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# Research Progress on Mechanism of Autophagy in Prevention and Treatment of Diabetic Peripheral Neuropathy by Traditional Chinese Medicine

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## Abstract:

Diabetic peripheral neuropathy (DPN) has a long course and poor prognosis, characterized by axonal degeneration and necrosis, segmental demyelination of nerve fibers, apoptosis of Schwann cells, and other neuronal cell injuries. Autophagy, the cleaning mechanism of nerve cells, eliminates damage caused by cellular stressors by clearing excess metabolites, thereby maintaining intracellular homeostasis. The sustained high glucose environment alters the level of autophagy in the body. Inhibition or excessive activation of autophagy can cause irreversible damage to nerve cells, accelerating the progression of DPN. Restoring autophagy balance is crucial for reducing nerve damage and treating DPN. Currently, there is no satisfactory treatment for DPN. Traditional Chinese medicine, with its unique advantages of multiple targets, multiple effects, and multiple components, has achieved good clinical effects in the prevention and treatment of DPN. Numerous animal and clinical studies on the treatment of DPN by traditional Chinese medicine have shown that autophagy is an important target for the treatment of DPN. Restoring autophagy balance can reduce nerve injury, delay the process of nerve cell death, and play a role in the prevention and treatment of DPN. However, its specific mechanism remains unknown. This paper focuses on the regulation of autophagy and its mechanism of action in the pathogenesis of DPN, as well as the intervention effect of single traditional Chinese medicine or compound effective ingredients on autophagy. It further explores the pathogenesis of DPN and potential therapeutic targets of traditional Chinese medicine intervention in autophagy, providing a reference for further improving the efficacy of traditional Chinese medicine in treating DPN.

## Keywords:

Autophagy
Diabetic peripheral neuropathy
Schwann cell
Traditional Chinese medicine
Regulation and control mechanism
Review

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# 1. Introduction

Diabetic peripheral neuropathy (DPN) is a chronic complication of diabetes characterized by symmetrical numbness, tingling, muscle weakness, and hyperalgesia in the distal limbs [1]. Research by the International Diabetes Federation has shown that the prevalence of DPN can be as high as 70% to 90% [2–3]. DPN often has no symptoms in the early stage, making it prone to missed diagnosis or misdiagnosis. As the condition progresses, it can lead to severe consequences such as diabetic foot ulcers, fractures, necrosis, or amputation, thus imposing a heavy economic burden on patients and severely affecting their quality of life [4]. The pathogenesis of DPN is complex. Metabolic imbalances caused by long-term hyperglycemia can damage the peripheral nervous system through various mechanisms such as neuroinflammation, advanced glycation end products, mitochondrial dysfunction, oxidative stress, and dyslipidemia, leading to neuronal material and metabolic disturbances [5]. Autophagy is an important pathway for nerve tissue to clear harmful substances, preventing harmful substances from damaging nerve cells by degrading protein aggregates and removing damaged organelles [6]. The relationship between the disruption of autophagy pathways in diabetes and the progression of DPN has also been confirmed, making the restoration of autophagy particularly important for the treatment of DPN [6]. Clinically, there is a lack of effective measures to delay or reverse DPN. Traditional Chinese medicine (TCM) has advantages in preventing and treating DPN, including multiple pathways and entry points, significant treatment effects, and few adverse reactions. TCM's bidirectional regulation of autophagy can timely clear senescent and damaged nerve tissue, reduce pathological damage to nerve cells, and slow down the process of nerve cell death [7]. Autophagy has become an important target for the prevention and treatment of DPN with TCM, both currently and in the future. However, its mechanism has not been fully elucidated. Therefore, this article provides a review of the regulatory mechanisms of autophagy and the intervention of TCM in autophagy for the treatment of DPN, aiming to provide new clinical ideas and methods for the treatment of DPN with TCM.

