

# The Emerging Role of the Interaction of Extracellular Vesicle and Autophagy—Novel Insights into Neurological Disorders

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**Abstract:** Eukaryotic cells release different types of extracellular vesicles (EVs), including exosomes, apoptotic bodies and microvesicles. EVs carry proteins, lipids and nucleic acids specific to cells and cell states. Autophagy is an intracellular degradation process, which, along with EVs, can significantly affect the development and progression of neurological diseases and, therefore, has been the hotspot. Generally, EVs and autophagy are closely associated. EVs and autophagy can interact with each other. On the one hand, the level of autophagy in target cells is closely related to the secretion and transport of EVs. In another, the application of EVs provides a great opportunity for adjuvant treatment of neurological disorders, for which autophagy is an excellent target. EVs can release their cargos into target cells, which, in turn, regulate the autophagic level of target cells through autophagy-related proteins directly and the non-coding RNA, signal transducer and activator of transcription 3 (STAT3), phosphodiesterase enzyme (PDE) 1-B, etc. signaling pathways indirectly, thus regulating the development of related neurological disorders.

**Keywords:** extracellular vesicle, autophagy, neurological disorders, non-coding RNA, STAT3, PDE1-B

## Introduction

Extracellular vesicles (EVs) are lipid-binding vesicles secreted by cells into the extracellular space, mainly composed of microvesicles (MVs), exosomes and apoptotic bodies.<sup>1,2</sup> They transport the cargos of proteins, nucleic acids, lipids, metabolites and organelles from the parent cell, and take an important role in biological functions.<sup>3,4</sup> EVs are released by nearly all cell types, such as oligodendrocytes, neurons, astrocytes, microglia and endothelial cells in the central nervous system (CNS).<sup>5-7</sup> EVs can stimulate neuronal development by facilitating neuron-glia contact and take a role in the pathophysiology of several neurological diseases, including stroke, multiple sclerosis (MS), Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), traumatic brain injury (TBI) and prion diseases.<sup>8-11</sup> Autophagy is a self-degradation process, which is a dynamic process that plays a key role in cell homeostasis by adapting cell metabolism to a stressful environment.<sup>12</sup> There are different types of autophagy, including typical macroautophagy, chaperone-mediated autophagy, and less studied microautophagy.<sup>13</sup> Meanwhile, autophagy is also associated with other inner membrane systems and signaling pathways to regulate endocytosis, exocytosis, and even the hydrolysis of biomolecules.<sup>14</sup> Growing evidence indicated that EVs participate in the excretion, degradation, and recovery of biomolecules, and play an important role in autophagy to promote cell survival.<sup>15,16</sup> Recent

studies have reported the ability of EVs to cooperate with autophagy flux for maintaining cell homeostasis. For example, adipose-derived mesenchymal stem cells (ADMSCs) or ADMSC-secreted EVs could induce neuroprotection by regulating autophagic flux through secreting EVs containing microRNA (miR) in ischemic stroke.<sup>8</sup> The present state of research into the involvement between EVs and autophagy in neurological disorders is summarized in this section, and describes the signaling pathways involved in mediating EVs-modulated autophagy.

## EVs and Neurological Disorders

### The Brief Course on Biogenesis of EVs

EVs, which serve as membrane-enclosed nanoscale particles, carry a variety of cargo, such as peptides, lipids, proteins nucleic acids (DNA, mRNA, short non-coding and long non-coding RNAs).<sup>17</sup> They act as regulation factors by their phenotypic effects on recipient cells and deliver information between cells both in a paracrine or endocrine manner.<sup>18</sup> These small bioactive molecules can also bear biomolecules of their parent cell of origin and may provide diagnostic and prognostic value in CNS diseases.<sup>19,20</sup> Multiple types of EVs exist, which can be categorized into 3 different types, the basis on their biogenesis, size, and isolation method: exosomes, microvesicles, and apoptotic bodies.<sup>21</sup> The exosomes with heterogeneous sizes (30–150nm) and compositions that elicit differential molecular and biological properties are generated from multivesicular bodies (MVB) in the endosomal system, which bud directly from the cell membrane.<sup>22</sup> Regarding the microvesicles (50–1000nm), they are generated by an outward budding from the plasma membrane of the cell and occur selectively in the lipid-rich microdomains of the membrane.<sup>21</sup> Apoptotic bodies (50–5000nm) are another type of EVs that are larger than exosomes and microvesicles, which are exclusively released from the cells undergoing apoptotic cell clearance during the last steps of the apoptosis process including cell dismantling and recycling of biomolecule building blocks.<sup>23,24</sup>

