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Current Neuropharmacology, 2021, *19*, 629-640

REVIEW ARTICLE

The Role of Autophagy in the Pathogenesis of Ischemic Stroke

Qianyu Shi[1], Quancheng Cheng[2] and Chunhua Chen[2,*]

[1]*School of Clinical Medical Sciences, Peking University Health Science Center, Beijing, 100191, China;* [2]*Department of Human Anatomy, Histology and embryology, School of Basic Medical Sciences, Peking University, Beijing, 100191, China*

ARTICLE HISTORY

Received: April 28, 2020
Revised: June 19, 2020
Accepted: July 23, 2020

DOI:
10.2174/1570159X18666200729101913

Abstract: Autophagy is a strictly regulated process that degrades and recycles long-lived or misfolded proteins and damaged organelles for the maintenance of energy and function homeostasis of cells. Insufficient oxygen and glucose supply caused by cerebral ischemia lead to a higher ratio of AMP/ATP, which will activate the AMPK pathway to initiate the process of autophagy. Accumulating evidence shows that autophagy participates in the pathogenesis of ischemic stroke as a double-edged sword. However, the exact role of autophagy in the pathogenesis of ischemic stroke is controversial and yet to be elucidated. In this review, the autophagy pathway, both in physiological conditions and in ischemic stroke, is expounded. The focus was also on discussing the double-edged sword effect of autophagy in brain ischemia and its underlying mechanisms. In addition, potential therapeutic strategies for ischemic stroke targeting autophagy pathway were also reviewed.

Keywords: Autolysosome, autophagy, double-edge sword, ischemic stroke, pathogenesis, therapeutic strategies.

1. INTRODUCTION

Stroke is assumed to be the leading cause of death and disability globally [1-3]. It hits 15 million people globally annually, with 5 million deaths and another 5 million left permanently disabled, causing a heavy economic burden on family and society [4]. Stroke is a neurological disease caused by insufficient cerebral circulation because of infarction or hemorrhage of cerebral vessels. According to the pathological changes of cerebral vessels, a stroke could be an ischemic stroke (IS) or a hemorrhagic stroke (intracerebral hemorrhage and subarachnoid hemorrhage) [3].

IS is initiated by the infarction of cerebral vessels because of thromboembolism and thrombus formation. Cerebral vessel occlusion leads to insufficient oxygen and glucose supply for brain parenchyma, causing cascade responses, including blood-brain barrier dysfunction [5], neuroinflammation [6], oxidative stress [7], *etc.* Such responses are biological defense mechanisms under IS, yet can be deleterious to brain parenchyma when activated intensively and continuously.

Autophagy is a natural, regulated process which helps cells to eliminate unnecessary or dysfunctional components, including long-lived proteins, insoluble proteins, and even whole organelles [8, 9]. Autophagy is composed of microautophagy, macroautophagy, and chaperone-mediated autophagy (CMA) according to the way of cargo delivery to the lysosomes. Microautophagy is a non-selective lysosomal degradative process that engulfs cytoplasmic constituents *via* the invagination of the lysosomal membrane [10, 11]. Through engulfment of long-lived and membrane proteins, microautophagy exerts the functions of maintaining organellar size, membrane homeostasis, and cell survival under nitrogen restriction [11]. Macroautophagy is the most prevalent form of autophagy that forms a double-membrane compartment known as phagophore enfolding the cargo, which then matures into an autophagosome [12, 13]. The combination of autophagosome and lysosome generates autolysosome, in which the cargo is degraded and the degradation products are released into the cytoplasm for reuse [12, 14, 15]. CMA is the only selective lysosomal degradative process, which targets the substrates by heat shock cognate 70 (HSC70) selective binding [13, 16]. Proteins bound by chaperone then are delivered to the lysosomes, and the transportation into lysosomes is mediated by the specific recognition and combination of the lysosome-associated membrane protein type 2a (LAMP2a) receptor and the chaperone-bound proteins [13, 17]. Through the three mechanisms mentioned above, autophagy is the main regulatory catabolic process to degrade long-lived proteins and damaged organelles in eukaryotic cells [18].

Autophagy also exerts a neuroprotective effect in IS by controlling neural survival and death through variable mechanisms [19]. Many efforts have been made to understand the pathogenesis of IS since the first identification hemorrhagic and IS [20]. However, it remains obscure and unthorough, and thrombolytic therapy is the only drug treatment recommended by the American Heart Association/American Stroke Association, which includes only two drugs (alteplase and tenecteplase) with a limitation of short therapeutic time window [21, 22]. Accumulating evidence has supported that autophagy plays a crucial role in IS. Therefore, understanding the role of autophagy in the pathogenesis of IS can

* Address correspondence to this author at the Department of Human Anatomy, Histology and embryology, School of Basic Medical Sciences, Peking University, Beijing, 100191, China; Tel: (8610) 8280 1427;
Fax: (8610) 8280 1164; E-mail: cch@bjmu.edu.cn