# 2. Overview of autophagy

The term "autophagy" was first coined by Belgian scientist Christian de Duve, referring to the biological process where cells degrade, recycle, and reuse their internal metabolites through lysosomes to maintain cellular homeostasis when exposed to external stimuli such as hypoxia, starvation, and metabolic abnormalities [8]. Under normal physiological conditions, cells in the body maintain a certain level of autophagy, eliminating the harm caused by cellular stressors by clearing excess metabolites, thus maintaining the balance of the intracellular environment. However, under pathological conditions, inadequate autophagy can lead to abnormal accumulation of senescent organelles and damaged proteins, causing harm to the entire cell. On the other hand, excessive activation of autophagy can lead to cell self-digestion, inducing programmed cell death [9]. Autophagy is a series of cellular events involving multiple autophagy-related genes (*Atg*). The specific process includes:

(1) The formation and nucleation of phagocytes controlled by autophagy-related gene 6 (*Atg6*) homologs, programmed cell death-1 (*Beclin-1*) complexes, or vesicular transport proteins in response to stress signaling pathways on the endoplasmic reticulum and other membranes;

(2) The autophagy markers *Atg5–Atg12* complex and light chain 3 (*LC3*) bind to the autophagosome membrane and participate in the extension of the autophagic membrane through a series of phosphorylations;

(3) The extended double-membrane vesicle structure encapsulates damaged senescent organelles and abnormal proteins, forming autophagosomes;

(4) Fusion of autophagosomes with lysosomes and proteolytic degradation of engulfed molecules by lysosomal proteases [8].

Autophagy is divided into various types, including selective and non-selective autophagy based on the specificity of the degraded substrate, and macroautophagy, microautophagy, and chaperone-mediated autophagy based on the pathway of substrate delivery to lysosomes. Macroautophagy is the most widely studied type [10,11], particularly in diseases, and is the focus of this article.

## 3. Relationship between autophagy and diabetic peripheral neuropathy

The long-term presence of diabetic glucose and lipid metabolism disorders alters cellular autophagy activity, contributing to the development of DPN [12]. As highly differentiated cells with poor regenerative capacity and constantly exposed to a complex environment of electrical and biological signals, nerve cells rely on autophagy as a crucial cellular biological pathway to clear harmful substances [13]. Among them, autophagy in myelin Schwann cells (SCs) plays a particularly critical role in DPN. Under normal physiological conditions, SCs maintain high autophagy activity to clear damaged myelin cell fragments and assist in nerve regeneration and repair [14]. Studies have found that SC autophagy can clear 60% of damaged fragments within 72 hours after injury, while knocking down autophagy-related genes such as *Atg7* not only exacerbates nerve fiber myelin sheath rupture but also slows down axon regeneration after injury [15]. However, DPN model rats and SCs cultured in high glucose exhibit reduced autophagy function [16]. Yang *et al.* (2014) also found that STZ-induced diabetic rats had reduced autophagosome formation and downregulation of the autophagy-inducing factor *Beclin-1* in the sciatic nerve, accompanied by axonal terminal swelling and Purkinje cell degeneration. Adding the autophagy inducer rapamycin increased *Beclin-1* expression in the sciatic nerve, enhanced SC autophagy ability, improved myelin degeneration, and reduced axonal atrophy [17,18]. This suggests that under glucolipotoxic conditions, the inhibition of SC autophagy is involved in the pathogenesis and development of DPN. Additionally, excessive activation of autophagy can also cause cell apoptosis and neuronal damage. Towns observed mitochondrial dysfunction, increased autophagosomes, enhanced autophagy capacity, upregulated expression of autophagy regulatory proteins *Beclin-1* and *LC3-II*, and pathological vacuolar changes in axons in dorsal root neurons of STZ-induced diabetic rats [19]. Upregulation of autophagy in spinal dorsal horn neurons leads to persistent pain in diabetic rats [20], confirming this point. Thus, it is evident that a certain level of autophagy plays a positive role in nerve repair and regeneration, while autophagy imbalance, whether inhibition or excessive activation, can cause neuropathological damage and accelerate the progression of demyelinating neuropathies.

