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REVIEW

The role of autophagy in ischemic brain injury

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ABSTRACT

Ischemic brain injury occurs in many clinical settings, including stroke, cardiac arrest, hypovolemic shock, cardiac surgery, cerebral edema, and cerebral vasospasm. Decades of work have revealed many important mechanisms related to ischemic brain injury. However, there remain significant gaps in the scientific knowledge to reconcile many ischemic brain injury events. Brain ischemia leads to protein misfolding and aggregation, and damages almost all types of subcellular organelles including mitochondria, endoplasmic reticulum, Golgi apparatus, lysosomes, etc. Irreparably damaged organelles and insoluble protein aggregates are normally removed by autophagy. The build-up of common autophagic components, such as LC3, p62, and ubiquitinated proteins, are generally observed in brain tissue samples in animal models of both global and focal brain ischemia, but the interpretation of the role of these autophagy-related changes in ischemic brain injury in the literature has been controversial. Many pathological events or mechanisms underlying dysfunctional autophagy after brain ischemia remain unknown. This review aims to provide an update of the current knowledge and future research directions regarding the critical role of dysfunctional autophagy in ischemic brain injury.

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Introduction of ischaemic brain injury

Ischemic brain injury can manifest in various clinical situations including stroke, cerebral vasospasm, cerebral edema, cardiac surgery, shock, and cardiac arrest [1–4]. Animals typically cannot survive a global brain

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ischemic episode exceeding 20–30 minutes [5]. However, they can endure prolonged focal brain ischemia with or without reperfusion, mirroring that of humans with ischemic stroke untreated or treated with revascularization therapies through thrombolytic or endovascular thrombectomy [6]. Brief global brain ischemia with reperfusion can cause irreversible and delayed neuronal death in hippocampal, striatal, and neocortical regions during reperfusion in both animals and humans [6]. Conversely, prolonged focal brain ischemia (stroke) progresses from early striatal infarction to delayed dorsolateral cortical infarction overlying the striatum [7].

Focal brain ischemia produces two regions of injury: the ischemic core and the penumbra. In the ischemic core, where brain tissue has extremely low blood flow, about 80% of the neurons die within hours [8,9]. The surrounding penumbra, characterized by transient ischemia or relatively low blood flow, experiences a more gradual neuronal death, with about 40% of the neurons dying over several days [7,10]. Focal brain ischemia can also lead to reperfusion injury in the penumbra even without external reperfusion, as blood flow can be re-established via collateral circulation [11]. The full extent of damage evolves over time, with initial ionic edema followed by cytotoxic edema, as well as blood–brain barrier breakdown, whereas microglia activation, monocyte infiltration, and astrogliosis were often not observed until 12–24 hours after focal brain ischemia [10].

Autophagy initiation and autophagosome biogenesis

Eukaryotic cells have two major intracellular degradation mechanisms: the ubiquitin–proteasome system and autophagy [12–14]. The ubiquitin-proteasome system degrades intracellular soluble ubiquitinated proteins, while autophagy primarily handles insoluble proteins through macroautophagy, microautophagy, and chaperone-mediated autophagy [12–14]. Macroautophagy, often simply called autophagy, is the most studied form as it removes “large” cellular components. It plays a key role in both cell survival and maintenance and is implicated in various human diseases [15–18]. Autophagy has a sophisticated organizational hierarchy that constantly monitors cellular waste and damaged components, adjusting their numbers and compositions under both normal and pathological conditions [13].

There are numerous autophagy-related proteins and regulators. Figure 1 illustrates the general autophagy pathway. Autophagy is generally activated by conditions of nutrient deprivation, but its activation has also been associated with both physiological and pathological processes, such as development, differentiation, ischemia, traumatic brain injury, neurodegenerative diseases, stress, infection, and cancer [17,19]. Furthermore, autophagy-related proteins and regulators may be expressed differentially among

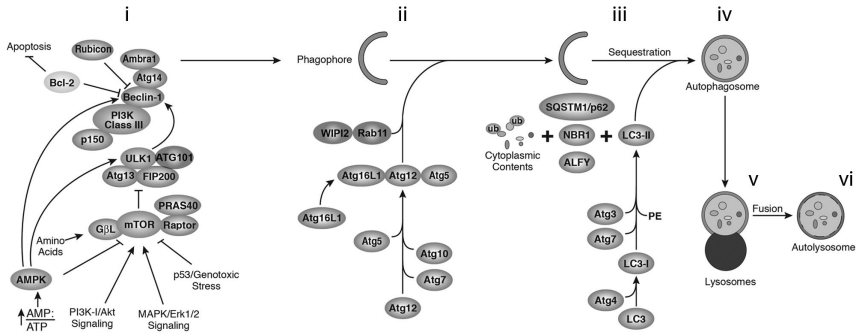

Figure 1. Schematic overview of general autophagy pathway. In biological conditions, the autophagy pathway is under precise control by multiple regulatory proteins which can either induce or inhibit autophagy. When autophagy is activated via the mTOR regulatory pathways (i), phagophore formation is initiated by ATG complexes (ii). LC3 is lipidated by Atg7 and Atg3, thereby becoming LC3-II, and incorporated into the phagophore membrane (iii) to sequester ubiquitinated proteins via the ubiquitin binding domain of its binding partner SQSTM1/p62. As more LC3-II is incorporated, the phagophore membrane continues to expand and engulf cellular components for subsequent degradation before it eventually seals to form an autophagosome (iv). The autophagosome then undergoes membrane fusion with lysosomal structures containing functionally active proteases (v) to form autolysosomes which are capable of degrading waste cargo (vi). Abbreviations: ALFY = autophagy-linked FYVE protein; AMP = adenosine monophosphate; AMPK = AMP-activated protein kinase; Atg = autophagy related; ATP = adenosine triphosphate; Bcl-2 = B-cell lymphoma-2 protein; Beclin1 = mammalian ortholog of yeast Atg6; FIP200 = FAK family kinase-interacting protein of 200 kDa; LC3 = microtubule-associated protein 1A/1B-light chain 3; LC3-I = cytosolic microtubule-associated protein 1A/1B-Light Chain 3; LC3-II = microtubule-associated protein 1A/1B-light chain 3-phosphatidylethanolamine conjugate; MAPK/Erk1/2 = mitogen-activated protein kinase/extracellular signal-regulated kinase-1/-2; mTOR = mammalian target of rapamycin; NBR1 = neighbor of Brca1 gene; p150 = mammalian homolog of yeast VPS34; p53 = tumor protein p53; PE = phosphatidylethanolamine; PI3K-1/Akt = phosphatidylinositol 3-kinase/protein kinase B; PRAS40 = proline-rich AKT substrate of 40 kDa; Rab11 = Ras-related protein Rab-11; Raptor = regulatory-associated protein of mTOR; Rubicon = run domain Beclin-1-interacting and cysteine-rich domain-containing protein; ub = ubiquitin; ULK1 = UNC-51-like kinase 1; WIPI2 = WD-repeat protein interacting with phosphoinositides.

various cell types in the brain in both physiological and pathological conditions [20,21].

The autophagy pathway has been described in greater detail in other publications [22,23]. Briefly, mammalian target of rapamycin (mTOR) kinase is a critical regulator of autophagy (Figure 1). mTOR activation by phosphoinositide 3-kinase (PI3K)/Akt and mitogen-activated protein kinase (MAPK) signaling promotes suppression of autophagy, whereas mTOR inhibition by AMP-activated protein kinase (AMPK) and p53 signaling promotes autophagy [24–26]. Three related serine/threonine kinases, referred to as UNC-51-like

kinase-1, -2, and -3 (ULK1, ULK2, UKL3), act downstream of the mTOR complex in which ULK1 and ULK2 form a large complex with ATGs 13 and 101 and the scaffold protein known as FIP200 (an ortholog of yeast Atg17) [27]. The ULK1/ATG complex promotes initiation of autophagy via its phosphorylation of Beclin-1 (mammalian homolog of yeast Atg6) within the Class III PI3K complex which consists of the human type III phosphatidylinositol 3-kinase (also known as hVps34), p150 (a mammalian homolog of yeast Vps15), and Atg14-like protein (Atg14L or Barkor) [28,29]. Alternatively, Bcl-2 can inhibit Beclin-1-dependent autophagy in a variety of cell types, including yeast, mammalian cells, and mouse heart muscle [30–32]. Additionally, autophagy and apoptosis may be connected both positively and negatively, and extensive crosstalk exists between the two processes [33].