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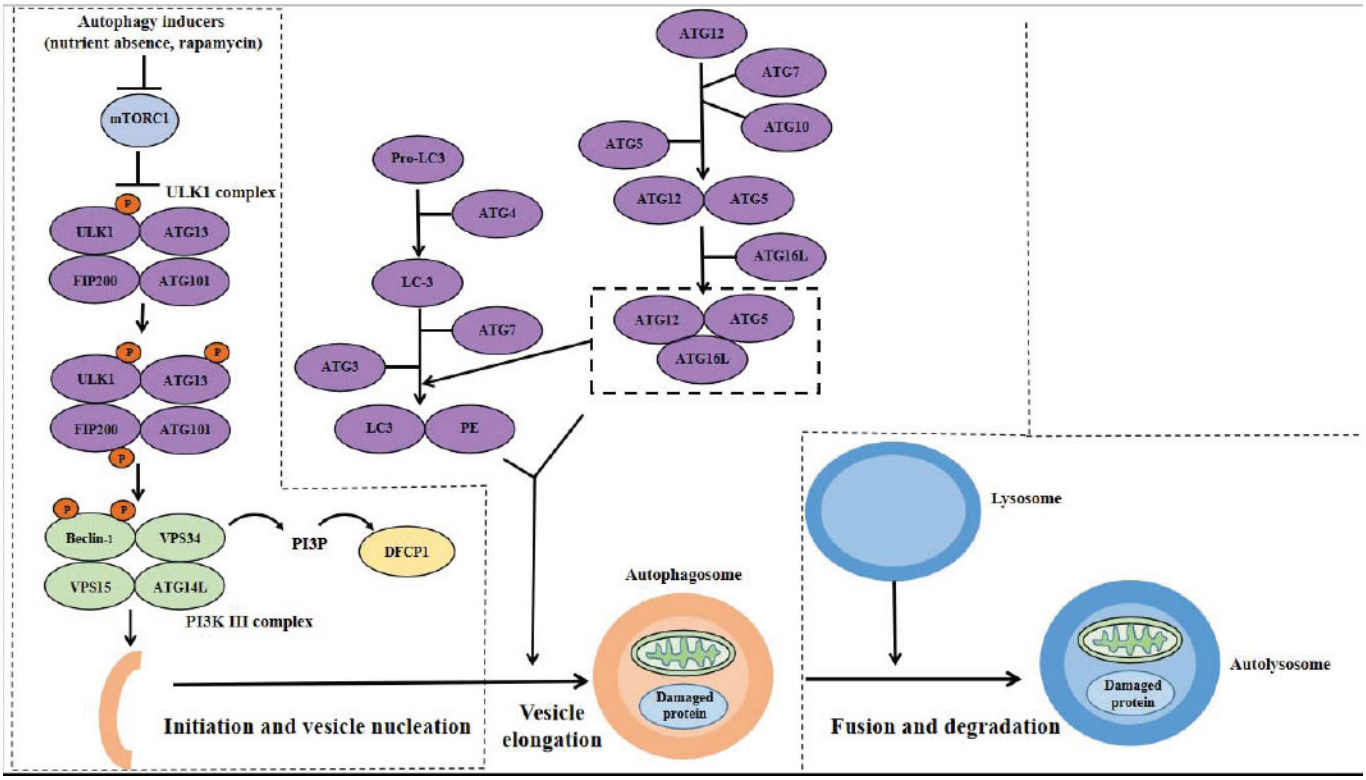

Fig. (1). Schematic illustration of the autophagy pathway. Autophagy is strictly controlled through multiple pathways. Autophagy inducer, such as nutrient absence or rapamycin, activates the ULK1 complex through phosphorylating ULK1, which leads to the activation of PI3K III complex. Activated PI3K III complex produces phosphatidylinositol 3-phosphate by VPS34. Phosphatidylinositol 3-phosphate then recruits DFCP1 to accelerate the process of vesicle nucleation. ATG12 is conjugated to ATG7, ATG10, and ATG5 in order, forming the ATG12-ATG5 conjugate with the help of ATG7 and ATG10. Combined with ATG16L, the ATG12-ATG5-ATG16L conjugate is formed. Mediated by ATG4, Pro-LC3 is converted to LC3, which is conjugated to PE by ATG3, ATG7, and ATG12-ATG5-ATG16L conjugate, forming LC3-PE conjugate. Both ATG12-ATG5-ATG16L conjugate and LC3-PE conjugate participate in the elongation of autophagosomes. After elongation, autophagosomes are fused with lysosomes forming autolysosomes, which is mediated by SNARE syntaxin 17, PLEKHM1, and Atg14. Cargos in autolysosomes are digested by lysosomal enzymes. Nutrients generated by digestion act as activators and inhibitors for mTOR and AMP-activated protein kinase (AMPK), respectively, which lead to the inactivation of ULK1 complex and termination of autophagy. Abbreviation: mTOR, mammalian target of rapamycin; ULK1, Unc-51-like kinase 1; ATG, autophagy related proteins; FIP, focal adhesion kinase family interacting protein; VPS, vacuolar protein sorting; PI3P, phosphatidylinositol-3-phosphate. *(A higher resolution / colour version of this figure is available in the electronic copy of the article)*.