### The Vital Role of EVs in Neurological Disorders

Studies on EVs have contributed to broadening our current acknowledge of the physiology and pathology of nervous system disease. Depending on a cell of origin, microenvironment and the status of the disease, EVs can present a variety of functions. EVs, derived from neurons, astrocytes, microglia and oligodendrocytes, serve as regulators and mediate cell to cell communication.<sup>25</sup> Under neurophysiological conditions, neuronal EVs can regulate the differentiation of neural axons by transferring neuron-specific cargoes to a variety of glial cells and regulating relevant functions.<sup>26</sup> For instance, miR-124-3p can be delivered by neuronal EVs from primary neurons to astrocytes and increase the expression of the glutamate transporters.<sup>27</sup> Moreover, EVs secreted by microglia can transfer nervous growth factors to neurons.<sup>28</sup> Microglia also absorbs myelin debris through oligodendrocytes-derived EVs.<sup>29</sup> Nevertheless, EVs show several different properties under pathological conditions. On the one hand, EVs exert protective effects on neurons and remove pathological deposition proteins. For example, the relationship between alternative EVs molecules and prognosis of stroke has already been explored.<sup>30</sup> This characteristic can be utilized to distinguish several subtypes of stroke with high accuracies, such as spontaneous intraparenchymal hemorrhage, aneurysmal subarachnoid hemorrhage and ischemia stroke.<sup>31</sup> Additionally, EVs have great potential in the treatment of stroke, compared to the narrow time window of the traditional method. Neural progenitor cell (NPCs)-EVs, as a critical factor, participate in neurogenesis and neural restoration in post-stroke.<sup>32</sup> Furthermore, the effect of NSC-EVs has also been evaluated in an animal ischemia stroke model.<sup>33</sup> The result of pathologic and symptomatic status improved significantly after administrating EVs after 24 hours, including alleviating lesion volume and brain swelling. Likewise, Xin et al have demonstrated that gene-modified EVs can enhance neuroplasticity and function recovery after MCAO treatment in rat.<sup>34</sup> On the other hand, EVs can accelerate the pathological process of neurological disorders including inflammation, apoptosis, and autophagy. Microglia transfers EVs to astrocytes during neuroinflammation, which initiates the trigger to activate A1 astrocytes, leading to neuronal damage.<sup>35</sup> Besides, ceramide-containing EVs from astrocytes can cause neural apoptosis during AD pathological proteins.<sup>36</sup>

## Autophagy and Neurological Disorders

### The Brief Course on Autophagy

Autophagy is a general term that mainly refers to self-eating cellular catabolism,<sup>37</sup> which is a process induced by changes in the internal conditions of cells,<sup>38</sup> such as starvation, hypoxia nutrient deficiencies, and infection,<sup>39</sup> resulting in degrade toxic proteins, damaged organelles and invading pathogens via the lysosomal pathway.<sup>40</sup> It plays a vital role in stabilizing cell homeostasis by orderly degradation and recycling cellular components. As much as we know, three different kinds of autophagy are found in mammalian cells according to their methods of substrate delivery: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA).<sup>41</sup> Macroautophagy is an intracellular degradation system for the majority of proteins and some organelles,<sup>42</sup> which means it can break down cargo and then allow for the recycling of the resulting macromolecules, and the recycling of substrates through macroautophagy has an important role in maintaining cellular homeostasis.<sup>43</sup> Microautophagy, in which the lysosomal degradative process uses autophagic tubes to mediate both invagination and vesicle scission into the lumen by direct engulfment of cytoplasmic cargo.<sup>44</sup> It also exerts an effect on cellular homeostasis. The major role of CMA in cellular metabolism is supposed to supply amino acids after protein degradation. However, some recent researchers find that the energy metabolism can also be significantly altered after the function of CMA impairment.<sup>45</sup>