## 4. The regulatory mechanism of autophagy in diabetic peripheral neuropathy (DPN)

Research has indicated that abnormalities in autophagy mediated by autophagy regulatory signaling pathways are closely related to the development and progression of DPN [21]. These mainly include the mammalian target of rapamycin (mTOR) signaling pathway, AMP-activated protein kinase (AMPK) signaling, and induced putative kinase (PINK)/E3 ubiquitin ligase (Parkin) signaling pathway. Additionally, autophagy in the state of DPN is also regulated by autophagy-related factors, non-coding RNAs, and oxidative stress.

### 4.1. Major signaling pathways

#### 4.1.1. mTOR signaling pathway

As a negative regulator of autophagy, mTOR plays a critical role in regulating neuronal autophagy and can mediate multiple signaling pathways involved in the regulation of autophagy [22]. mTOR is a 289-kDa serine/threonine protein kinase that mainly consists of two protein complexes, mTORC1 and mTORC2. mTORC1 is highly sensitive to the agonist rapamycin, which not only inhibits the autophagy-initiating kinase ATG1 but also blocks the key autophagy inducer Unc-51-like kinase 1 (ULK1) at the P757 site, thereby suppressing the occurrence of autophagy [23]. Investigations have found that high glucose levels can upregulate the expression of mTOR, leading to the phosphorylation of downstream effectors such as p70 ribosomal protein S6 kinase (p70S6K), which promotes mRNA translation and inhibits the formation of autophagosomes by blocking the detachment of endoplasmic reticulum membranes [24]. Additionally, the phosphorylation of mTOR is also related to the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway. Oxidative stress induced by high glucose releases large amounts of reactive oxygen species (ROS), stimulating cell proliferation and leading to the phosphorylation of Akt under the action of PI3K. This inhibits the activation of its downstream tuberous sclerosis complex (Tsc) and promotes the phosphorylation of downstream factors

including mTOR. Regulation of the PI3K/Akt/mTOR signaling pathway can reduce excessive autophagy of RCS96 Schwann cells induced by high glucose, slow down cell apoptosis, and improve DPN [25].

#### 4.1.2. AMPK signaling pathway

Contrary to the inhibition of autophagy by the mTOR signaling pathway, activation of the AMPK signal induces autophagy. AMPK is an intracellular adenosine nucleotide level sensor that maintains intracellular energy homeostasis by promoting ATP energy catabolism pathways and downregulating ATP energy consumption. Under long-term high glucose conditions, lysosomes are damaged, leading to the binding of TGF-β-activated kinase 1 (TAK1) with galectin-9, which phosphorylates AMPK. Additionally, AMPK can activate ULK1, thereby inducing autophagy [26,27]. Abdelkader *et al.* (2022) showed that in an STZ-induced DPN model, the phosphorylation level of AMPK decreased. Administration of the SGLT-2 inhibitor EMPA increased AMPK phosphorylation levels, along with enhanced expression of autophagy proteins *Beclin-1* and *LC3-II*, significantly improving peripheral nerve cell damage caused by high glucose [28].

#### 4.1.3. PINK/Parkin signaling pathway

The PINK/Parkin signaling pathway plays a central role in diabetic neuropathic pain by mediating mitochondrial autophagy [29]. PINK1 is mainly expressed in mitochondria and degraded/cleaved in the inner mitochondrial membrane. Under persistent high glucose conditions, mitochondrial damage occurs, and protease hydrolytic activity is inhibited, leading to the accumulation of PINK1 on the outer mitochondrial membrane and phosphorylation of the mitochondrial autophagy regulator Parkin, thus initiating mitochondrial autophagy [30]. He *et al.* (2022) found that Parkin increased in the spinal cord of diabetic mice, accompanied by an increase in mitochondrial autophagy. Hypoxia-inducible factor 1 (HIF-1) improved mitochondrial autophagy and hyperalgesia in diabetic mice by mediating Parkin-related mitochondrial dysfunction [31]. Thus, Parkin plays a neuroprotective role by increasing mitochondrial autophagy.