provide a new perspective in the research and contribute to the development of new treatment in the clinic. Multiple researches have demonstrated that drugs aimed at autophagy have therapeutic potential in IS. For example, a mitochondrial fusion protein, mitofusin 2, which is capable of enhancing the formation of autophagosomes and facilitate the fusion of autophagosomes and lysosomes, is suggested to alleviate ischemia-reperfusion induced damage [23]. Hamartin, a product of the tuberous sclerosis complex 1 gene, is demonstrated to confer neuroprotection against IS *via* induction of autophagy [24]. Melatonin, an endogenous hormone, is suggested to be neuroprotective against IS by inhibiting endoplasmic reticulum stress-dependent autophagy *via* PERK and IRE1 signaling pathways [25]. In this study, a comprehensive overview of the role of autophagy in the pathogenesis of IS is provided, and an attempt is made to shed new light on the design of new strategies against IS.

2. AUTOPHAGY PATHWAY UNDER A NORMAL PHYSIOLOGICAL SITUATION

The autophagy pathway is strictly controlled by variable regulatory constituents. Currently, the autophagy pathway is divided into four steps, including initiation, vesicle nucleation, vesicle elongation, fusion and degradation (Fig. **1**) [26]. Through these processes, autophagy accomplishes the metabolic and renewal needs of cells, degrading and recycling the long-lived or misfolded proteins and damaged organelles.

2.1. Initiation of Autophagy

Autophagy is initiated by autophagy inducers (nutrients absence, rapamycin, *etc.*), which dephosphorylate the mammalian target of rapamycin (mTOR) mTORC1 and cause the

disassociation of mTORC1 and the serine–threonine protein kinase, Unc-51-like kinase 1 (ULK1) [18]. The ULK1 complex comprises of ULK1, the autophagy-related proteins 13 (ATG13), ATG101, and focal adhesion kinase family interacting protein (FIP200) [19, 27-29]. Following the separation from mTOR, ULK1 then undergoes autophosphorylation and activates ATG13 and FIP200 through phosphorylation, thus initiating autophagy [18].

2.2. Vesicle Nucleation

The class III phosphatidylinositol 3-kinase (PI3K III) complex, including Beclin-1, vacuolar protein sorting 34 (VPS34), VPS15, and ATG14L, is responsible for vesicle nucleation [19, 30]. After the initiation of autophagy, the PI3K III complex is recruited to the location of autophagosome formation by the activated ULK1 complex [31]. The ULK1 kinase targets and activates the PI3K III complex by phosphorylating Beclin-1 [32], which facilitates VPS34 to produce phosphatidylinositol 3-phophate [33-37]. Phosphatidylinositol 3-phosphate then recruits the DFCP1 to accelerate the process of vesicle nucleation [37].

2.3. Vesicle Elongation

The vesicle elongation is mediated by two ubiquitin-like conjugates, ATG12-ATG5-ATG16L and ATG8 (LC3)-phosphatidylethanolamine (PE) [38]. ATG12 is conjugated to ATG7, ATG10, and ATG5 in order, forming the ATG12-ATG5 conjugate with the help of ATG7 and ATG10 [39, 40]. Integrated with ATG16-like (ATG16L), the ATG12-ATG5 conjugate becomes the ATG12-ATG5-ATG16L conjugate, which promotes cargo recruitment and vesicle elongation [41]. The conversion of Pro-LC3 to LC3 is mediated by ATG4. LC3 then conjugates with PE with the help of ATG3, ATG7, and ATG12-ATG5-ATG16L conjugate, forming LC3-PE conjugate that is responsible for the elongation of autophagosomes [42, 43].