### A Double-Edged Sword Role of Autophagy and Possible Signaling Pathway

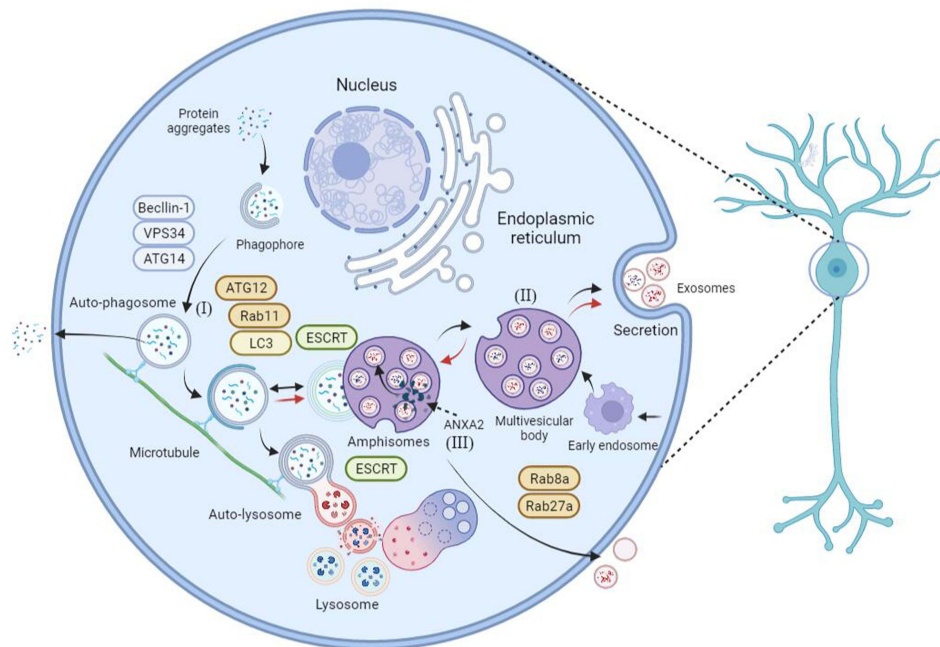
In mammals, cells in the CNS are especially vulnerable to damage. Autophagy is the main response against aggregate-prone proteins, defective organelles and insoluble protein aggregates, which plays an important housekeeping role in the CNS.<sup>46</sup> Despite it has been unclear whether autophagy plays a potential beneficial or harmful role in the CNS, there is no doubt that autophagy critically contributes to neuronal fate.<sup>47</sup> Under the ischemic condition, neurons are particularly vulnerable and autophagy is thought to be activated by the clearance of protein aggregates and damaged mitochondria and preservation of energy balance.<sup>48</sup> Meanwhile, numerous direct evidence has been verified that autophagy could be a therapeutic target in ischemic stroke. Researchers reported that rapamycin stimulates autophagy, which then improves mitochondrial function and reduces infarct volume, brain edema, and motor impairments resulting from ischemia.<sup>49,50</sup> However, this function could be reversed by administrating 3-methyl-adenine (3-MA) through intracerebroventricular injection, an autophagy inhibitor.<sup>51</sup> Some other studies find that autophagy is also related to the promotion of cell death. It is possible that excessive up-regulation of autophagy and long-term autophagy eventually result in self-digestion or have harmful effects.<sup>52</sup> Interestingly, in some AD and PD patients, accumulation of autophagy vesicles (autophagosomes and lysosomes) has been observed, leaving the question of whether the suppressive or excessive autophagy leads to the accumulation of vesicles.<sup>53,54</sup> Multiple signaling pathways can be activated under the stressed condition that subsequently feeds into the autophagy pathway<sup>47</sup> mainly in neurodegenerative diseases. ULK1/2 forms a complex with ATG13, ATG101, and focal adhesion kinase family-interacting protein of 200 kDa (FIP200). Mammalian target of rapamycin complex 1 (mTORC1) and AMP-dependent protein kinase (AMPK) can be activated by nutrients, growth factors, and AMP/ATP respectively, which, regulate the ULK1/2 complex through a series of phosphorylation events,<sup>55</sup> thereby in response to the autophagy activation. Some studies suggest that the PI3K/Akt/mTOR pathway could regulate ischemia injury.<sup>56,57</sup> One research also has elaborated that knockout of p50 (NF- $\kappa$ B1) enhanced autophagy by repression of mTOR in cerebral ischemic mice<sup>58</sup> and hypoxia-inducible factor 1 (HIF-1), reactive oxygen species (ROS), and AMP-activated protein kinase (AMPK) are all involved in response to hypoxia during cerebral ischemia.<sup>59,60</sup> One analysis of brain tissue from AD patients found the role of Keap1 in autophagosomes.<sup>61</sup> Besides, in HD models, overexpression of IRE1 kinase leads to impaired autophagic flux<sup>62</sup> and IRE1 can recruit TRAF2/ASK same as activation of JNK, disrupting its interaction with BECN1, therefore activating autophagy.<sup>63</sup>

### The Crosstalk of Impact of Autophagy on EVs

Cells use EVs to communicate with the environment and surrounding cells by carrying information such as lipids, proteins, or nucleic acids.<sup>64,65</sup> Autophagy plays a vital role in the synthesis and degradation of EVs. The mechanism of EVs secretion and transport seems to be closely related to the formation of autophagosomes.<sup>64</sup> It has been reported that

extracellular components can be degraded through the endo/exosome pathway, while intracellular components can be delivered to lysosomes through the process of autophagy, ultimately leading to the degradation of lysosomal contents.<sup>66</sup> In addition, autophagosomes not only have a strong ability to fuse with lysosomes but also can fuse with multivesicular bodies to form amphiphiles, which eventually fuse with lysosomes and dissolve the inner material of intraluminal vesicles (ILVs).<sup>64</sup> Therefore, intracellular autophagy plays a decisive role in the secretion and transport of EVs. Elevated levels of autophagy significantly inhibited EVs release due to increased fusion of multivesicular bodies with autophagic vacuoles.<sup>67</sup> In contrast to the release of EVs, the precursor MVBs of exosomes can be cleared through the autophagy-lysosome pathway by direct fusion with lysosomes or autophagosomes. This is because multivesicular bodies directly enter the autophagy pathway after autophagy activation, and then significantly reduce the release of EVs<sup>68</sup> (Figure 1).