The ATG proteins control autophagosome formation through the Atg12-Atg5 and LC3-II (Atg8-II) complexes. Atg12 is conjugated to Atg5 by Atg7- and Atg10-mediated consecutive enzymatic reactions similar to the processes of protein ubiquitination mediated by ubiquitin–activating enzyme (E1) and ubiquitin-conjugating enzyme (E2) [34]. The Atg12-Atg5 conjugate then interacts noncovalently with Atg16 to form a large complex for attaching to the phagophore membrane [35]. LC3/Atg8 is cleaved at its C-terminus by the protease Atg4 to generate the cytosolic LC3–I [36]. LC3-I is conjugated to phosphatidylethanolamine (PE) by Atg7 and Atg3 also via the E1- and E2-like consecutive enzymatic reactions, thus becoming the autophagosome membrane-bound form of LC3 known as LC3-II [37]. Phagophore expansion requires the incorporation of more LC3-II complexes that interact with autophagy receptors such as sequestosome 1 (SQSTM1), also known as p62, for engulfing protein aggregates and damaged organelles. These autophagy receptors share a common ubiquitin-binding domain (UBD) and an LC3-interacting region (LIR), where the UBD recognizes ubiquitinated protein aggregates and the LIR interacts with LC3-II on the phagophore membrane (Figure 2) [38,39]. As the phagophore engulfs cellular components, it expands and eventually seals to form an autophagosome (AP) (Figure 2).

Autophagic degradation or autophagic flux involves the lysosomal cycle

The AP must enter a lysosomal cycle (L–cycle) for degradation of waste cargo. Lysosomes were initially discovered in 1955 by Christian de Duve's laboratory in Louvain, Belgium, through isolation of a subcellular fraction containing common acid hydrolases with broad substrate specificity [40]. de Duve later shared the 1974 Nobel Prize for discoveries concerning cellular structural and functional organization [41]. From their initial discovery, we now know all L-cycle structures (late endosome-LE, autolysosome-AL, terminal lysosome-L), as well as their respective transitional

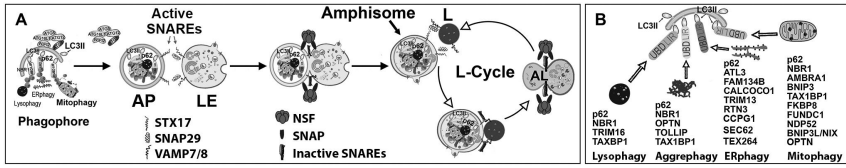

Figure 2. (A) Schematic diagram of autophagosome formation and lysosomal degradation cycle (L–Cycle). Abbreviations: AL = autolysosome; AP = autophagosome; ATG = autophagy-related protein; L = terminal lysosome; LC3-II = microtubule-associated protein 1A/1B-light chain 3-phosphatidylethanolamine conjugate; NSF = N-ethylmaleimide sensitive factor ATPase; p62 = sequestosome 1 or p62/SQSTM1; SNAP = soluble NSF attachment protein; SNAREs = SNAP receptors; STX17 = syntaxin-17; SNAP29 = synaptosome-associated protein-29; VAMP = vesicle-associated membrane protein; WIPI2 = WD-repeat protein interacting with phosphoinositides. (B) Schematic diagram of organelle-specific autophagy receptors. Abbreviations: AMBRA1 = autophagy and beclin 1 regulator 1; ATL3 = atlastin GTPase 3; BNIP3 = BCL2 interacting protein 3; BNIP3L/NIX = BNIP3-like protein or NIX; CALCOCO1/NDP52 = calcium binding and coiled–coil domain 1; CCPG1 = cell cycle progression 1; FAM134B = family with sequence similarity 134 member B; FKBP8 = FKBP prolyl isomerase 8; FUNDC1 = FUN14 domain containing 1; NBR1 = neighbor of Brca1 gene; OPTN = optineurin; RTN3 = reticulon 3; SEC62 = translocation protein SEC62; TAX1BP1 = Tax1-binding protein 1; TEX264 = testis expressed protein 263; TOLLIP = toll interacting protein; TRIM13 = tripartite motif-containing protein 13; TRIM16 = tripartite motif-containing protein 16.

intermediate structures contain similar acid hydrolases and lysosomal structural proteins [6]. Therefore, they can collectively be referred to as lysosomes or lysosomal structures [42,43]. However, these lysosomal structures differ in intraluminal pH: about 5.5 in LE, 4.5–5.5 in AL, and 4.5 in L [44–47]. The difference in pH is regulated by differing densities or activities of a multi-subunit proton pumps, called Vacuolar H^+-ATPases (V-ATPases), located on the membranes of these lysosomal structures [48,49].

Autophagic flux measures both AP formation and degradation and is used for evaluating autophagic degradation activity [50]. Normally, the lysosomal system has an excessive capacity to handle increased AP loads, rapidly degrading the entire AP structure including all AP components (e.g., LC3-II, p62, etc.) together with their cargos, even under stress conditions. Lysosomal inhibitors such as bafilomycin A1 and chloroquine can effectively block autophagic flux under normal conditions. This suggests that autophagic flux is an active, ongoing process and the L-cycle is a key rate–limiting step in the process [51]. Consequently, elevated levels of AP structural proteins, such as LC3

and p62 often indicate reduced autophagic flux, suggesting deficient L–cycle activity [50,52].

The L-cycle is shared by autophagic, endocytic, and Golgi apparatus biosynthetic pathways

The L-cycle involves three main structures (LE, AL, L) and begins when an AP fuses with a LE to form an intermediate/hybrid organelle known as the amphisome (Figure 2) [53–55]. This amphisome then fuses with a terminal L, creating a more acidic AL where the entire AP structure (including LC3-II, p62, and cargo content) is degraded. After degradation, the AL must convert to a new terminal L for the subsequent round of the L-cycle (Figure 2, L-cycle). This AL-to–L conversion likely involves protein and lipid complexes, though the exact mechanism remains unclear [42,43,53]. The L-cycle repeats continuously, removing protein aggregates and damaged organelles [54].

As shown in Figure 3, the lysosomal system integrates traffic from the autophagic, endocytic, and Golgi apparatus biosynthetic pathways. In the endocytic pathway, endocytic materials are engulfed by invagination of the cell membrane, forming an intracellular vesicle called the early endosome (EE). These EEs, through a maturation process, become LEs to enter the L-cycle. The Golgi pathway delivers lysosomal proteins (hydrolases, V-ATPases, and lysosomal structural proteins) to the LE. In the autophagic pathway, protein aggregates and damaged or surplus organelles are engulfed into APs that fused with LEs to become amphisomes, as described earlier (Figure 2) [6].

LEs receive cargo from EEs and APs, and hydrolases from the Golgi (Figure 3). The hydrolases in the LEs and amphisomes cannot efficiently degrade waste cargo due to their high intraluminal pH (5.0–6.0). They must first fuse with more acidic Ls to become autolysosomes/endolysosomes (AL/EL) with more acidic pH (~4.5) for waste cargo degradation (Figures 2 and 3). After degradation, AL/ELs must convert to new Ls to participate in the next round of LE/AP- or amphisome-to-L fusion (Figures 2 and 3) [6,42,43,53]. Among lysosomal structures, AL/ELs are the principal degradation site containing both cargo to be degraded and fully active acid hydrolases due to the optimal acidic pH. LEs cannot degrade the cargo efficiently due to the higher intraluminal pH. The terminal lysosome, derived from post-degradation AL/ELs, acts mainly as intraluminal acidic environment providers, delivering additional V-ATPases and mature hydrolases to LEs during the L-cycle [6,42,43,53].