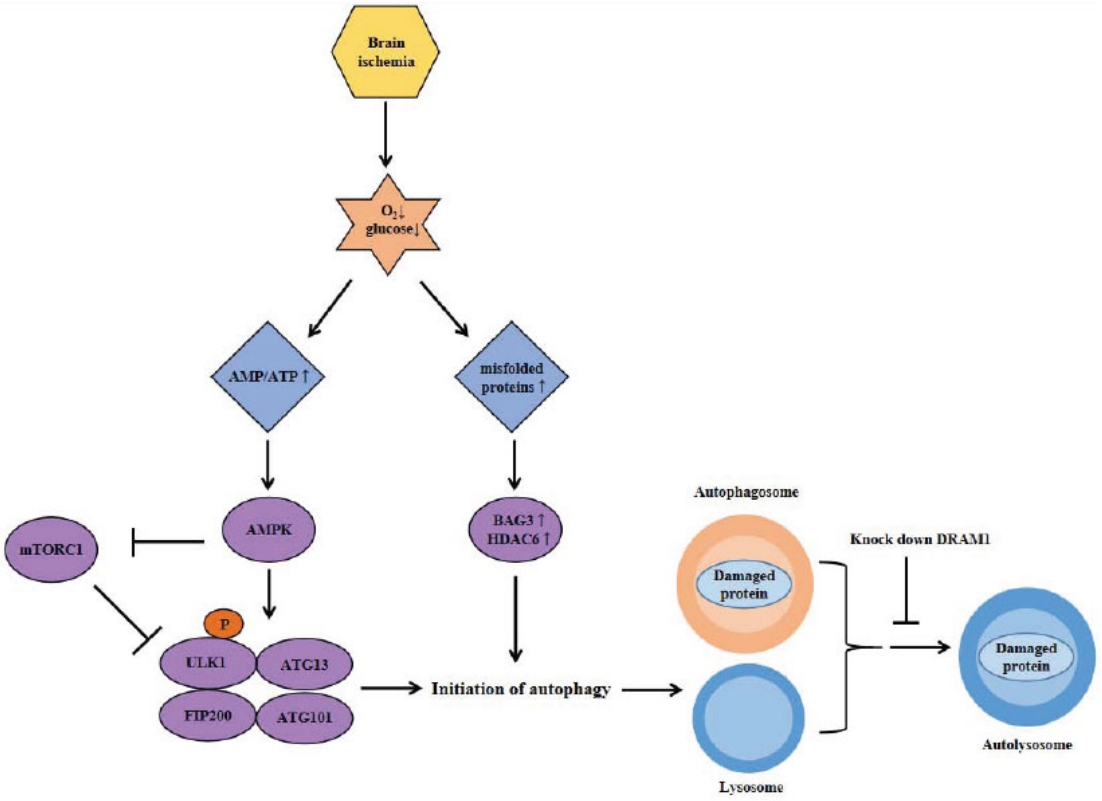

Fig. (2). Regulation of autophagy pathway in ischemic stroke. Brain ischemia leads to diminished oxygen and glucose supply to the brain, causing multiple metabolic changes. Exposed in nutrient-depleted condition, the ratio of AMP/ATP in brain cells becomes higher, which is a major stimulus for AMPK activation. Activated AMPK initiates autophagy through a direct and indirect pathway. In a direct pathway, AMPK activates the ULK1 complex by phosphorylating ULK1 at several serine residues, while in indirect pathway, AMPK activates it by inhibiting mTORC1. In IS, accumulated misfolded proteins disrupt the balance between the ubiquitin-proteasome and autophagy-lysosome pathway. Increased BAG3 and HDAC6 initiate the conversion of ubiquitin-proteasome pathway to autophagy-lysosome pathway. A study shows that knockdown DRAM1 inactivates the autophagy pathway through blocking the fusion of autophagosome and lysosome, indicating the role of DRAM1 in the autophagy pathway. Abbreviation: mTOR, mammalian target of rapamycin; AMPK, 5'-Adenosine monophosphate-activated protein kinase; ULK1 Unc-51-like kinase 1; ATG, autophagy related proteins; FIP, focal adhesion kinase family interacting protein; DRAM1, DNA damage-regulated autophagy modulator protein 1. *(A higher resolution / colour version of this figure is available in the electronic copy of the article)*.

Table 1. *In vivo* and In *vitro* Role of Autophagy in the Pathogenesis of Ischemic Stroke.

Animals	Model	Phenotypes	Role of Autophagy in Ischemic Stroke	References
Adult male Wistar rats	4VO	Hamartin induced autophagy	Protective	[24]
Adult male SD rats	tMCAO	Melatonin inhibited autophagy	Harmful	[25]
-	pMCAO	Metformin induced autophagy	Protective	[52]
-	4VO	Rapamycin induced autophagy	Protective	[58]
-	pMCAO and OGD/R*	3-MA or Wort inhibited autophagy	Harmful	[59]
-	pMCAO and tMCAO	Carnosine inhibited autophagy	Harmful	[60]
-	pMCAO	Compound C inhibited autophagy	Protective	[61]
-	pMCAO	3-MA inhibited autophagy	Harmful	[62]
-	dMCAO	3-MA or Becn1-shRNA inhibited autophagy	Harmful	[63]
-	pMCAO	3-MA or bafliomycin A1 inhibited autophagy	Harmful	[64]
-	tMCAO and OGD/R*	Eugenol induced autophagy	Protective	[65]
Adult SD rats	tMCAO	Homocysteine induced autophagy	Harmful	[66]
Adult male C57BL/6 J mice	tMCAO and OGD/R*	IL-17A induced autophagy	Harmful	[67]
-	pMCAO	Silibinin inhibited autophagy	Harmful	[68]
-	pMCAO	N-*acetylserotonin* inhibited autophagy	Harmful	[69]
-	tMCAO and OGD/R*	Schizandrin inhibited autophagy	Harmful	[70]
Adult male C57BL/6 mice	tMCAO	circHECTD1 knockdown inhibited autophagy	Harmful	[71]
-	tMCAO	3-MA inhibited autophagy	Harmful	[72]
-	OGD/R*	Rapamycin induced autophagy	Protective	[73]
GPR30$^{-/-}$ mice	tMCAO	GPR30 knockout inhibited autophagy	Protective	[74]
IL-21$^{-/-}$ mice	tMCAO	IL-21 knockout inhibited autophagy	Harmful	[75]
GPR37$^{-/-}$ mice	tMCAO	GPR37 knockout induced autophagy	Harmful	[76]
TIGAR$^{-/-}$ mice and TIGAR-transgenic mice	tMCAO and OGD/R*	TIGAR knockout induced autophagy and TIGAR transgene inhibited autophagy	Harmful	[77]
Male arrb1$^{-/-}$ and arrb2$^{-/-}$ mice	tMCAO	ARRB1 knockout inhibited autophagy	Protective	[78]
ATF6 knock-in mice	tMCAO	ATF6 knock-in induced autophagy	Protective	[79]
Irgm1$^{-/-}$ mice	pMCAO	IRGM1 knockout inhibited autophagy	Protective	[80]
Neonatal Wistar rats	ischemia-hypoxia	Lithium inhibited autophagy	Harmful	[81]