In neurodegenerative diseases, the aberrant protein aggregation of  $\alpha$ -synuclein is thought to be critical.<sup>69</sup> EVs are considered to serve as a vehicle for this abnormal protein to transport from cell to cell. The current studies showed that EVs with  $\alpha$ -synuclein are fully capable of inducing abnormal protein aggregation in recipient neurons, impairing autophagic flux by upregulating PELI1, which in turn leads to the degradation of LAMP-2.<sup>69–71</sup> Willén et al indicated that AD-linked  $\beta$ -amyloid ( $A\beta$ ) causes EV enlargement and that amyloid fibrils can act in the endocytic pathway of neurons. In turn, altering EVs can also lead to the accumulation and aggregation of  $A\beta$ .<sup>72</sup> In addition, the EVs containing  $\alpha$ -synuclein and beta-amyloid, as well as other pathogenic proteins, are involved in mediating autophagy and inflammation, and a similar discovery of their potential role is also confirmed in AD.<sup>73–77</sup> Houtman et al<sup>77</sup> and Ahmed et al<sup>76</sup> found that the inflammatory response promoted by the NLRP3-Caspase-1 inflammasome pathway triggers autophagy dysfunction and  $A\beta$  accumulation, and can be amplified and regulated by LC3-positive vesicles, further confirming the important role of autophagy in AD pathological progression. Furthermore, recent evidence suggests that aggregation of  $\alpha$ -synuclein and the resulting cytotoxicity are hallmarks of PD. The secretory pathway of  $\alpha$ -synuclein oligomers is



**Figure 1** EVs biogenesis and autophagy in neurological diseases. Existing studies have shown that abnormal protein accumulation and aggregation are hallmarks of various neurological diseases, while exosome release and autophagic degradation are two ways to clear them, and there are multiple possible interactions between autophagy and exosome biogenesis: (I) Macroautophagy begins with phagophore formation and expansion: phagocytosis of cytoplasmic proteins and organelles when VPS34, beclin-1 and ATG14 form a complex and initiate phagophore nucleation and formation. Then, with the assistance of ATG12, Rab11 and LC3 proteins, it leads to the formation of autophagosomes. The autophagosome moves along microtubules, during which it can fuse with MVBs and exchange substances to form two bodies. Both autolysate and amphibian formation are controlled by ESCRT proteins. It then fuses with the lysosome to degrade the engulfed contents.<sup>120</sup> (II) Maturation of early endosomes produces MVBs, late endocytic compartments containing numerous ILVs. Fusion of MVBs to the plasma membrane results in the release of ILVs into the extracellular space as exosomes. (III) Amphisomes can fuse with the plasma membrane and secrete their contents. Shown is the autophagy-dependent secretion of ANXA2, where the amphisome intermediate is required for ANXA2 release in exosomes. This image is adapted from previous studies<sup>64,120,121</sup> published under the Creative Common Attribution License. **Abbreviations:** ANXA2, annexin A2; ESCRT, endosomal sorting complexes required for transport proteins; MVBs, multivesicular bodies; ILVs, intraluminal vesicles.

strongly influenced by autophagic activity. Compared with free  $\alpha$ -synuclein oligomers, exosome-associated  $\alpha$ -synuclein oligomers are more readily taken up by recipient cells and can induce more toxicity. Therefore, preventing  $\alpha$ -synuclein exosomes release and regulating autophagy may be a novel approach to preventing disease transmission in PD.<sup>69,78,79</sup> More importantly, by purifying microglia/macrophage EVs from the cerebrospinal fluid of PD patients, they confirmed the presence of  $\alpha$ -synuclein oligomers in CD11b<sup>+</sup> exosomes and that such exosomes inhibit autophagy by impairing autophagic flux, thereby disrupting intracellular homeostasis and exacerbating neurotoxicity, further supporting the involvement of exosomes in  $\alpha$ -synuclein-related pathological processes.<sup>80</sup> Taken together, the level of autophagy in target cells is closely related to the secretion and transport of EVs.