Discrepancies in describing lysosomal structures exist in brain ischemia research literature [56–58]. Some studies describe lysosome as terminal digestive organelles, rather than dynamic structures undergoing the L-cycle. Often, LEs and AL/ELs are vaguely batched together as “lysosomes” [6]. These inconsistencies occur because all three basic lysosomal structures (LE, AL/EL,

and L) contain virtually identical proteins, although in different quantities. Furthermore, the LE is considered a precursor to AL/EL and L, as lysosomal hydrolytic enzymes and structural proteins are delivered from the trans-Golgi network to LE, then to AL/EL and L. Common lysosomal markers, such as cathepsins and lysosomal membrane-associated proteins (LAMPs) are major components of all three structures. Distinguishing these structures is challenging due to no specific protein markers. M6PR is present in the LE but not in AL/EL and L. However, its utility as a specific marker for LEs is somewhat limited by the fact that it is also located in the Golgi and cell membrane [6,59]. Rab7 is currently known to be present in LEs and AL/ELs, but not in Ls [6,60].

The L-cycle is regulated by membrane fusion events

The bilayer lipid membrane fusions along the autophagic, endocytic, and Golgi apparatus biosynthetic pathways shown in Figure are mediated by multiple protein complex machineries [61]. The core complex consists of N-ethylmaleimide-sensitive factor ATPase (NSF), soluble NSF attachment protein (SNAP), and SNAP receptors (SNAREs) (Figure 2). SNAREs, either directly anchored or indirectly associated to lipid membranes, execute the membrane-to-membrane fusion through interactions between SNAREs on opposing membranes (e.g., Figure 2, AP and LE) [62]. After fusion, SNAREs form an inactive complex (Figure 2, inactive SNAREs) and must be reactivated for the subsequent LE/AP-to-L membrane fusions (Figure 2, active SNAREs). This reactivation is mediated by NSF ATPase and its co-factor SNAP (Figure 2) [62–65]. Notably, NSF inactivation impedes membrane fusion events in the autophagic, endocytic, and Golgi apparatus biosynthetic pathways disrupting autophagic flux after brain ischemia [6].

Two autophagy-specific SNARE complexes STX17-SNAP29-VAMP8 and YKT6-SNAP29-STX7 are involved in both the AP-to-LE fusion (forming amphisomes) and amphisome-to-lysosome fusion (forming ALs) (Figure 2) [66–68]. However, it remains to be investigated whether other SNAREs are involved for these membrane fusion processes. In addition to the SNARE complexes, other protein complexes, such as Rab GTPases, tethering complexes, and the ESCRTs (endosomal sorting complexes required for transport) also play an essential role in these membrane fusion events. However, the role and specificity of these complexes for the autophagic processes remain to be elucidated.

Apart from their role in the autophagic pathway, the NSF-SNAP-SNARE machinery also plays an essential role in neurotransmitter release and synaptic vesicle (SV) recycling. Like the AP-to-LE and LE-to-L fusions shown in Figure 2, with synaptic stimulation, neurotransmitter-filled SVs are moved to and then fused with the presynaptic plasma membrane, resulting in neurotransmitter release. Synaptic SNAREs are the core protein complexes

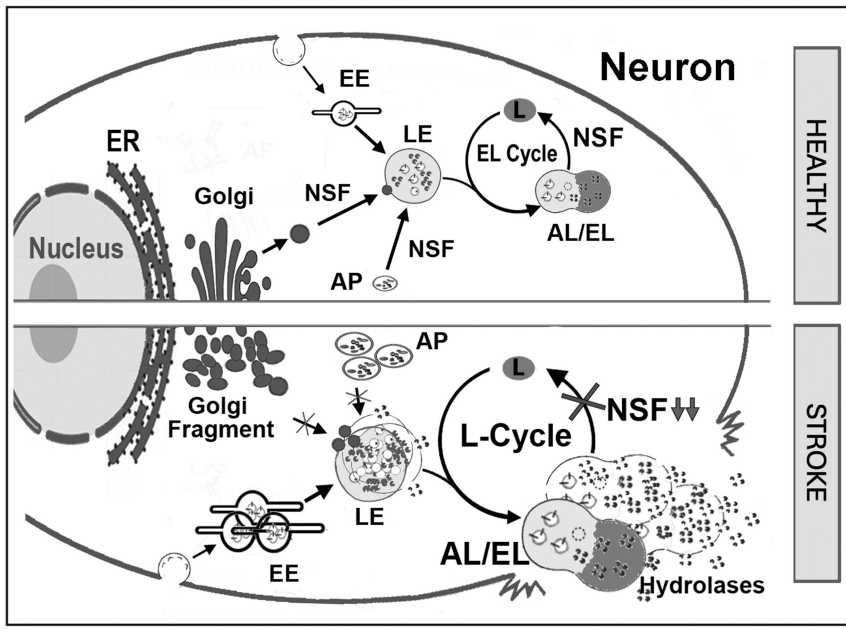

Figure 3. Schematic diagram of post–**s**troke hydrolase release. Upper: In healthy neurons, lysosomal hydrolytic enzymes and lysosomal structural proteins are synthesized on the ER-associated polyribosomes, modified in the ER and the Golgi lumen, and transported via vesicles to the late endosome (LE). The LE also receives incoming waste cargos from early endosome (EE) of the endocytic pathway and autophagosome (AP) of the autophagic pathway. The enzyme- and waste cargo-loaded LE then enters the "L–Cycle" by fusing with an acidic terminal lysosome (L) for the formation of an autolysosome (AL) or endolysosome (EL) where the cargo can be degraded in an acidic environment (~pH 4.5). After degradation of waste cargo, EL converts into a new L via an NSF–mediated mechanism for the next round of LE-to-L fusion. Lower: middle cerebral artery occlusion stroke model leads to NSF deficiency in neurons, thus causing disruption of the AL's/EL's conversion to a new L. This results in the endocytic and autophagic traffic jams at the step of the AL's or EL's conversion to L. Consequently, both the size and number of all the structures related to the endocytic and autophagic pathways before the EL-to-L conversion phase, are increased. This cascade of events eventually damages the hydrolase-containing structures to release hydrolases into the cytoplasm or extracellular space contributing to stroke brain injury.

responsible for mediating the SV–to–presynaptic membrane fusion [69]. Although both abundant and redundant, SNARE proteins can be relatively organelle specific. For example, STX17, SNAP29, VAMP8, and YKT6 are major endolysosomal SNAREs as described above, whereas STX1, SNAP25, synaptobrevin, etc., are synaptic SNAREs responsible for the SV–to–presynaptic membrane fusion which is an exocytic process [69]. After exocytic neurotransmitter release, synaptic proteins, including synaptic SNAREs (STX1, SNAP25, synaptobrevin, etc.), are retrieved into presynaptic terminals

via endocytosis. Following SV-to-presynaptic membrane fusion, SNAREs form an inactive complex on the presynaptic plasma membrane to be disassembled by NSF ATPase and its co-factor SNAP [70]. Disassembled SNAREs and other synaptic proteins are then internalized into the presynaptic terminals mostly via clathrin-mediated endocytosis where SNAREs can be reused to mediate subsequent SV-to–presynaptic plasma membrane fusion events [71]. Recycling of synaptic proteins, mediated by the disassembly of inactive SNARE complexes by NSF ATPase and SNAP, is a critical process required for sustaining high rates of synaptic transmission without exhausting the supply of synaptic vesicles.