### 4.2. Autophagy-related factors

Autophagy is a complex dynamic process that is strictly regulated and involves various autophagy-related factors. *Beclin-1*, a homolog of the yeast autophagy gene *Atg*/*Vps30*, participates in the formation of autophagosomes and autophagic vacuoles, and its expression is positively correlated with autophagy levels [32]. *LC3* is a marker protein for autophagy and exists in two interchangeable forms. Extracellular *LC3* is catalyzed, modified, and processed by autophagy-related enzymes, covalently binding to phosphatidylethanolamine on the autophagosome surface to form *LC3-I* and *LC3-II*. The conversion of *LC3-I* to *LC3-II* indicates the formation of autophagosomes and is considered an important marker for detecting autophagy [33]. p62 protein (62-kDa protein, p62) is an autophagy receptor with a ubiquitin-binding domain that binds to *LC3-II* protein through specific amino acid sequences to form a complex, which is degraded with the formation of autolysosomes. Therefore, the content of p62 is negatively correlated with the degree of autophagy [34]. Numerous animal and cell experiments have shown that *Beclin-1* expression is significantly reduced in DPN models, accompanied by decreased *LC3-II* expression and increased p62 content [35,36].

### 4.3. Non-coding RNAs

In recent years, with the deepening of research on non-coding RNAs, it has been found that non-coding RNAs are involved in the regulation of autophagy [37]. Long non-coding RNAs (lncRNAs) are transcripts longer than 200 nucleotides that can directly target autophagy-related genes after transcription, enhancing the autophagy process [38]. MicroRNAs (miRNAs) are endogenous small non-coding RNAs that specifically bind to the 3' untranslated region (3'UTR) of target mRNAs post-transcriptionally, regulating autophagy target genes such as *Beclin-1* and *LC3*. Additionally, miRNAs also target and regulate upstream signaling pathways of autophagy, including PI3K/Akt/mTOR, to participate in the autophagy process [38]. Liu *et al.* (2021) reported that the lncRNA X-inactive specific transcript (XIST) induces autophagy and prevents apoptosis in high glucose-cultured Schwann cells through the miR-30d-5p/SIRT1 axis, alleviating DPN [39].

### 4.4. Oxidative stress

Reactive oxygen species (ROS) generated by glucolipotoxicity are crucial signaling molecules for the induction of autophagy. On the one hand, ROS can control the activity of *Atg4*, which is essential for autophagosome formation. On the other hand, ROS can also induce poly ADP-ribose polymerase 1 (PARP-1), activating AMPK through AMP activation to promote autophagy [22]. The ULK-Atg13-FIP200 complex plays a key role in the formation of autophagosomes and autophagic double-membrane vesicles [40]. ROS can inhibit mTOR signaling proteins, affecting the dephosphorylation of *Atg13*, activation of ULK, and activity of FIP200, thereby influencing the autophagy process. Zhou *et al.* (2015) also confirmed that ROS-induced JNK activation leads to autophagy and apoptosis, which is associated with caspase-independent autophagic cell death [41].

As shown in **Figure 1**, the revelation of the relationship between the regulatory mechanism of autophagy in the state of DPN and the occurrence and development of DPN has led more scholars to recognize that autophagy is involved in the "point of no return" of DPN. Regulating autophagy provides a new opportunity for the treatment of DPN. Currently, evidence for the use of autophagy modulation methods such as caloric restriction, mTOR agonists, and metformin in the treatment of DPN is limited, and their side effects are relatively significant, preventing them from achieving the desired therapeutic effect.