Abbreviations: SD, Sprague Dawley; 4VOs, 4 vessels occlusion; tMCAO/pMCAO/dMCAO, transient/permanent/distal middle cerebral artery occlusion; OGD/R, oxygen glucose deprivation/reperfusion; * means *in vitro*.

2.4. Fusion and Degradation

Autophagy receptor or adaptor proteins, including SNARE syntaxin 17 [44], PLEKHM1 [45], and Atg14 [46], mediate the fusion of autophagosomes and lysosomes for the formation of autolysosomes where target proteins or organelles are digested by lysosomal enzymes. Through such a process, substances like amino acids, lipids, and nucleosides, are produced for reuse by the cells [22]. Recently, it was discovered that some proteins (*e.g.* autophagy related proteins 14) are required not only for the initiation of autophagy but also for the fusion of autophagosomes with lysosomes [46].

2.5. Termination of Autophagy

Nutrients generated by autolysosomes act as activators for mTOR [38, 47, 48], leading to the inactivation of ULK1 complex and the termination of autophagy. Moreover, reactivated mTOR leads to the regeneration of lysosomes by generating proto-lysosomal tubules and vesicles, which are extruded from autolysosomes [38, 47, 48].

Except for mTOR reactivation, autophagy can achieve self-inhibition regardless of stress conditions. Such a mechanism is mediated by CULLINs, the E3 ubiquitin ligases, which participate in the degradation of autophagy proteins [48]. Through the ubiquitin-proteasome pathway, CULLINs mediate the degradation of members of ULK and VSP34 complex and lead to autophagy termination eventually [48].

3. AUTOPHAGY PATHWAY IN ISCHEMIC STROKE

Occlusion of cerebral arterial circulation leads to diminished oxygen and glucose supply to the brain, causing various subsequent metabolic changes. Following infarction, cells in the brain are exposed to nutrient-depleted conditions, manifesting higher ratio of AMP/ATP, which is a ma-

jor stimulus for AMPK activation [49]. Activated AMPK initiates the autophagy pathway by activating the ULK1 kinase complex through direct and indirect pathways (Fig. **2**) [50].

In a direct pathway, AMPK activates the ULK1 kinase complex by phosphorylating ULK1 at several serine residues, while in an indirect pathway, AMPK activates it by inhibiting mTORC1 [50]. Several studies suggest that the activation of AMPK mediates the autophagy-related neuroprotection [51-53]. In addition, the number of misfolded proteins surges in IS, which overloads the ubiquitin-proteasome pathway and disrupts the balance between the ubiquitin-proteasome and autophagy pathway [9]. Increased BAG3 and HDAC6 initiate the conversion from the ubiquitin-proteasome pathway to the autophagy pathway [54], which leads to the initiation of the autophagy pathway in IS (Fig. **2**). There is a study demonstrating that the autophagy pathway is activated by the expression of DNA damage-regulated autophagy modulator protein 1 (DRAM1), which is expressed in IS [55]. And knockdown of DRAM1 inactivates the autophagy pathway through blocking the fusion of autophagosome and lysosome and intensifies the cell death in IS [55]. In conclusion, autophagy inducers (such as nutrient deprivation and misfolded proteins accumulation) initiate autophagy through various mechanisms.

4. ROLES OF AUTOPHAGY IN THE PATHOGENESIS OF ISCHEMIC STROKE

Accumulating data have indicated that autophagy is involved in the pathogenesis of IS, and regulating the activity of autophagy can change the outcomes of IS. However, whether autophagy is beneficial or detrimental for the survival of neuronal cells in IS is controversial, and currently, it is thought of as a double-edge sword [56, 57]. After searching in PubMed and other resources, most of the current *in vivo* and *in vitro* studies are listed in Table **1**.