Organelle-specific autophagy

Autophagy was long thought to be a non-specific bulk degradation process, although emerging evidence shows that it can also be selective through a growing number of newly discovered organelle-specific autophagic receptors (Figure 2B) [72–77]. This selectivity has led to terms like mitophagy, ER-phagy, lysophagy, Golgiphagy for the removal of damaged mitochondria, ER, lysosomes, and Golgi apparatus, respectively. Brain ischemia damages almost all subcellular organelles, making organelle-specific autophagy studies highly relevant to understanding autophagy's role in ischemic brain injury. Mitophagy has been widely studied due to mitochondria's dynamic nature and potential to produce deleterious reactive oxygen species and release apoptotic proteins when damaged [78]. In comparison, despite their significance, research on other organelle-specific autophagy remains limited in the field of brain ischemia.

General changes in autophagy genes after brain ischaemia

Brain ischemia with a duration longer than 5 minutes can result in tremendous cellular and tissue damage [79]. Aside from inducing organelle damage and the associated cellular consequences, such as releasing deleterious reactive oxygen species and apoptotic proteins as in the case of mitochondrial damage, several studies have identified another consequence of cerebral ischemia: accumulation of neurotoxic protein aggregates [80–84]. Among brain cell types, neurons are the most susceptible to damage from brain ischemia. Furthermore, neurons are highly specialized, non-divisible cells where replacement of healthy neurons is not a viable option. For these reasons, sophisticated internal (e.g., intra–neuronal) and external (e.g., phagocytosis of cellular debris) quality control mechanisms are essential to support cellular and tissue homeostasis after brain ischemia [85]; one of the major such quality control mechanisms is autophagy. Therefore, levels of autophagic genes and proteins are significantly altered after brain ischemia.

For example, Becn1 gene expression is upregulated at 2 days and then gradually decreased following ischemia in both hippocampal cortex and CA1 region [86–88]. Bnip3 gene expression is upregulated in CA1 region at 2 days and then downregulated at 7- and 30 days following ischemia [86,87]. In another report, both Becn1 and Bnip3 gene expressions were found to be significantly upregulated at 12 months and 24 months post-ischemia in the CA3 region [89]. Furthermore, autophagy-related proteins are expressed differentially among neurons and non–neuronal cell types in post-ischemic brains which are further elaborated below in the following sections. These studies highlight significant spatial and temporal effects of cerebral ischemia on autophagy-related gene expression in various brain cell populations, further emphasizing the complexity of autophagy's role in ischemic brain injury and stressing the need to analyze autophagic changes in ischemic injury carefully with respect to the entire autophagic flux process, as well as different cell populations and recovery time following ischemic insult.

Neuronal autophagy flux after brain ischaemia

Autophagy has been extensively studied in animal ischemic brain injury models [6,53,55]. AP components like LC3-II, ATG5, p62, and lysosomal proteins, such as LAMPs and cathepsin B generally show progressive and persistent increases in their protein levels in neurons undergoing the cell death process after brain ischemia [6,90,91]. However, these increases may not reflect increased autophagic activity but rather indicate reduced autophagic flux caused by deficient lysosomal degradation (L-cycle activity) after brain ischemia [6,91–93]. This phenomenon is observed in brain ischemia models, myocardial ischemia models, as well as in some human lysosome storage diseases [6,94–96]. As highlighted in a guideline for studying autophagy, autophagic activity should be evaluated by examining the entire autophagic process, i.e., autophagic flux [53]. Therefore, increased autophagosome numbers or levels of LC3 and p62 likely result from deficient lysosomal degradation of AP structural proteins and cargos rather than increased autophagy initiation [6,73,91–93].

Chemical inhibitors of autophagy are often used to study autophagy in animal brain ischemia models. However, most current chemical autophagy inhibitors or modulators lack specificity, as highlighted in a review by Mizushima et al. [97]. For example, a group of phosphoinositide 3-kinase inhibitors, such as 3-methyladenine and wortmannin are widely used as autophagy inhibitors despite not being autophagy specific. 3-methyladenine can increase autophagic flux under certain conditions [98]. At high concentrations, it can target other kinases and cellular processes, including glycogen metabolism, lysosomal acidification, endocytosis, and mitochondrial

permeability transition. Therefore, the effects of chemical autophagy modulators on brain ischemia should be confirmed with multiple, non-overlapping methods.

Autophagic changes in the post-ischemic brain tissue may be influenced by different types of cells, including infiltrated inflammatory cells [99–102]. Most studies of autophagic gene expression are performed using brain tissues with mixed cell types and thus may not distinguish if increased autophagic proteins are from neurons, non-neuronal cells, and infiltrated inflammatory cells. Autophagic flux can differ, or even behave inversely, between neurons and other cells like astrocytes, microglia cells, or infiltrated inflammatory cells [6,90]. Consequently, changes in LC3-II level in tissue homogenate or subcellular fractions may reflect impaired AP degradation (reduced autophagic flux) in neurons or the mixed changes from different cell types after brain ischemia. Understanding autophagy's role after brain ischemia requires studying the entire autophagic flux process in different brain cell types. Furthermore, basal levels and steady-state regulation of autophagy may significantly differ between *in vivo* brain cells and in cultured cells. Thus, results from cell culture studies should be confirmed by *in vivo* studies [6].

Electron microscopy (EM) is an effective tool to quantify APs and ALs after brain ischemia [91]. Previous EM studies show that transient cerebral ischemia leads to a dramatic build–up of intracellular vesicular structures in vulnerable CA1 neurons [103–111]. Some of these vesicular structures likely represent APs and ALs [5,6,85]. A common feature indicating impairment of the autophagic pathway is the build-up of abnormal protein aggregates and aberrant organelles [112–114]. These are the predominant ultrastructures in CA1 neurons destined to undergo delayed neuronal death following global brain ischemia [5,6]. The build-up of damaged organelles, along with autophagic and lysosomal structures, indicates deficient AP degradation or decreased autophagic flux in post-ischaemic neurons [6].

Autophagic flux is generally considered as a pro-survival response in post-ischemic neurons to maintain neuronal homeostasis by removing toxic protein aggregates and damaged organelles [115–119]. However, some studies have interpreted it as detrimental [120–123] due to, for instance, its removal of some of salvageable organelles after brain ischemia. Meanwhile, a handful of others acknowledge the exact role of autophagic flux in post-ischemic neurons to be somewhat ambiguous or situationally beneficial/detrimental [124–129]. These discrepancies may be due to differences in autophagy across different cell types, variation in the extent of autophagic activity, as well as differences in the spatial and temporal dynamics of autophagic activity [6,129]. This controversial role of autophagic flux in post-ischemic neurons may also be partially due to the issue of misinterpretation of results,

which has been extensively discussed in previous publications [130–132]. The controversial role of autophagy may also suggest that our current knowledge of autophagy in pathological conditions is likely partial and that specific tools or treatments for the enhancement of the entire autophagic flux are limited to understand the final outcomes.

Inactivation of neuronal NSF ATPase after brain ischaemia

As described above in Figures 2 and 3, the NSF-SNAP-SNARE machinery is the prerequisite for the AP-to-LE and LE/AP-to-L fusion, as well as Golgi transport vesicle-to-LE fusion for delivering lysosomal hydrolases and structural proteins. After fusion, SNAREs form an inactive cis-complex on the single resulting membrane. The inactive SNARE cis-complex must be dissociated or reactivated by NSF ATPase for subsequent membrane fusions [66–68]. Because mammalian cells possess only a single form of NSF, deficiency in NSF halts all membrane fusion activities [6]. This handicaps both the delivery of lysosomal hydrolases and lysosomal structural proteins from the trans-Golgi network and impedes the LE-to-L fusion forming AL/EL (Figure 2). Consequently, lysosomal degradation activity becomes deficient, reducing autophagic flux after brain ischemia [6,90–93].

