trimers^{30,35–37}. In addition, adiponectin could also decrease autophagy *via* AMPK in the liver. This inhibition of autophagy was known as suppressor of glucose form autophagy (SOGA)^{38,39}. One relative study reported that SOGA knockdown might activate autophagy or proteolysis to prevent hepatocytes glucose production by adiponectin stimulation^{40,41}. Another experiments conducted by Kundu et al.⁴² demonstrated that hyperglycemia impairs AMPK phosphorylation, which depended on LKB1 and CaMKK activation. Inactive AMPK also inhibited autophagy through mTOR upregulation and accumulation of matrix protein. In addition, the downstream regulation is similar with the normal autophagy activation pathway like AMPK-ULK1⁴³. So LKB1, CaMKK and SOGA could be the more significant discovery as the present potential sensors for AMPK and autophagy glucose production or lipid synthesis in hepatocytes, especially in hyperglycemic liver. Autophagy plays a positive protective role in liver glucose and lipid regulation in metabolic diseases as well.

2.4. AMPK and autophagy in diabetic cardiomyopathy

Diabetic cardiomyopathy has become a major cause of diabetes-related morbidity and mortality. It is characterized by ventricular dysfunction that develops in diabetic patients without coronary artery disease or hypertension. Autophagy has been known as an important myocardial adaptive response to conserve energy. Dieter et al.¹⁴ reported that improving cardiac autophagy in patients with diabetes might prevent the impact of diabetic cardiomyopathy development. Restoration of autophagy could block the accumulation of dysfunctional organelles and cytotoxic protein aggregates. Autophagy is strongly controlled by the mammalian target of rapamycin (mTOR)-dependent signaling pathway. Most cardiac studies confirm that mTOR is a negative regulator of autophagy as well (*via* mTORC1-ULK1/2 phosphorylation or mTORC2-Akt-FoxO phosphorylation). Inhibition of mTOR is linked to autophagy induction in isolated cardiomyocytes and myocardial tissue^{44,45}. The AMPK-mTOR pathway has been considered an important mechanism in autophagy regulation in response to energy stress and glucose starvation. In mammals, compromised cellular energy production inhibits mTOR through activation of AMPK and subsequently, phosphorylation of the TSC. Since mTOR negatively regulate autophagy, it is likely that diabetes activates TSC-mTOR signaling through inactivation of AMPK, which inhibits the ULK kinase complex, preventing the initiation of autophagy. Nonetheless, over activation of autophagy in diabetes has also been found to be harmful to development of

cardiomyopathy and cardiac function to induce excessive cell stress⁴⁶. Whether the autophagy in cardiomyocytes should be a protective or detrimental mechanism is still a debate at this time⁴⁷. Therefore, it might be better to balance the status of autophagy activation in diabetic cardiomyopathy. Besides autophagy of cardiomyocytes, myocardial fibroblasts have also been mentioned in the rapamycin-regulated process of diabetic cardiomyopathy. However, most studies of pathological myocardial fibroblasts focused on cell apoptosis, extracellular matrix increasing, expression of collagen increasing and so on. There are no original reports about autophagy dysfunction of myocardial fibroblasts in diabetic cardiomyopathy. Additionally, according to the pathological process of hepatic fibrosis and renal fibrosis, autophagy is involved in increasing extracellular matrix and collagen degradation. Thus autophagy dysfunction of myocardial fibroblasts in cardiomyopathy or myocardial fibrosis might be a potential mechanism of diabetic cardiomyopathy, and a critical point and a target for diabetic cardiomyopathy treatment⁴⁴.

2.5. AMPK and autophagy in diabetic nephropathy

Diabetic nephropathy (DN) is a serious complication of diabetes. In the early stages of diabetes, patients exhibit hyperfiltration with high glomerular filtration rates (GFRs) and infrequent occurrence of microalbuminuria. Glomerular damage, along with proteinuria, and subsequent tubulointerstitial lesions induced by diabetes finally lead to end stage renal disease⁴⁸. Several studies have suggested that autophagy is involved in the pathogenesis of DN. AMPK as a regulator of autophagy has also been suggested to be involved in the pathogenesis of DN. It has been reported that podocytes and proximal tubular cells are the main cells affected in DN. Autophagy plays a crucial role in maintaining the function of podocytes and proximal tubular cells. Fang et al.⁴⁹ reported that in high-glucose conditions in cultured podocytes and streptozotocin (STZ)-induced type 1 diabetic rats, autophagy was suppressed with a decrease of the expression of Beclin-1, ATG12-5, and LC3, and lead to the impaired filtration barrier function of podocytes. Interestingly, Cina et al.⁵⁰ reported that mTORC1 is highly activated and may be involved in the mechanisms of autophagy inhibition in podocytes of diabetic mice and patients. In addition, several studies have reported that AMPK activation by AICAR or adiponectin shows podocyte protective effects against various nephrotoxic conditions. It seems that autophagy activation is intricate in AMPK-mediated podocyte protection. AMPK can activate autophagy *via* two independent mechanisms: suppression of mTORC1 activity and direct control of ULK1 phosphorylation. Between these two independent mechanisms of AMPK on the regulation of autophagy, suppression of mTORC1 activity might contribute to the protection of AMPK in podocytes of DN. Furthermore, the results from Yamahara et al.⁵¹ suggested that hyperactivation of mTORC1 signaling in proximal tubular cells was involved in obesity-mediated autophagy suppress. However, proximal tubular cells are correlated with SIRT1 (sirtuin type 1) or p62/SQSTM1 (sequestosome 1) protein in autophagy. As an example, in proximal tubular cells accumulation of p62/SQSTM1 protein occurred in type 2 diabetes patients, which suggested that deficiency of autophagy might affect diabetic kidney disease in humans^{48,51}. These findings lead us to hypothesize that autophagy is altered in diabetic kidneys, and autophagy deficiency should contribute to the pathogenesis of diabetic nephropathy, related with not only podocytes but also proximal tubular cells. Therefore,