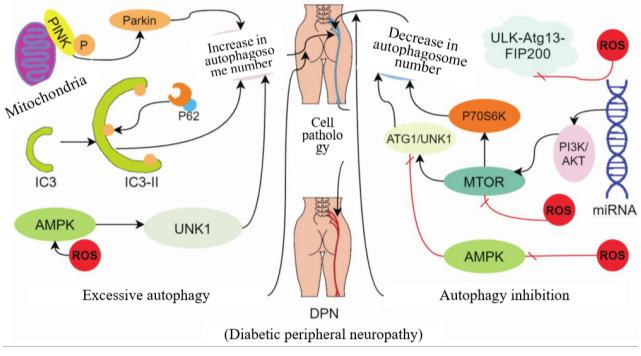


**Figure 1.** The mechanism of autophagy in DPN.

## 5. Regulation of autophagy by traditional Chinese medicine for the prevention and treatment of DPN

Recent studies have shown that traditional Chinese medicine (TCM) exhibits significant advantages in the treatment of DPN, including multi-effect, multi-component, multi-target, and precise efficacy with almost no adverse reactions. Among them, the bidirectional regulation of autophagy by TCM for the treatment of DPN is particularly prominent. This article systematically summarizes the use of monomeric and compound active ingredients from TCM to target autophagy for the treatment of DPN.

### 5.1. Monomers

#### 5.1.1. Flavonoids

Quercetin is a flavonoid compound with pharmacological effects such as anti-oxidation, anti-cancer, anti-diabetes, and anti-inflammation. It also has strong neuroprotective and immunomodulatory effects. Studies by Qu *et al.* (2014) [42] have found that under high glucose conditions, autophagosomes in RSC96 cells decrease, proliferative activity is inhibited, and the expression of *Beclin-1*, *LC3*, and other autophagy molecular markers is significantly reduced. However, after intervention with quercetin, the expression of *Beclin-1* and *LC3* increases significantly, reversing cell damage.

Puerarin is a type of isoflavone monomeric compound with anti-oxidative stress and neuroprotective effects. Li *et al.* (2020) [43] found that adding 0.5 mmol/L of puerarin to high glucose-induced RSC96 cell injury can inhibit autophagy at 1 hour, reduce apoptosis at 6 hours, down-regulate the expression of autophagy-related proteins *Beclin-1* and *LC3-II*, and increase the content of p62 protein.

Isoliquiritigenin is a flavonoid compound with various biological activities such as anti-inflammatory and anti-oxidative stress effects. Yerra *et al.* (2017) reported that isoliquiritigenin can induce the AMPK signaling pathway through silent mating type information regulation 1 (SIRTI) to exert anti-oxidative stress and promote mitochondrial autophagy, further protecting nerves and improving symptoms of diabetic neuropathy [44].

#### 5.1.2. Saponin compounds

Dioscin, a saponin compound found in the traditional Chinese medicine *Discorea nipponica*, has various pharmacological effects including lipid-lowering, anti-

oxidation, hypoglycemic, and analgesic properties. Zhang (2020) [45] discovered that dioscin can increase the expression of PI3K, Akt, and mTOR in mouse nerves. By activating the classical autophagy signaling pathway PI3K/Akt/mTOR, it repairs damaged nerves, thereby treating painful diabetic peripheral neuropathy. Astragaloside IV, the main component of *Astragalus membranaceus*, promotes autophagy, has anti-cancer effects, and inhibits angiogenesis. Yin *et al.* (2021) reported that astragaloside IV can enhance the expression of *Beclin-1* and *LC3* in the sciatic nerve of DPN rats, boost autophagy activity in RSC96 cells, and inhibit cell apoptosis. This reduces peripheral nerve myelin sheath damage. Further investigation revealed that the mechanism behind this phenomenon is related to the miR-155-mediated PI3K/Akt/mTOR signaling pathway [46].