## EVs are Involved in Regulating Autophagy in Neurological Disorders

### Stroke

It is known that EVs play multiple roles in ischemic stroke, including inhibiting neuronal apoptosis, regulating autophagy, and reducing inflammation.<sup>9,34,81–84</sup> Especially, recent researches show that EVs and EV-cargos can be used as biomarkers for the different ischemic stroke stages and inhibit abnormal autophagy to improve neuroregeneration in preclinical ischemic stroke model.<sup>34,85–87</sup> Hence, we summarized EVs secreted from several kinds of cells, such as astrocytes, adipose-derived stem cells (ADSC), MSC and microglia, can alleviate neurological deficits by regulating autophagy in ischemic models (Table 1). Most studies showed these effects of EVs by using a rat or mouse model in their researches. Pei et al reported that EVs derived from astrocytes could enhance neuron viability, and inhibit OGD-induced apoptosis and levels of TNF- $\alpha$ , IL-6, and IL-1 $\beta$  via regulating autophagy.<sup>88</sup> In addition, inhibition or knockdown of PDE1-B significantly enhanced the autophagic flux in BV2 cells, promoted M2, and inhibited the M1 phenotype.<sup>89</sup> These EVs derived from conditioned microglia are expected to regulate cortical neuronal survival under ischemic conditions. On the other hand, many EV-miRNAs that improve ischemia-induced neuronal damage via regulating autophagy have been found. Chen et al demonstrated exosomes secreted from ischemic-preconditioned astrocyte (IPAS-EXOs) cultured with neurons exert neuroprotection.<sup>90</sup> They observed that these effects depend on circSHOC2 in IPAS-EXOs, suppressed neuronal apoptosis, and ameliorated neuronal damage by regulating autophagy and acting on the miR-7670-3p/SIRT1 axis in vivo model.<sup>90</sup> In addition, other researchers also indicated that EVs derived from astrocytes transferred miR-190b or miR-361 could inhibit OGD-induced autophagy and neuronal damage by targeting Atg7 or downregulating the AMPK/mTOR pathway, respectively.<sup>91,92</sup> Besides, EVs secreted from ADSCs have been widely researched and shown to exert beneficial effects in reducing infarct size by an intravenous injection.<sup>8,9</sup> Kuang et al indicated that miR-25-3p is the highest expressed miRNA in ADMSC-EVs that interacts with the p53 pathway, and miR-25-3p oligonucleotide mimics reduce cell death while anti-oligonucleotides regulate P53-BNIP3 signaling in primary neurons to increase autophagy flux and cell death in cerebral ischemia. Hence, AD-MSCs induce neuroprotection by improved autophagic flux through secreted EVs containing miR-25-3p.<sup>8</sup> In addition, miR-30d-5p-enhanced EVs derived from ADSCs inhibit autophagy-mediated microglia polarization to M1, promote M2 microglia/macrophage polarization and reducing infarct size and brain damage.<sup>9</sup> Finally, EVs derived from humans are also effective for ischemic impairment. miR-21-3p is significantly down-regulated in human umbilical vein endothelial cell (HUVECs)-EVs during hypoxia/reoxygenation (H/R). The miR-21-3p knockdown can activate autophagy and inhibit cell apoptosis, showing a protective effect on neuron cells treated by H/R.<sup>93</sup> Xia et al also illustrated that EVs secreted by MSCs inhibited autophagy and promoted angiogenesis by regulating and activating signal transducer and activator of transcription 3 (STAT3), reducing the infarct size, enhancing angiogenesis and reducing long-term neurological deficits.<sup>85</sup>

### Neurodegenerative Diseases

Current research has demonstrated that EVs are related to disease progression in neurodegenerative diseases. These EVs may have multiple and important physiological functions in AD and PD, from deregulating synaptic activity, promoting demyelination, modulating autophagy impairment and regulating microglia activity.<sup>94,95</sup> Recently, research into the effects of EVs on autophagy and the therapy of AD and PD has also been extensively studied (Table 2). EVs isolated from umbilical cord MSCs conditioned medium (ucMSCs-CM) exposed to BV2 microglial cells, showed that ucMSCs-EVs

**Table 1** Preclinical Studies Assessing the Effect of EVs on the Activation of Autophagy in Stroke

Author, Year	Country	Species	Model	Route	Cell Source	Effect	Autophagy Marker	Mechanism
Pei et al <sup>88</sup> 2019	China	Mice, Cells	MCAO, OGD	IV, co- incubation	AS	Inhibition	LC3, Beclin-1, P62	NA
Zang et al <sup>89</sup> 2021	China	Mice, Cells	MCAO, OGD	SI, co-incubation	BV2	Inhibition	LC3, P62	PDE1-B
Liu et al <sup>113</sup> 2021	China	Mice, Cells	MCAO, OGD	IV, co-incubation	M2 microglia	Inhibition	LC3, LAMP-1, Beclin-1, P62	miRNA-135a-5p/TXNIP/NLRP3
Chen et al <sup>90</sup> 2020	China	Mice, Cells	MCAO, OGD	IV, co-incubation	IPAS	Inhibition	LC3, Beclin-1, P62	miR-7670-3p/SIRT1
Pei et al <sup>91</sup> 2019	China	Cells	OGD	Co-incubation	AS	Inhibition	LC3, Beclin-1, P62	miR-190b
Kuang et al <sup>8</sup> 2020	Germany	Mice, Cells	MCAO, OGD	IV, co-incubation	ADMSCs	Inhibition	LC3	miR-25-3p
Jiang et al <sup>9</sup> 2018	China	Rats, Cells	MCAO, OGD	IV, co- incubation	ADSCs	Inhibition	LC3, Atg5, Beclin-1, P62	miR-30d-5p
Jiang et al <sup>93</sup> 2018	China	Cells	H/R	Co-incubation	HUVECs	Inhibition	LC3, Atg12, Beclin-1	miR-21-3p
Xia et al <sup>85</sup> 2020	China	Rats, Cells	MCAO, OGD	IV, co-incubation	MSC	Inhibition	LC3, Beclin-1, P62	STAT3

**Abbreviations:** MCAO, middle cerebral artery occlusion; OGD, oxygen-glucose-deprivation; ADSCs, adipose-derived stem cells; H/R, hypoxia/reoxygenation; HUVECs, human umbilical vein endothelial cells; AS, astrocytes; IPAS, ischemic-preconditioned astrocyte; ADMSCs, Adipose-derived mesenchymal stem cells; iPSC, induced pluripotent stem cells; MSC, mesenchymal Stem Cells; IV, intravenous injection; SI, stereotaxic injection.