There are a few *in vivo* studies referencing the NSF-SNAP-SNARE machinery after brain ischemia [5,91,108,133]. One study found that cytosolic NSF was depleted in the hippocampal CA1 neurons as early as 30 minutes post-reperfusion following global brain ischemia due to its deposition into inactive Triton X-100-insoluble protein aggregates [108]. This depletion of active NSF persisted until neuronal death occurred at 2–3 days post-reperfusion. In comparison, NSF remained active in surviving dentate gyrus neurons exposed to the same ischemic episode [108]. The inactive deposition of NSF was accompanied by complete fragmentation of Golgi stacks and accumulation of numerous intracellular vesicular structures (partly due to Golgi fragmentation) in hippocampal CA1 neurons [108]. The authors speculated that persistent depletion of active NSF likely contributes to delayed neuronal death in CA1 neurons after a brief period of global brain ischemia.

Yuan et al. [5] found that 20 minutes of global brain ischemia led to the selective and complete inactivation of NSF ATPase in neurons destined for delayed neuronal death. This inactivation resulted in massive accumulation of Golgi fragments, intracellular vesicles, and cargo-laden late endosomes in affected neurons. They also observed upregulation of lysosomal hydrolase cathepsin B (CTSB) immunoreactivity as larger, diffused, and irregularly shaped puncta, with some CTSB immunoreactivity evenly distributed in the cytoplasm of post-ischemic neurons destined to die [5]. This suggested CTSB release from the Golgi apparatus and lysosomal structures into the neuronal cytoplasm after transient global brain ischemia [5]. Importantly, CTSB release

was mostly restricted within neurons, suggesting a microscale event. The authors also suggested that limited cathepsin release might not cause catastrophic destruction of neuronal structures or indiscriminate tissue infarction. Instead, it could activate cell death signaling pathways after a brief period of global brain ischemia [5,92].

Yuan et al. [91] also demonstrated irreversible NSF depletion or inactive deposition in virtually all penumbral neurons after 2 hours of middle cerebral artery occlusion in a rat stroke model. The irreversible inactivation of NSF ATPase led to large-scale CTSB release, causing indiscriminate neuronal structural damage. As a result, post-ischemic neurons ruptured, releasing contents including CTSB, ubiquitinated protein aggregates, and p62–protein aggregates into the extracellular space. Hu et al. [93] found that CTSB knockout mice compared to wild-type mice had significantly smaller injury area in the hippocampus, increased neuronal survival in the striatal core area, and improved physical and functional performance post-ischemia in the mouse stroke model [93]. These studies support the hypothesis that NSF inactivation in post-ischemic neurons leads to deficient lysosomal degradation activity or autophagic flux, build-up of lysosomal structures, and release of CTSB after brain ischemia [6,93]. It remains to be further investigated if and how the cascade of NSF inactivation and CTSB release leads to neuronal death after brain ischemia.

Autophagy in non–neuronal cells after brain ischaemia

Normal neurons exhibit predominant autophagic and lysosomal degradation activities in the brain, evidenced by the localization of p62, cathepsin-B, -L and -D, as well as LAMP-1 and -2 primarily in neurons of non-ischemic mouse brain regions [6,91,93]. This may explain why neuronal autophagy changes have been extensively characterized in different neurological diseases, including brain ischemia [6,91,134]. However, autophagy is likely altered in other cell types in the post-ischemic setting, including oligodendrocytes, microglial and infiltrated inflammatory cells, and blood–brain barrier (BBB) related cells [6]. This is of particularly significant interest as autophagy in non-neuronal cells plays a key role in brain tissue remodeling and repair after ischemic stroke [131].

Kotoda et al. [135] studied focal cerebral ischemia in Atg5flox/flox LysMCre + mice (autophagy deficient in myeloid lineage cells) and in "wildtype" Atg5flox/flox control mice. Myeloid lineage cells are derived from a common myeloid progenitor and include macrophages, monocytes, granulocytes, neutrophils, eosinophils, microglia, and mast cells. They found that mice lacking ATG5 in myeloid lineage cells had a lower 14-day post-ischemia survival rate after ischemia (20% versus 70%; $p < 0.05$). Although there was no difference in infarct volume at 12 hours, autophagy-deficient mice had larger

infarct volumes at 3- and 7-days post-reperfusion and worsened behavioural performances. Relative to those in the wild-type control mice, there was also a higher proportion of inflammatory cells expressing M1 marker CD16/32 and higher levels of proinflammatory cytokines in autophagy-deficient mice after 12 hours of reperfusion. The authors concluded that ATG5 deficiency in myeloid cells exacerbates secondary injury by shifting the inflammatory balance toward the proinflammatory response after ischemic stroke [135].

BBB impairment is significant in animal stroke models [136]. Studies have shown that autophagy dysfunction may contribute to this impairment. Adult mice with endothelial cell-specific ATG3 depletion exhibit BBB leakage [137]. Furthermore, both autophagy-related mRNA and protein expression are repressed in the endothelial cells from older mice (24-month) and older human male subjects (>68 years) after stroke [138]. These findings suggest a potential link of dysfunctional autophagy with BBB impairment after brain ischemia.

White matter is the axonal tracts that interconnect neuronal cell bodies in the central nervous system, and is comprised of myelinated and unmyelinated axons, myelinating oligodendrocytes, astrocytes, microglial cells, and blood vessels. In humans, white matter injury is an important predictor of long-term functional outcome after ischemic stroke [139,140]. However, this aspect is significantly understudied in rodent ischemia models. At the writing of this review (December 2024), PubMed searches reveal only 313 publications for "mouse ischemia white matter injury" and 215 publications for "mouse ischemia axonal injury". In comparison, there are 6,960 publications in the PubMed for "mouse ischemia brain injury" during the same time period. This disparity highlights the limited focus on white matter or axonal injury in animal brain ischemia studies. One factor contributing to this research gap is the significant difference in white and gray matter volume ratios between rodents and humans [141].

Mature oligodendrocytes (oligodendrocytes hereafter) are myelin sheath-producing cells. The myelin sheath wraps white matter axons and is responsible for electrical impulse propagation and protects the nerve fibers from injury. Oligodendrocytes are fragile, fibrous, and fixed in place, irreplicable, with high sensitivity to brain ischemia likely due to their high energy needs for myelination [142]. Each oligodendrocyte can wrap up to 50 neuronal axons. Oligodendrocytes originate from oligodendrocyte progenitor cells, which are derived from stem cells, proliferating throughout life to self-renew, and constitutes about 5% of the cells in the adult central nervous system [143]. In response to myelin damage, oligodendrocyte progenitor cells migrate to the lesion area, differentiate into mature oligodendrocytes, and produce new myelin [144].

During the early phase of brain ischemia, a proportion of oligodendrocytes in the ischemic territory is initially damaged, often followed by axonal and

oligodendrocyte degeneration and regeneration processes. Martín–Lopez et al. [142] reported a significant increase in the number of oligodendrocyte precursor cells at 2 days, followed by a significant decrease at 5 and 21 days in a rat model of focal brain ischemia, compared to sham control. The study observed reduced levels of myelin basic protein and of Black Gold II-stained white matter at 2 and 5 days, returning to control levels by 21 days. EM confirmed marked demyelination at 5 days, with spontaneous remyelination approaching control levels at 21 days after brain ischemia. The authors concluded that a spontaneous oligodendrocyte remyelination mechanism occurs between 5 and 21 days after brain ischemia, potentially explaining the recovery of sensorimotor function from initial deficits in rodents after brain ischemia [142].

Autophagy plays a key role in adult oligodendrocyte function, particularly in modifying myelin wraps [145]. The importance of this process in stroke recovery is highlighted by studies showing that genetic deletion of Atg5 in oligodendrocytes impairs functional recovery after brain ischemia [146]. Moreover, autophagy inhibition reduces both the number and length of myelin segments on axons [147]. Emerging evidence suggests that autophagic activity in oligodendrocytes may be key to understanding white matter injury and remyelination processes after brain ischemia [148,149]. However, the detailed investigations into autophagy pathway components and their regulations in oligodendrocytes after brain ischemia remain limited, highlighting a significant area of future research.