#### 5.1.3. Alkaloids

Lycorine is an alkaloid isolated from the Amaryllidaceae family, known for its anti-inflammatory, antiviral, and antitumor activities [47]. Yuan *et al.* (2022) found that lycorine not only improves peripheral nerve function and autophagy-related proteins in diabetic mice but also enhances the expression of *Beclin-1*, *Atg3*, and *LC3-II* in RSC96 cells cultured in high glucose conditions *in vitro*. The possible mechanism revealed involves activating the AMPK pathway and downregulating MMP9, thereby inducing the conversion of *LC3-II* in DPN to promote Schwann cell autophagy [48]. Berberine, an isoquinoline alkaloid of the protoberberine type also known as coptisine, is mainly present in the roots, rhizomes, and stem bark of the traditional Chinese medicine *Coptis chinensis*. Yerra *et al.* (2018) [49] discovered that berberine can reverse the decreased expression of p-AMPK(Thr172) in sciatic nerves under high glucose conditions, enhance mitochondrial autophagy, improve mitochondrial function defects, and inhibit neuronal damage and neuroinflammation.

#### 5.1.4. Others

Emodin is a free anthraquinone derivative found in traditional Chinese medicines such as rhubarb, *Polygonum multiflorum*, and *Polygonum cuspidatum*. Modern pharmacology has revealed its antiviral, antitumor, antidiabetic, and neuroprotective effects [50]. Fan *et al.* (2018) [51] reported that emodin can upregulate p62 protein expression and downregulate autophagy markers *LC3*I/*LC3*II and *Beclin-1* protein expression, alleviating hyperglycemia-induced excessive autophagy. Further research suggested that its neuroprotective activity might be achieved by upregulating miR-9 and regulating PI3K/AKT and nuclear factor kappa-B (NF-KB) signaling pathways. *Lycium barbarum* polysaccharide, a water-soluble polysaccharide extracted from *Lycium barbarum*, is known for its immunity-boosting, hypoglycemic, hypolipidemic, antioxidant, and antitumor effects. Liu *et al.* (2018) found that *Lycium barbarum* polysaccharide inhibits the activity of the mTOR/p70S6K pathway in the sciatic nerve of diabetic rats, thereby promoting the expression of autophagy proteins *LC3* and *Beclin-1*. This prevents demyelination of peripheral nerves in diabetic rats and protects the sciatic nerve [52]. Salvianolic acid B, a phenolic acid isolated from *Salvia miltiorrhiza*, is a new generation of natural water-soluble biologically active compounds with antioxidant, anti-myocardial ischemia, and anti-inflammatory pharmacological effects [53]. Wang *et al.* (2019) [54] showed that salvianolic acid B inhibits the development of DPN by suppressing the c-Jun N-terminal kinase (JNK) pathway and preventing autophagy and cell apoptosis. Musketone, a dry secretion from the hair follicles of male musk deer skin, exhibits anti-fibrotic, anti-inflammatory, anti-apoptotic, and antitumor pharmacological effects. Dong *et al.* (2019) study reported that musketone upregulates Akt and mTOR expression, activating the Akt/mTOR signaling pathway to reduce HG-induced autophagy and apoptosis in RSC96 cells, thereby improving DPN [25].

### 5.2. Compound prescriptions

Increasing research indicates that traditional Chinese medicine decoctions and proprietary Chinese medicines also play a significant role in the treatment of patients with DPN. Yin (2020) [15] discovered in both *in vitro* and *in vivo* experiments with rats that Qigui Tangtongning granules (composed of Astragalus, Angelica, Pueraria, Rehmannia, Corydalis, Clematis, and Millettia) can alleviate Schwann cell apoptosis in a high-glucose environment and upregulate the expression of miRNA-155-5P to target and inhibit the PI3K/Akt/mTOR signaling pathway, thereby promoting autophagy in Schwann cells