**Table 2** Preclinical Studies Assessing the Effect of EVs on the Activation of Autophagy in Neurodegenerative Diseases

Author, Year	Country	Species	Animal Model	Route	Cell Source	Effect	Autophagy Marker	Mechanism
Xu et al <sup>96</sup> 2018	China	Cells	NA	Co-incubation	ucMSCs	Inhibition	LC3, P62, Beclin I	Autophagy - A $\beta$ 25-35
Guo et al <sup>80</sup> 2020	China	Cells	NA	Co-incubation	Microglia	Inhibition	LC3, P62, LAMP2	a-synuclein transmission
Xia et al <sup>98</sup> 2019	China	Mice, Cells	NA	SI, co-incubation	Plasma	Inhibition	LC3, Beclin-1, P62	a-synuclein transmission
Zhou et al <sup>99</sup> 2019	China	Cells	NA	Co-incubation	SH-SY5Y	Inhibition	LC3, P62	miR-19a-3p
Li et al <sup>100</sup> 2020	China	Mice, Cells	MPTP	IP, co-incubation	ADSC	Inhibition	LC3, P62	miR-188-3p

**Abbreviations:** LAMP2, lysosome-associated membrane protein 2; ADSC, adipose-derived stem cell; IP, intraperitoneal injection;  $\alpha$ -syn, alpha-synuclein; IN, intranasal administration; SI, stereotaxic injections.

promoted the phagocytosis of BV2 cells, affected the level of autophagy-related proteins (LC3, Beclin-1 and p62) and finally inhibited the proliferation and decreased cell death of BV2 cells induced by A $\beta$ 25–35.<sup>96</sup> Furthermore, injection of EVs derived from hypoxic MSCs could decrease the activation of astrocytes and microglia, down-regulate proinflammatory cytokines (TNF- $\alpha$  and IL-1 $\beta$ ), and up-regulate anti-inflammatory cytokines (IL-4 and IL-10) via regulating the activation of signal transducer and activator of transcription 3 (STAT3) and NF- $\kappa$ B.<sup>97</sup> On the other hand, a study uncovered that  $\alpha$ -synuclein-containing EVs released by microglia were able to induce protein aggregation in recipient neurons, whereas depleting microglia dramatically suppressed the transmission of  $\alpha$ -synuclein in PD. Moreover,  $\alpha$ -synuclein also impaired autophagy flux by upregulating PELI1, resulting in degradation of LAMP2 in activated microglia.<sup>80</sup> These results suggest that regulation of autophagy, blocking microglial delivery of  $\alpha$ -synuclein via exosomal pathways, has the potential to serve as a therapeutic target for PD.<sup>98</sup> Zhou et al indicated that enhancing the expression of miR-19a-3p in exosomes derived from SH-SY5Y cells inhibited the autophagy of recipient microglia through the AKT/mTOR signaling pathway.<sup>99</sup> Furthermore, using miR-188-3p-enriched EVs derived from ADSC could suppress autophagy and Pyroptosis, whereas increased proliferation via targeting CDK5 and NLRP3 in mice and MN9D cells.<sup>100</sup> Other studies have also revealed that  $\alpha$ -synuclein secretion in EVs can be affected by regulating autophagy.<sup>70,101–103</sup> Finally, substances such as rapamycin, curcumin and manganese can also affect the release of EVs from dopaminergic neurons by regulating autophagy, thereby reducing the aggregation of  $\alpha$ -synuclein.<sup>71,101,104</sup>

## TBI

There is growing evidence that EVs and autophagy play important roles in TBI, therefore, abnormal EVs secretion and autophagy may lead to further neuronal damage over time.<sup>105–107</sup> Recent studies have shown that the autophagy pathway is continuously activated after TBI, which may lead to aggravated neural damage (Table 3). For example, the level of miR-21-5p in neuronal exosomes increased from the acute phase of TBI. The use of this miR-21-5p-enriched neuronal exosomes could inhibit neuronal autophagy activity by inhibiting neuronal autophagy targeting Rab11a, thereby

**Table 3** Preclinical Studies Assessing the Effect of EVs on the Activation of Autophagy in Traumatic Brain Injury

Author	Year	Country	Species	Route	Source	Autophagy Marker	Effect	Mechanism
Wang et al <sup>107</sup>	2020	China	Rat, Cells	NA	Plasma	NA	Inhibition	NA
Li et al <sup>105</sup>	2019	China	Mice, Cells	Co-culture, IV	HT22, BV2	P62, LC3	Inhibition	miR-21
Li et al <sup>106</sup>	2019	China	Cells	IV	HT22	P62, LC3	Inhibition	miR-124-3p