Autophagy may be involved in astrocyte proliferation, but current understanding is limited in stroke animal models [150–152]. Most *in vivo* studies of astrocyte autophagy use relatively non-specific inhibitors, making it challenging to draw definitive conclusions. As a result, the role of astrocyte autophagy after brain ischemia in animal models remains to be defined [153].

Concluding remarks

Information accumulated through many years of autophagy research has provided a broader understanding of the entire autophagy process in neurological diseases, including brain ischemia. However, significant knowledge gaps still exist. The regulations of cell-specific autophagy and organelle-specific autophagy after brain ischemia remain only partially understood. Furthermore, our knowledge regarding relationships between autophagic activity and other cell injury pathways after brain ischemia is limited.

Neurons require exceptionally high levels of autophagic activity to maintain their homeostasis because of numerous neuronal processes. A typical neuron has about 7,000 distal synaptic connections to other neurons [154]. Reduction of the lysosomal acidification with the V–ATPase inhibitor bafilomycin A1 leads to a build–up of APs

exclusively within the neuronal soma region, suggesting that the digestive lysosomal structures are mainly located in regions near the neuronal soma [155–159]. This is consistent with observations of cathepsins and LAMP1/2 immunoreactivities being predominantly located in the neuronal soma region in non–ischemic neurons, which dramatically increase post–ischemia [5,91,93]. Accumulating evidence suggests that autophagic cargo-loaded structures from these thousands of synaptic connections may need to be transported from the remote site of the processes to the soma for degradation. Dysfunctional autophagy has been observed in neurons across a variety of neurological disorders, including stroke, traumatic brain injury, Alzheimer disease, Parkinson disease, Huntington disease, and amyotrophic lateral sclerosis [160]. Notably, lysosomal storage disorders also favorably affect neurons [94–96]. Understanding the details of autophagic flux changes after brain ischemia and across neurological diseases is crucial for developing therapeutic strategies against autophagy–related brain injuries.

While changes in autophagic flux occur in both neuronal and non-neuronal cells after brain ischemia, current studies of non-neuronal cell autophagy are relatively limited but highly relevant to neurological function recovery. Studies have shown that autophagy deficiency in specific non-neuronal cells can significantly impact the recovery of neurological function after brain ischemia [135,146]. These studies provide strong evidence that autophagic activities in non-neuronal cell types are required for the recovery after brain ischemia, emphasizing the necessity for further in-depth studies in this area.

General autophagy enhancement, such as via rapamycin treatment might not be an effective therapeutic strategy and has potential for harm in pathological conditions, as it broadly induces autophagy via mTOR inhibition. In the setting of brain ischemia, a more attractive treatment strategy would specifically target and correct the defect in autophagic flux rather than simply broadly enhance autophagy. For example, inactivation of NSF ATPase may be a critical defective step within the entire autophagy pathway resulting in interruption of endolysosomal trafficking and build-up of toxic, damaged organelles and protein aggregates in post-ischemic neurons [5,90,91]. Furthermore, cell-specific autophagic flux enhancement might also be considered when developing therapeutic strategy in pathological conditions. For example, phagocyte-specific autophagic flux enhancement might be a double-edge sword: eliminating damaged cellular structures and debris to maintain tissue homeostasis, whereas the over-enhancement might lead to collateral removal of salvageable cells. Therefore, a complete understanding of the autophagy pathway, and its defects in the setting of pathological conditions, such

as cerebral ischemia, is necessary to guide the development of better treatment strategies.

Disclosure statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Abbreviations

AL/EL:	autolysosome/endolysosome
AP:	autophagosome
ATG:	autophagy related
BBB:	blood brain barrier
CTSB:	cathepsin B
EE:	early endosome
EM:	electron microscopy
L:	lysosome (terminal)
L-cycle:	lysosomal degradation cycle
LAMP:	lysosomal membrane–associated protein
LC3:	microtubule–associated protein light chain 3
LC3–II:	lipidated form of LC3
LE:	late endosome
NSF:	N–ethylmaleimide sensitive factor
SNAP:	soluble NSF attachment protein
SNARE:	SNAP receptor
SQSTM1/p62:	sequestosome 1
V–ATPase:	vacuolar H^+–ATPase

Author contributions

Bingren Hu wrote the original draft of the manuscript. Emily Osterli, Yujung Park, and Kurt Hu edited and revised the manuscript. Gary Kasof and Thorsten Wiederhold contributed to Figure 1, its associated description, discussion, and revisions of the manuscript. Chunli Liu contributed to the editing, discussion, and revisions of the manuscript.

Ethical approval

This article does not contain any studies with human subjects. All experimental procedures involving animal use were approved by the Animal Use and Care Committees at the University of California San Diego.

References

[1] Penketh J, Nolan JP. In–hospital cardiac arrest: the state of the art. Crit Care. 2022 Dec 6;26(1):376.
[2] Nistor M, Behringer W, Schmidt M, et al. A systematic review of neuroprotective strategies during hypovolemia and hemorrhagic shock. Int J Mol Sci. 2017 Oct 26;18(11):2247.
[3] Hogue CW, Gottesman RF, Stearns J. Mechanisms of cerebral injury from cardiac surgery. Crit Care Clin. 2008 Jan;24(1):83–98.
[4] Sandroni C, Cronberg T, Sekhon M. Brain injury after cardiac arrest: pathophysiology, treatment, and prognosis. Intensive Care Med. 2021 Dec;47(12):1393–1414.
[5] Yuan D, Liu C, Wu J, et al. Inactivation of NSF ATPase leads to cathepsin B release after transient cerebral ischemia. Transl Stroke Res. 2018 Jun;9(3):201–213.
[6] Hu K, Gaire BP, Subedi L, et al. Interruption of endolysosomal trafficking after focal brain ischemia. Front Mol Neurosci. 2021;14:719100.
[7] Carmichael ST. Rodent models of focal stroke: size, mechanism, and purpose. NeuroRx. 2005 Jul;2(3):396–409.
[8] Lipton P. Ischemic cell death in brain neurons. Physiol Rev. 1999 Oct;79(4):1431–1568.
[9] Puig B, Brenna S, Magnus T. Molecular communication of a dying neuron in stroke. Int J Mol Sci. 2018 Sep 19;19(9):2834.
[10] Fifield KE, Vanderluit JL. Rapid degeneration of neurons in the penumbra region following a small, focal ischemic stroke. Eur J Neurosci. 2020 Aug;52(4):3196–3214.
[11] Traystman RJ. Animal models of focal and global cerebral ischemia. ILAR J. 2003;44(2):85–95.
[12] Cooper GM. The cell: a molecular approach. protein degradation. 2nd ed. Sunderland (MA): Sinauer Associates; 2000.
[13] Dikic I. Proteasomal and autophagic degradation systems. Annu Rev Biochem. 2017 Jun 20;86:193–224.
[14] Zhao L, Zhao J, Zhong K, et al. Targeted protein degradation: mechanisms, strategies and application. Signal Transduct Target Ther. 2022 Apr 4;7(1):113.
[15] Wong E, Cuervo AM. Autophagy gone awry in neurodegenerative diseases. Nat Neurosci. 2010 Jul;13(7):805–811.
[16] Mariño G, Madeo F, Kroemer G. Autophagy for tissue homeostasis and neuroprotection. Curr Opin Cell Biol. 2011 Apr;23(2):198–206.
[17] Schneider JL, Cuervo AM. Autophagy and human disease: emerging themes. Curr Opin Genet Dev. 2014 Jun;26:16–23.
[18] Kim M, Sandford E, Gatica D, et al. Mutation in ATG5 reduces autophagy and leads to ataxia with developmental delay. Elife. 2016 Jan 26;5:e12245.
[19] Mizushima N, Levine B. Autophagy in mammalian development and differentiation. Nat Cell Biol. 2010 Sep 1;12(9):823–830.