4.1. Detrimental Role of Autophagy in Ischemic Stroke

Studies, including *in vivo* and *in vitro* findings, suggest that autophagy may exhibit a detrimental influence on the outcome of IS. Autophagy inhibitors, 3-methyladenine (3-MA) or wortmannin (Wort), reduced infarction volume in rats of middle cerebral artery occlusion (MCAO) model [59, 62-64, 72]. Atg5 is an indispensable component in the vesicle elongation process of autophagy and showed a neuroprotective effect of astrocytes. Atg5 gene knockout mice had less cortical injury [59]. TP53-induced glycolysis and apoptosis regulator (TIGAR), a regulator of the autophagy pathway, was shown to ameliorate cerebral ischemia/reperfusion (I/R)-induced neuronal injury [77]. In $TIGAR^{-/-}$ mice, I/R-induced injury is partly prevented by 3-MA, an autophagy inhibitor [77]. While in $TIGAR^{+/+}$ mice, the neuroprotective effect of TIGAR was partly abolished by rapamycin, an autophagy activator [77]. IL-21 contributed to the neuronal injury after IS and IL-21 promoted autophagy expression in neuronal cells after hypoxia/ischemia, suggesting that autophagy might exert harmful impact on IS [75]. GPR37 knockout mice showed increased infarction and autophagic cell death compared with wild-type mice after IS, which suggested that autophagy participates in the pathogenesis of IS and its effect is detrimental [76]. Moreover, multiple agents, including carnosine [60], melatonin [25], lithium [81], schizandrin [70], silibinin [68], homocysteine [66], bafilomycin [64], N-acetylserotonin [69], were reported to either exert neuroprotection by inhibition of autophagy or aggravate ischemic brain damage by its' activation.

4.2. Beneficial Role of Autophagy in Ischemic Stroke

Many studies also suggested that autophagy may have a neuroprotective effect on IS. In a 4 vessel occlusion (4VO) model of mice, administration of autophagy activator rapamycin showed less infarction volume, neuronal injury, and motor deficits [58]. Such a result was also found in an *in vitro* oxygen glucose deprivation/reperfusion (OGD/R) model [73]. Metformin, a famous first-line diabetes drug, initiates autophagy by activating AMPK, thus exerting a protective role in IS [52]. IRGM1 knockout mice were reported to have more severe brain damage in IS and lower level of autophagy activity than wild type mice [80]. Other genetically engineered models of mice, such as ATF6 knock-in mice [79], ARRB1 knockout mice [78], and GPR30 knockout mice [74], also demonstrated that autophagy has a protective role in IS. Various agents, including hamartin [24], compound C [61], and eugenol [65], were reported to be either neuroprotective by activation or deleterious by inhibition of autophagy.

Many studies did not take sex as a variable when analyzing the experimental outcomes of IS research. However, accumulating data shows that sex differences play an important role in the regulation of the autophagy pathway and may lead to the different outcomes between males and females [82-84]. There are several mechanisms through which sex differences occur in IS and the most widely accepted are gonadal hormones and chromosomal makeup hypotheses. Gonadal hormones, including androgens and estrogens, modulate the autophagic pathway *via* their receptors, androgen receptor (AR), estrogen receptor 1 (ESR1), and estrogen receptor 2 (ESR2). Increasing evidence indicates that AR, together with ESR1 and ESR2, is involved in the translational modulation of many autophagy related genes which act in the processes of vesicle nucleation and elongation in humans [82]. By binding to introns of target genes, AR regulates the transcription of ULK2, ATG3, ATG5, BCL2, AMBRA1 (autophagy, and beclin 1 regulator 1), and PIK3C3 (phosphatidylinositol 3-kinase catalytic subunit type 3) [82]. There are 19 autophagy genes regulated by ESR1, including ULK2, ATG5, LC3B (light chain 3 beta), PIK3C3 (phosphatidylinositol 3-kinase catalytic subunit type 3), and SQSTM1, and 12 autophagy genes regulated by ESR2, including ULK2, ATG7, ATG13, UVRAG (UV radiation resistance associated), and AMBRA1 (autophagy and beclin 1 regulator 1) [82]. In addition, accumulating data indicate that many genes located on the X chromosome participate in autophagic pathway or regulate its' activity, including ATP6AP2 (ATPase H+ transporting accessory protein 2) and LAMP2 (lysosomal associated membrane protein 2) [82].

Such genes located on the X chromosome may explain the sex differences between menopausal female and age-matched male morbidity, fatality rate, and disabling rate in IS.

Recent studies showed that autophagy also participates in the regulation of microglia activation in neuroinflammatory diseases. Shibutani *et al.* and Deretic *et al.* found that autophagy suppresses the activation of inflammasomes in microglia through the proteolytic elimination of its adaptor protein ASC or its substrate pro-IL-1β, as well as clearance of damaged mitochondria by mitophagy [22, 85-87]. Accordingly, autophagy is proved to participate in the regulation of microglial inflammation in a rodent model of brain ischemia [22, 88-90]. However, whether autophagy promotes or inhibits inflammatory response after IS is controversial and yet to be elucidated. Apart from the inhibition of inflammasome activation, autophagy can also modulate the phenotypic transformation of microglia *via* the nuclear factor-κB pathway, which is conducive for the recovery of neural tissue after ischemia [22].