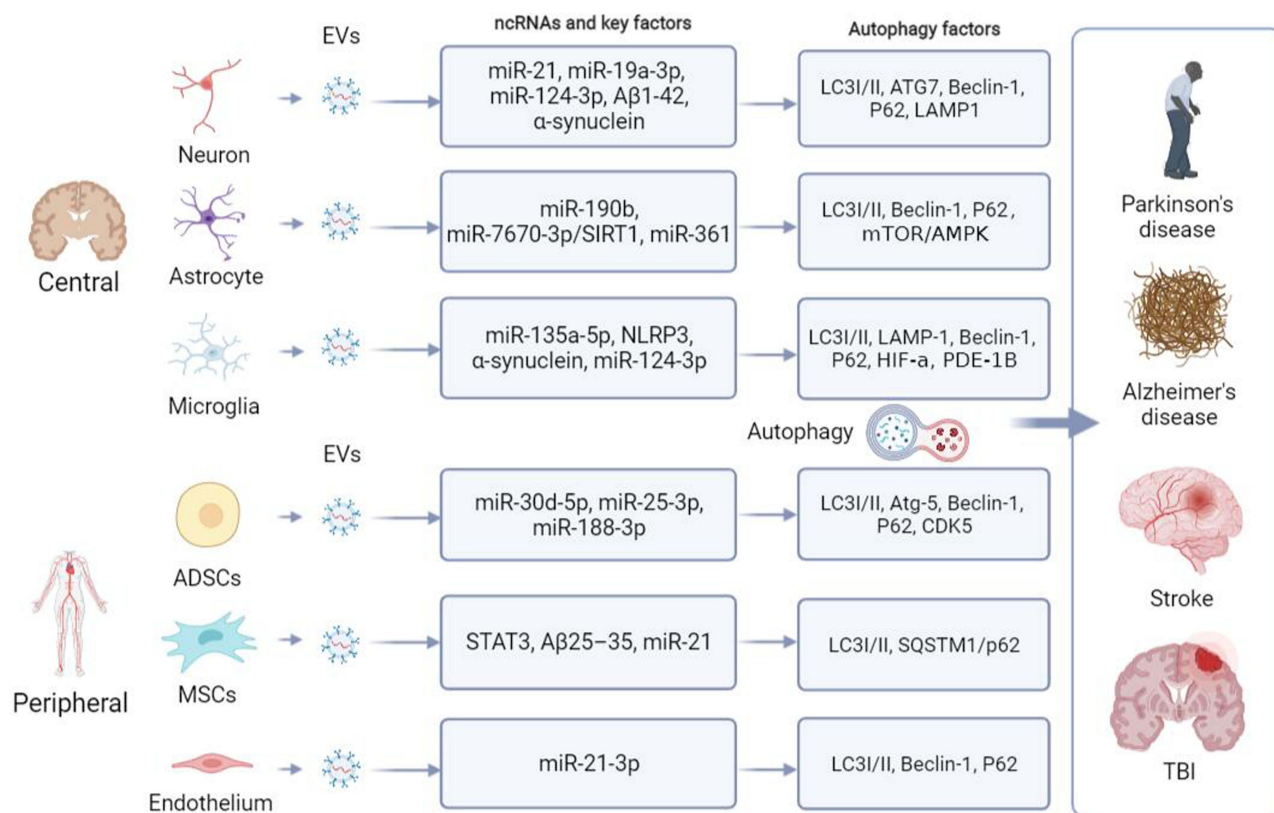
**Abbreviations:** TBI, traumatic brain injury, IV, intravenous injection; NA, not available.

attenuating trauma-induced autophagy-mediated neural damage in vitro.<sup>105</sup> Similarly, the increase of miR-124-3p in microglial exosomes after TBI might inhibit neuronal autophagy and prevent neuronal damage by translocating it into neurons.<sup>106</sup> Overall, treatment with these miR-enriched EVs may represent a new therapeutic strategy for the treatment of nerve damage after TBI. A schematic illustration of EVs derived from different cell sources and their respective roles in neurological diseases is shown in Figure 2.

## The Mechanism of EVs in the Effect of Regulating Autophagy

### ncRNAs in EVs are Key Players in Regulating Autophagy

Non-coding RNAs (ncRNAs), such as miRs, long non-coding RNAs and circular RNAs (circRNAs), are a large class of RNA transcripts that are transcribed from the genome, however, lack the function of encoding proteins.<sup>108</sup> At the RNA level, they can conduct their respective biological effects and play a vital role in cell growth, differentiation, replication, and apoptosis.<sup>108,109</sup> ncRNAs are also uncovered in the extracellular milieu, plenty of ncRNAs selectively sorted into EVs potentially regulate specific aspects of autophagy, thus protecting from degradation. Current shreds of evidence have demonstrated that ncRNAs are effective treatment candidates owing to their capacity of promoting neuronal recovery. Studies have demonstrated the effects of autophagy regulation of specific ncRNAs, especially for miRs, which are highly expressed in EVs.<sup>110</sup> miRs, the biggest family of ncRNAs containing 20–25 nucleotides, play key roles in the remodeling process under neurological disorders.<sup>110</sup> It is reported that miR-21-3p can attenuate brain injuries via multiple mechanisms.<sup>111,112</sup> Interestingly, the miR-21-3p expression was downregulated in HUVECs after a hypoxic condition, suggesting that miR-21-3p might be a potential effector.<sup>93</sup> To explore its involvement in autophagy pathways played by miR-21-3p in the protective effect of HUVECs-exosomes on hypoxia-treated neural cells, the pathway mediating the



**Figure 2** Schematic illustration of EVs derived from different cell sources and their respective roles in neurological diseases. In the central and peripheral system, different donor cells including neurons, microglia, and astrocyte can modulate their respective recipient cells by transferring various extracellular vesicle-cargos through modulating autophagy, thus regulating central nervous system diseases progression and recover.