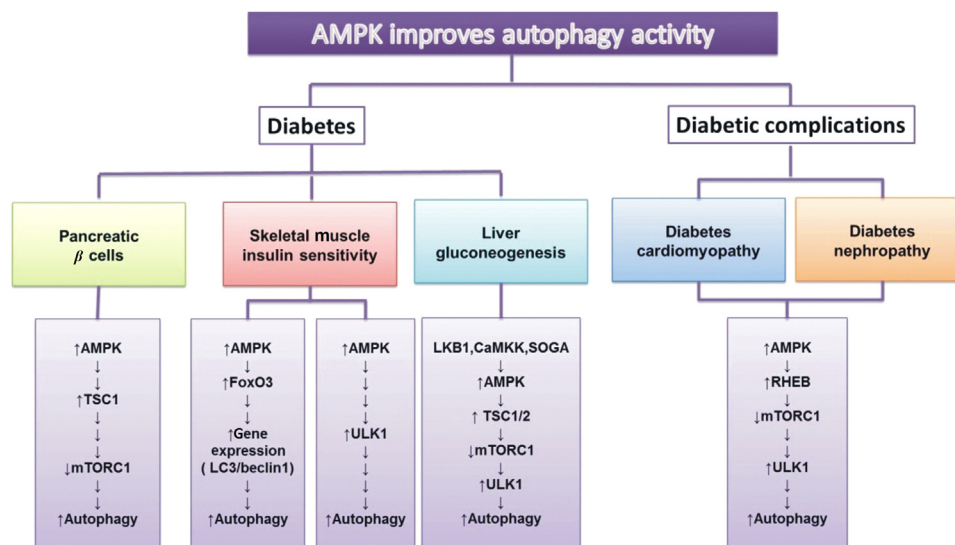


Figure 2 Activation of autophagy could occur in several diabetes-related diseases, including pancreatic β cells, muscle insulin sensitivity and liver gluconeogenesis related syndromes or diabetes cardiomyopathy and diabetes nephropathy. Subsequently, mTORC1 and TSC1 expression were included in the regulation of pancreatic β cells. In skeletal muscle, ULK1 or FoxO3 and LC3 might affect insulin sensitivity as well as in liver gluconeogenesis, which has a strong association with autophagy activation. Additionally, LKB1, CAMKK and SOGA are the special sensors involved in liver gluconeogenesis studied recently. And AMPK-RHEB-TOC1 is the significant signaling pathway in diabetes cardiomyopathy and nephropathy. All of these are linked with energy sensor, AMPK.

if pharmacological AMPK activation really acts as an autophagy activator, a drug that stimulates AMPK may be a potential therapy for diabetic nephropathy⁵². Several studies have reported that AMPK activation shows renoprotective effects in diabetic nephropathy. Autophagy may be involved in AMPK mediated renoprotective action.

3. Conclusions and perspective

It has been found for diabetes mellitus that autophagy is a significant factor with AMPK, an important regulatory signaling pathway. The autophagy induced by cellular lesions could affect a variety of tissues, glucose or lipid metabolism and insulin secretion. Hence, these conditions might lead to serious chronic disease as diabetes complications (Fig. 2). At times, diabetes itself may not be such a serious status. But continuing or additional tissue damage could lead to a critical mass of cell injuries and imbalance homeostasis. Accordingly, autophagy induced by AMPK is involved in calorie production and lipogenesis mainly with other protein signals, such as mTORC1, LKB1, ULK and LC3. The crosstalk between autophagy and AMPK might be a possible therapeutic target. In future research, blocking other regulatory pathways of autophagy like mTORC1 might be significant, including AMPK upstream and downstream regulation. Likewise, in recent decades, medicine, therapeutic approaches and pathogenesis would tend to depend on individuals. Therefore, combining with genetic and protein levels in AMPK and autophagy stimulation pathways might be a tendency for diabetes and complications regulatory analysis.

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