Although the exact role of autophagy in the pathogenesis of IS is ambiguous and inconsistent, it is unanimously believed that moderate activation of autophagy is neuroprotective, while excessive autophagy activation is harmful [22]. The inconsistent results of these studies may be due to several reasons. First, the time of activation or inhibition of autophagy was different among these studies. As a self-protective mechanism, activation of autophagy at the early stage of IS is neuroprotective *via* degrading misfolded proteins and damaged organelles to maintain the intracellular environment [91]. Interestingly, several studies suggested that autophagy participates in the ischemic preconditioning-inducing neuroprotection [61, 92-94]. In these studies, activation (Sphingosine kinase 2 [92] and conventional protein kinase C γ [93]) or inhibition (compound C [61] and 3-MA [94]) of autophagy, enhanced or diminished the neuroprotective effects of the ischemic preconditioning. Therefore, activation of autophagy at the early stage of IS or before IS is neuroprotective. However, if the ischemic stress continues for a long time, continuously activated autophagy will cause damage. Second, the extent of autophagy activation varied among these studies due to the different models and modulators. As mentioned before, moderate autophagy activation is protective, while excessive autophagy activation is deleterious in IS. Last but not the least, the agents used in some studies are not specific to autophagy. For example, 3-MA is widely used as an inhibitor for autophagy in various studies. However, 3-MA is not specific for targeting the autophagy pathway. 3-MA acts as an autophagy inhibitor [95] through inhibiting PI3K pathway, which participates in other biological processes, including necrosis and apoptosis [96, 97], besides autophagy activation [98, 99]. Rapamycin, an autophagy activator, initiates autophagy *via* inhibiting mTOR activity. But the mTOR signaling pathway is also suggested to have immunosuppressive and antiproliferative impacts [100, 101]. Therefore, it is hard to tell which signaling pathway, caused by these agents, actually influences the outcome of IS. To solve these problems, standardized protocols of IS models and more specific agents for autophagy need future studies.

5. POTENTIAL THERAPEUTIC STRATEGIES FOR ISCHEMIC STROKE TARGETING AUTOPHAGY PATHWAY

Multiple potential therapeutic agents for IS targets the autophagy pathway (Table **2**). These agents modulate different processes of autophagy to exert a neuroprotective effect through variable signaling pathways. The first category is targeting the initiation of autophagy. Rapamycin, an autophagy activator, initiates autophagy by inhibiting mTORC1 to disinhibit the ULK1 complex, which is the key component of autophagy initiation [102]. It has been reported that the administration of rapamycin in rodent models of focal ischemia can diminish infarct volume and augment behavior outcomes [103]. Interestingly, it was found that lower doses of rapamycin were associated with better and improved outcomes, which is potentially due to an optimal level of autophagy activation. Hamartin, a protein encoded by the tuberous sclerosis complex 1 gene (TSC1), has been reported to exert neuroprotection through the mechanism, which is similar to rapamycin [24]. Metformin, the first-line medicine for type 2 diabetes treatment [104, 105], activates AMPK to initiate autophagy. It is suggested that metformin preconditioning is beneficial for the outcomes of cerebral ischemia [52]. Other agents targeting autophagy initiation include fingolimod [106], N-acetyl-serotonin [69], minocycline [107], and carnosine [60]. The second category is targeting vesicle nucleation of autophagy. Melatonin, an endogenous hormone, is demonstrated to alleviate neuronal injury after IS by impeding the expression of Beclin-1 [25, 108], which is critical for vesicle nucleation of autophagy. Interestingly, N-acetyl-serotonin, a precursor of melatonin, is reported to be neuroprotective similar to melatonin but with different mechanisms [69]. N-acetyl-serotonin exerts its effects through inhibiting the initiation of autophagy while melatonin plays its roles through suppressing the vesicle nucleation of autophagy [25, 69, 108]. Bexarotene, an FDA-approved agent, is suggested to have neuroprotective potential in stroke [109]. By suppressing the degradation of the autophagosome, a double-membrane vesicle enfolding substrates needs degradation, bexarotene acts on vesicle elongation to promote autophagy [109]. 3-MA, a classic autophagy inhibitor, inhibits ischemia-induced LC3 expression to repress vesicle elongation, thus diminishes autophagy-induced injury in IS [64].