**Abbreviations:** ADSCs, adipose-derived stem cells; MSCs, mesenchymal stem cells; EVs, extracellular vesicles; AD, Alzheimer's disease; TBI, traumatic brain injury.



effect of miR-21-3p was explored by focusing on the activity of ATG12 protein. Jiang et al conducted a dual-luciferase assay and revealed that miR-21-3p can directly bind to the 3'UTR sequence of the ATG12 gene and suppresses ATG12 signaling.<sup>93</sup> Likewise, Li et al indicated that the expression of miR-21-5p was increased in EVs derived from HT-22 neurons extracted from the TBI mice brain. EV-miR-21-5p produced a protective effect by suppressing autophagy in HT-22 neurons after scratch injury via directly targeting the Rab11a 3'UTR region to inhibit Rab11a-mediated neuronal autophagy.<sup>105</sup> Besides that, multiple miRNAs, such as miR124-3p,<sup>106</sup> miR19a-3p,<sup>99</sup> miR188-3p,<sup>100</sup> miR-30d-5p,<sup>9</sup> miR-190b,<sup>91</sup> miR-7670,<sup>90</sup> miR-25<sup>8</sup> and miR-135a,<sup>113</sup> shuttled in respective EVs are involved in regulating autophagy various cell as shown in Tables. Hence, a plethora of miRNAs are found in EVs of which the precise signaling cascades that are regulated are not fully known, yet.

## mRNA, Such as STAT3 and PDE1-B, Play an Important Role in the Effect Regulating Autophagy

Phosphodiesterase enzyme (PDE) 1-B is the best characterized among 11 groups of the superfamily of PDE enzymes in mammals.<sup>114</sup> PDE, a calcium and calmodulin-dependent phosphodiesterase, limits the intracellular levels of cyclic nucleotides by catalyzing the hydrolysis of cAMP and cGMP. Recently, it has been revealed that PDE1-B is involved in the regulation of autophagy and exosome release.<sup>89</sup> Zang et al<sup>89</sup> demonstrated a novel mechanism by which vinpocetine, an inhibitor of PDE1-B, regulated microglia-neuron communication via altering autophagy in BV2 cells. Firstly, the PDE1-B expression in microglia was progressively elevated in the peri-infarct region after MCAO. Using vinpocetine can inhibit BV2 microglia M1, promote M2 phenotype, and enhance autophagy in OGD-conditioned BV2 cells, which is associated with the release of exosomes that protects neurons against OGD-induced damage. With regard to signal transducer and activator of transcription 3 (STAT3), located on chromosome 17q21, which was first reported as a transcriptional enhancer of acute-phase genes activated by interleukin 6.<sup>115</sup> STAT3, the only embryonic lethal family member of the STAT family, is a prominent nuclear transcription factor that regulates more than 1000 gene expressions.<sup>116</sup> STAT3 protein becomes transcriptionally activated primarily by tyrosine phosphorylation, in turn, translocates to the nucleus and targets sequence-specific DNA elements for consequent transcription of target genes.<sup>115,116</sup> A great number of studies have endorsed the growing evidence of an important role of STAT3 in autophagy regulation.<sup>117–119</sup> Interestingly, the current study has demonstrated that EVs can regulate autophagy as the upstream of STAT3. For example, Xia et al<sup>85</sup> indicated that the expression of the LC3-II/LC3-I and Beclin-1 was significantly increased while the P62 protein level was decreased after the stroke model. However, MSCs-EV markedly reversed the levels of autophagy-associated protein levels induced by MCAO. Besides that, the specific autophagosomes characterized by double-membrane structure were increased after MCAO. Notably, the EV group found fewer autophagosomes by TEM. Furthermore, EVs can significantly activate STAT3 after stroke. Xia et al<sup>85</sup> used stat3, a STAT3 inhibitor, to further confirm the role of STAT3 in the preventative effects of EVs on stroke-induced autophagy, and revealed suppression of STAT3 can abolish EV-induced inhibition of autophagy in vivo and in vitro. MSCs-EVs might therefore contribute to inhibiting autophagy by activating STAT3. The ability of EVs from other cells to suppress autophagy, information regarding this aspect, however, is scarce and appears to be limited, for which additional and reliable data is urgently necessary.

## Conclusions and Prospects

Generally, EVs and autophagy are closely associated. Cells use EVs to communicate with the environment and surrounding cells by carrying information such as lipids, proteins, or nucleic acids. Autophagy plays a vital role in the synthesis and degradation of EVs, which is closely related to the formation of autophagosomes. In another, the application of EVs provides a great opportunity for adjuvant treatment of neurological disorders, for which autophagy is an excellent target. Although EVs exist in extracellular fluid, they can release their cargos into target cells via endocytosis, receptor-ligand binding, and membrane fusion, which, in turn, regulate the autophagic level of target cells through autophagy-related proteins directly and the ncRNA, STAT3, PDE1-B, etc. signaling pathways indirectly, thus regulating the development of related neurological disorders.

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## Disclosure

Wei Wei and Yongli Pan should be considered co-first authors. The authors declare that they have no competing interests.

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