[20] Maday S, Holzbaur EL. Compartment–specific regulation of autophagy in primary neurons. J Neurosci. 2016 Jun 1;36(22):5933–5945.
[21] Kulkarni A, Chen J, Maday S. Neuronal autophagy and intercellular regulation of homeostasis in the brain. Curr Opin Neurobiol. 2018 Aug;51:29–36.
[22] Yu L, Chen Y, Tooze SA. Autophagy pathway: cellular and molecular mechanisms. Autophagy. 2018;14(2):207–215.
[23] Yamamoto H, Zhang S, Mizushima N. Autophagy genes in biology and disease. Nat Rev Genet. 2023 Jun;24(6):382–400.
[24] Kim J, Kundu M, Viollet B, et al. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat Cell Biol. 2011 Feb;13(2):132–141.
[25] Heras–Sandoval D, Pérez–Rojas JM, Hernández–Damián J, et al. The role of PI3K/AKT/mTOR pathway in the modulation of autophagy and the clearance of protein aggregates in neurodegeneration. Cell Signal. 2014 Dec;26(12):2694–2701.
[26] Cui D, Qu R, Liu D, et al. The cross talk between p53 and mTOR pathways in response to physiological and genotoxic stresses. Front Cell Dev Biol. 2021 Nov 18;9:775507.
[27] Hosokawa N, Sasaki T, Iemura S, et al. Atg101, a novel mammalian autophagy protein interacting with Atg13. Autophagy. 2009 Oct;5(7):973–979.
[28] Funderburk SF, Wang QJ, Yue Z. The Beclin 1–VPS34 complex–at the crossroads of autophagy and beyond. Trends Cell Biol. 2010 Jun;20(6):355–362.
[29] Russell RC, Tian Y, Yuan H, et al. ULK1 induces autophagy by phosphorylating Beclin–1 and activating VPS34 lipid kinase. Nat Cell Biol. 2013 Jul;15(7):741–750.
[30] Pattingre S, Tassa A, Qu X, et al. Bcl–2 antiapoptotic proteins inhibit Beclin 1–dependent autophagy. Cell. 2005 Sep 23;122(6):927–939.
[31] Marquez RT, Xu L. Bcl–2:Beclin 1 complex: multiple, mechanisms regulating autophagy/apoptosis toggle switch. Am J Cancer Res. 2012;2(2):214–221.
[32] He C, Zhu H, Li H, et al. Dissociation of Bcl–2–Beclin1 complex by activated AMPK enhances cardiac autophagy and protects against cardiomyocyte apoptosis in diabetes. Diabetes. 2013 Apr;62(4):1270–1281.
[33] Maiuri MC, Zalckvar E, Kimchi A, et al. Self–eating and self–killing: crosstalk between autophagy and apoptosis. Nat Rev Mol Cell Biol. 2007 Sep;8(9):741–752.
[34] Noda NN, Fujioka Y, Hanada T, et al. Structure of the Atg12–Atg5 conjugate reveals a platform for stimulating Atg8–PE conjugation. EMBO Rep. 2013 Feb;14(2):206–211.
[35] Walczak M, Martens S. Dissecting the role of the Atg12–Atg5–Atg16 complex during autophagosome formation. Autophagy. 2013 Mar;9(3):424–425.
[36] Tanida I, Ueno T, Kominami E. Human light chain 3/MAP1LC3B is cleaved at its carboxyl–terminal Met121 to expose Gly120 for lipidation and targeting to autophagosomal membranes. J Biol Chem. 2004 Nov 12;279(46):47704–47710.
[37] Tanida I, Ueno T, Kominami E. LC3 conjugation system in mammalian autophagy. Int J Biochem Cell Biol. 2004 Dec;36(12):2503–2518.
[38] Pankiv S, Clausen TH, Lamark T, et al. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. J Biol Chem. 2007 Aug 17;282(33):24131–24145.
[39] Isogai S, Morimoto D, Arita K, et al. Crystal structure of the ubiquitin–associated (UBA) domain of p62 and its interaction with ubiquitin. J Biol Chem. 2011 Sep 9;286(36):31864–31874.

[40] Sabatini DD, Adesnik M. Christian de Duve: explorer of the cell who discovered new organelles by using a centrifuge. Proc Natl Acad Sci USA. 2013 Aug 13;110 (33):13234–13235.
[41] de Duve C. How I became a biochemist. IUBMB Life. 2006 Oct;58(10):614–618.
[42] Bright NA, Davis LJ, Luzio JP. Endolysosomes are the principal intracellular sites of acid hydrolase activity. Curr Biol. 2016 Sep 12;26(17):2233–2245.
[43] Sava I, Davis LJ, Gray SR, et al. Reversible assembly and disassembly of V–ATPase during the lysosome regeneration cycle. Mol Biol Cell. 2024 May 1;35(5):ar63.
[44] Mellman I, Fuchs R, Helenius A. Acidification of the endocytic and exocytic pathways. Annu Rev Biochem. 1986 Jul;55:663–700.
[45] Scott CC, Gruenberg J. Ion flux and the function of endosomes and lysosomes: pH is just the start: the flux of ions across endosomal membranes influences endosome function not only through regulation of the luminal pH. Bioessays. 2011 Feb;33(2):103–110.
[46] Mindell JA. Lysosomal acidification mechanisms. Annu Rev Physiol. 2012;74:69–86.
[47] Hu YB, Dammer EB, Ren RJ, et al. The endosomal–lysosomal system: from acidification and cargo sorting to neurodegeneration. Transl Neurodegener. 2015 Sep 30;4:18.
[48] Podinovskaia M, Prescianotto–Baschong C, Buser DP, et al. A novel live–cell imaging assay reveals regulation of endosome maturation. Elife. 2021 Nov 30; 10;10. doi:10.7554/eLife.70982
[49] De Luca M, Bucci C. A new V–ATPase regulatory mechanism mediated by the Rab interacting lysosomal protein (RILP). Commun Integr Biol. 2014 Oct;7(5). doi:10.4161/cib.29616.
[50] Klionsky DJ, Abdel–Aziz AK, Abdelfatah S, et al. Guidelines for the use and interpretation of assays for monitoring autophagy. 4th. Autophagy. 2021Jan;171:1–382
[51] Mauthe M, Orhon I, Rocchi C, et al. Chloroquine inhibits autophagic flux by decreasing autophagosome–lysosome fusion. Autophagy. 2018;14 (8):1435–1455.
[52] Runwal G, Stamatakou E, Siddiqi FH, et al. LC3–positive structures are prominent in autophagy–deficient cells. Sci Rep. 2019 Jul 12;9(1):10147.
[53] Zhao YG, Zhang H. Autophagosome maturation: an epic journey from the ER to lysosomes. J Cell Biol. 2019 Mar 4;218(3):757–770.
[54] Yim WW, Mizushima N. Lysosome biology in autophagy. Cell Discov. 2020 Feb 11;6(1):6.
[55] Ganesan D, Cai Q. Understanding amphisomes. Biochem J. 2021 May 28;478 (10):1959–1976.
[56] Repnik U, Česen MH, Turk B. The endolysosomal system in cell death and survival. Cold Spring Harb Perspect Biol. 2013 Jan 1;5(1):a008755.
[57] Inpanathan S, Botelho RJ. The lysosome signaling platform: adapting with the times. Front Cell Dev Biol. 2019 Jun 20;7:113.
[58] Zhang HY, Tian Y, Shi HY, et al. The critical role of the endolysosomal system in cerebral ischemia. Neural Regen Res. 2023 May;18(5):983–990.
[59] Lie PPY, Nixon RA. Lysosome trafficking and signaling in health and neurodegenerative diseases. Neurobiol Dis. 2019 Feb;122:94–105.
[60] Saftig P, Klumperman J. Lysosome biogenesis and lysosomal membrane proteins: trafficking meets function. Nat Rev Mol Cell Biol. 2009 Sep;10(9):623–635.