and reducing demyelination lesions in DPN. Zhao *et al.* (2020) [15] administered the traditional Chinese medicine Qizhi Kebitong formula (consisting of Astragalus, Mulberry Twig, Millettia, Clematis, Cyathula, Scorpion, and Siegesbeckia) to rats and found that compared to the model group, the DPN rat group showed significantly increased expression of *LC3*, *Beclin-1* proteins, and *LC3*, *Beclin-1*, ULK1 mRNA levels, while p62 expression decreased. This suggests that Qizhi Kebitong formula protects the sciatic nerve of diabetic mice by activating autophagy. Qu *et al.* (2016) [55,56] experiments revealed that the use of Jinmaitong (consisting of Dodder, Glossy Privet Fruit, Cassia Twig, Leech, Corydalis, and Asarum) significantly increased the expression of autophagy-related proteins *Beclin-1*, *LC3* protein, and *Beclin-1*-mRNA levels in the sciatic nerve of STZ-induced diabetic rats, significantly reducing the pathological morphology of the sciatic nerve tissue. Tu *et al.* (2019) [35] intervened with Tangtong formula (composed of Astragalus, Cassia Twig, Turmeric, Chuanxiong, Ground Beetle, Angelica, White Peony, and Asarum) on STZ-induced DPN model rats and found an upward trend in the expression of *Beclin-1* and *LC3* mRNA. Further research by He *et al.* (2022) [57] revealed that the mechanisms of Tangtong formula in improving nerve injury, reducing demyelination lesions, and protecting nerve fiber structure are related to activating the PI3K/Akt/mTOR signaling pathway and inhibiting excessive autophagy. Mu *et al.* (2018) [58] used Tangbikang (containing Astragalus, Cassia Twig, Glossy Privet Fruit, Leech, Red Peony, and Coptis) medicinal serum to intervene in rat RSC cells. The results showed that Tangbikang could increase the expression of *Beclin-1* and *LC3-II* proteins, promote autophagy of RSC cells in a high-glucose environment, reduce apoptosis, and exert neuroprotective effects.

## 6. Conclusion

In recent years, with the emergence of the concept of autophagy and the continuous deepening of research, traditional Chinese medicine has greatly demonstrated the correlation between autophagy and DPN in terms of autophagy regulation mechanisms such as the mTOR signaling pathway, AMPK signaling pathway, PLNK/Parkin signaling pathway, non-coding RNA, and oxidative stress. Significant progress has been made in related molecular mechanism research, highlighting the advantages of traditional Chinese medicine in improving and delaying DPN through multiple pathways, targets, and effects. This provides convenience and reduces the burden for patients. Commonly used clinical herbs such as Astragalus, Cassia Twig, Millettia, Glossy Privet Fruit, Asarum, and Leech, which have the functions of nourishing Qi, activating blood circulation, and dredging meridians, can exert neuroprotective effects through bidirectional regulation of autophagy, further improving and delaying the progression of DPN. These herbs have good application value and prospects in the prevention and treatment of DPN, providing a new scientific basis for deeply exploring the mechanism of traditional Chinese medicine in treating DPN diseases. However, there are still some issues in current research:

(1) Due to the complexity of traditional Chinese medicine components, it is unclear which specific herb or component targets a specific site to exert a therapeutic effect. Additionally, research mainly focuses on herbal extracts and compound prescriptions, while studies on single herbs are rare, which is not conducive to leveraging the advantages of traditional Chinese medicine. In the future, techniques such as network pharmacology, high-throughput mass spectrometry analysis, bioinformatics, multi-omics joint analysis, and molecular docking can be utilized to further investigate the material basis of traditional Chinese medicine targeting autophagy for the prevention and treatment of DPN, as well as to deeply explore the medicinal substances and their mechanisms of action.

(2) Both the inhibition and excessive activation of autophagy can cause certain nerve damage. Defining this "critical point" is a major challenge that can be addressed in future research.

(3) Current research is not sufficiently deep, and there are few studies on intervening in autophagy for the treatment of DPN, which are limited to cellular and animal levels. In the future, emphasis should be placed on conducting high-quality clinical research to accumulate evidence for assisting in the formulation of clinical treatment plans.

(4) The current research mainly focuses on macroautophagy, while studies on other types such as

microautophagy and chaperone-mediated autophagy are limited. In the future, the role and mechanism of other types of autophagy in DPN can be further investigated to open up new avenues for the treatment of DPN and better improve clinical efficacy.

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