Although agents targeting the autophagy pathway have therapeutic potential for IS, there are possible side effects that need to be considered. Long term administration of rapamycin, which activates autophagy pathway through inhibiting mTORC1, may also inhibit mTORC2 and cause immunosuppression and glucose intolerance [110]. Metformin initiates autophagy *via* activating AMPK. However, many patients can not tolerate adequate amounts of metformin because of its associated gastrointestinal adverse events, including diarrhea, nausea, flatulence, indigestion, vomiting, and abdominal discomfort [111]. As the first-line medicine for type 2 diabetes treatment [104], metformin may cause hypo-

Table 2. Potential Therapeutic Agents for Ischemic Stroke Targeting Autophagy Pathway.

Agents	Targeting Autophagy Pathway	Pharmacological Effects	References
Hamartin	Initiation of autophagy	Inhibition of mTORC1 to disinhibit ULK1 complex	[24]
Metformin	Initiation of autophagy	Activating ULK1 complex by activating AMPK	[52]
Carnosine	Initiation of autophagy	Reversing the inhibited phosphorylated levels of mTOR by ischemia	[60]
3-MA	Vesicle nucleation	Inhibition of ischemia-induced LC3 expression	[64]
N-acetyl-serotonin	Initiation of autophagy	Reducing the activation of autophagy	[69]
Rapamycin	Initiation of autophagy	Inhibition of mTORC1 to disinhibit ULK1 complex	[102]
Fingolimod	Initiation of autophagy	Activating mTOR/p70S6K pathway to suppress autophagy	[106]
Minocycline	Initiation of autophagy	Reducing the activation of autophagy	[107]
Melatonin	Vesicle nucleation	Impeding the expression of Beclin-1	[108]
Bexarotene	Vesicle elongation	Inhibition of autophagosome degradation	[109]

glycemia when administrated with a high dosage in a short period. Therefore, finding an optimal agent dosage is necessary for maximizing the neuroprotective effect while minimizing the possible side effects.

6. CONCLUSION AND FUTURE PERSPECTIVES

Autophagy is a strictly controlled multi-stages process and has been suggested to be involved in multiple diseases, including stroke [19, 57], rheumatology [112], cancer [113, 114], neurodegenerative disease [115, 116], cardiovascular disease [117, 118], and so on. As an evolutionarily conserved process, autophagy maintains cellular homeostasis by eliminating misfolded proteins and injured organelles [38]. Canonical autophagy pathway comprises initiation, vesicle nucleation, vesicle elongation, fusion and degradation [38, 119]. While during IS, diminished oxygen and glucose supply leads to the accumulation of AMP, which activates AMPK, thus initiating autophagy [49, 50]. In addition, the accumulation of misfolded proteins during IS causes upregulated BAG3 and HDAC6, leading to the conversion from the ubiquitin-proteasome pathway to the autophagy-lysosome pathway [9, 54]. The research community has reached a consensus that autophagy is responsible for the maintenance of cerebral homeostasis during IS [19]; however, it is still ambiguous about the exact role of autophagy in the pathogenesis of IS. On one hand, some research suggested that autophagy is detrimental to the outcomes of cerebral ischemia [25, 60, 67]. On the other hand, some studies showed a neuroprotective effect of autophagy during IS [24, 52, 58]. Whether autophagy is a friend or a foe in IS is hard to tell. But it is unanimously believed that moderate activation of autophagy is neuroprotective, while excessive autophagy activation is harmful [22]. The incongruous results of these studies may be due to different IS models and agent administration, which lead to different time and extent of modulation of autophagy.

Currently, the only medication that the FDA has approved for IS is limited to thrombolytic treatment [57], whose clinical application value is not promising due to its short therapeutic window and risk for intracerebral hemorrhage. Therefore, studies focused on potential therapeutic strategies for IS are urgently needed. Many researchers have found potential therapeutic strategies for IS, targeting the autophagy pathway [24, 52, 102]. But many agents targeting autophagy are not specific and even genetically engineered models of autophagy genes have limitations since autophagy-related genes also exert autophagy-independent functions [96, 99, 119]. Hence, more specific agents or genetic tools and unified disease models are needed to decipher the role of autophagy in the pathogenesis of IS in future studies. With a more comprehensive and profound understanding of the role of autophagy in the pathogenesis of IS, novel therapeutic strategies for IS are on their way.

LIST OF ABBREVIATIONS

3-MA = 3-methyladenine

AMPK = AMP-Activated Protein Kinase

AR = Androgen Receptor

ATG16L = ATG16-like

DRAM1 = Damage-Regulated Autophagy Modulator Protein 1

ESR1 = Estrogen Receptor 1

ESR2 = Estrogen Receptor 2

IS = Ischemic Stroke

mTOR = Mammalian Target of Rapamycin

OGD/R = Oxygen Glucose Deprivation/Reperfusion

PE = Phosphatidylethanolamine

PI3K III = Class III Phosphatidylinositol 3-kinase

TIGAR = TP53-induced Glycolysis and Apoptosis Regulator

ULK1 = Unc-51-like kinase 1; VPS34, vacuolar protein sorting 34

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

This work was supported by the National Natural Science Foundation of China grants (81873769).

CONFLICT OF INTEREST

The authors have no conflicts of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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