[61] Malhotra V, Orci L, Glick BS, et al. Role of an N–ethylmaleimide–sensitive transport component in promoting fusion of transport vesicles with cisternae of the golgi stack. Cell. 1988 Jul 15;54(2):221–227.
[62] Yoon TY, Munson M. SNARE complex assembly and disassembly. Curr Biol. 2018 Apr 23;28(8):R397–R401.
[63] Bombardier JP, Munson M. Three steps forward, two steps back: mechanistic insights into the assembly and disassembly of the SNARE complex. Curr Opin Chem Biol. 2015 Dec;29:66–71.
[64] Baker RW, Hughson FM. Chaperoning SNARE assembly and disassembly. Nat Rev Mol Cell Biol. 2016 Aug;17(8):465–479.
[65] Hong W, Lev S. Tethering the assembly of SNARE complexes. Trends Cell Biol. 2014 Jan;24(1):35–43.
[66] Mizushima N, Matsui T, Yamamoto H. YKT6 as a second SNARE protein of mammalian autophagosomes. Autophagy. 2019 Jan;15(1):176–177.
[67] Song X, Xi Y, Dai M, et al. STING guides the STX17–SNAP29–VAMP8 complex assembly to control autophagy. Cell Insight. 2024Feb2;3(2):100147.
[68] Zheng D, Tong M, Zhang S, et al. Human YKT6 forms priming complex with STX17 and SNAP29 to facilitate autophagosome–lysosome fusion. Cell Rep. 2024 Feb 27;43(2):113760.
[69] Milovanovic D, Jahn R. Organization and dynamics of SNARE proteins in the presynaptic membrane. Front Physiol. 2015 Mar 19;6:89.
[70] Burgalossi A, Jung S, Meyer G, et al. SNARE protein recycling by αSNAP and βSNAP supports synaptic vesicle priming. Neuron. 2010 Nov 4;68(3):473–487.
[71] Saheki Y, De Camilli P. Synaptic vesicle endocytosis. Cold Spring Harb Perspect Biol. 2012 Sep 1;4(9):a005645.
[72] Maejima I, Takahashi A, Omori H, et al. Autophagy sequesters damaged lysosomes to control lysosomal biogenesis and kidney injury. EMBO J. 2013 Aug 28;32(17):2336–2347.
[73] Anding AL, Baehrecke EH. Cleaning house: selective autophagy of organelles. Dev Cell. 2017 Apr 10;41(1):10–22.
[74] Lu LQ, Tang MZ, Qi ZH, et al. Regulation of the golgi apparatus via GOLPH3–mediated new selective autophagy. Life Sci. 2020 Jul 15;253:117700.
[75] Doblado L, Lueck C, Rey C, et al. Mitophagy in human diseases. Int J Mol Sci. 2021 Apr 9;22(8):3903.
[76] Mochida K, Nakatogawa H. ER–phagy: selective autophagy of the endoplasmic reticulum. EMBO Rep. 2022 Aug 3;23(8):e55192.
[77] Hickey KL, Swarup S, Smith IR, et al. Proteome census upon nutrient stress reveals golgiphagy membrane receptors. Nature. 2023 Nov;623(7985):167–174.
[78] Siesjö BK, Hu B, Kristìan T. Is the cell death pathway triggered by the mitochondrion or the endoplasmic reticulum? J Cereb Blood Flow Metab. 1999 Jan;19 (1):19–26.
[79] Lee JM, Grabb MC, Zipfel GJ, et al. Brain tissue responses to ischemia. J Clin Invest. 2000 Sep;106(6):723–731.
[80] Kato T, Hirano A, Katagiri T, et al. Neurofibrillary tangle formation in the nucleus basalis of meynert ipsilateral to a massive cerebral infarct. Ann Neurol. 1988 Jun;23(6):620–623.
[81] Pluta R, Kida E, Lossinsky AS, et al. Complete cerebral ischemia with short–term survival in rats induced by cardiac arrest. I. Extracellular accumulation of Alzheimer's beta–amyloid protein precursor in the brain. Brain Res. 1994 Jun 27;649(1–2):323–328.

[82] van Groen T, Puurunen K, Mäki HM, et al. Transformation of diffuse beta–amyloid precursor protein and beta–amyloid deposits to plaques in the thalamus after transient occlusion of the middle cerebral artery in rats. Stroke. 2005 Jul;36(7):1551–1556.
[83] Pluta R, Ułamek M, Jabłoński M. Alzheimer's mechanisms in ischemic brain degeneration. Anat Rec (Hoboken). 2009 Dec;292(12):1863–1881.
[84] Hatsuta H, Takao M, Nogami A, et al. Tau and TDP–43 accumulation of the basal nucleus of Meynert in individuals with cerebral lobar infarcts or hemorrhage. Acta Neuropathol Commun. 2019 Mar 28;7(1):49.
[85] Herrup K, Yang Y. Cell cycle regulation in the postmitotic neuron: oxymoron or new biology? Nat Rev Neurosci. 2007 May;8(5):368–378.
[86] Ułamek–Kozioł M, Kocki J, Bogucka–Kocka A, et al. Dysregulation of autophagy, mitophagy, and apoptotic genes in the medial temporal lobe cortex in an ischemic model of Alzheimer's Disease. J Alzheimers Dis. 2016 Jul 27;54 (1):113–121.
[87] Ułamek–Kozioł M, Kocki J, Bogucka–Kocka A, et al. Autophagy, mitophagy and apoptotic gene changes in the hippocampal CA1 area in a rat ischemic model of Alzheimer's disease. Pharmacol Rep. 2017 Dec;69(6):1289–1294.
[88] Ułamek–Kozioł M, Czuczwar SJ, Kocki J, et al. Dysregulation of autophagy, mitophagy, and apoptosis genes in the CA3 region of the hippocampus in the ischemic model of Alzheimer's Disease in the rat. J Alzheimers Dis. 2019;72(4):1279–1286.
[89] Pluta R, Bogucka–Kocka A, Bogucki J, et al. Apoptosis, autophagy, and mitophagy genes in the CA3 area in an ischemic model of Alzheimer's Disease with 2–year survival. J Alzheimers Dis. 2024;99(4):1375–1383.
[90] Liu C, Gao Y, Barrett J, et al. Autophagy and protein aggregation after brain ischemia. J Neurochem. 2010 Oct;115(1):68–78.
[91] Yuan D, Hu K, Loke CM, et al. Interruption of endolysosomal trafficking leads to stroke brain injury. Exp Neurol. 2021 Nov;345:113827.
[92] Yuan D, Liu C, Hu B. Dysfunction of membrane trafficking leads to ischemia–reperfusion injury after transient cerebral ischemia. Transl Stroke Res. 2018 Jun;9(3):215–222.
[93] Hu K, Park Y, Olivas Y, et al. Cathepsin B knockout confers significant brain protection in the mouse model of stroke. Exp Neurol. 2023 Oct;368:114499.
[94] Qureshi YH, Baez P, Reitz C. Endosomal trafficking in alzheimer's disease, parkinson's disease, and neuronal ceroid lipofuscinosis. Mol Cell Biol. 2020 Sep 14;40(19):e00262–20.
[95] Navarro–Romero A, Montpeyó M, Martinez–Vicente M. The emerging role of the lysosome in Parkinson's Disease. Cells. 2020 Nov 2;9(11):2399.
[96] Song Q, Meng B, Xu H, et al. The emerging roles of vacuolar–type ATPase–dependent Lysosomal acidification in neurodegenerative diseases. Transl Neurodegener. 2020 May 11;9(1):17.
[97] Mizushima N, Yoshimori T, Levine B. Methods in mammalian autophagy research. Cell. 2010 Feb 05;140(3):313–326.
[98] Wu Y–T, Tan H–L, Shui G, et al. Dual role of 3–methyladenine in modulation of autophagy via different temporal patterns of inhibition on class I and III phosphoinositide 3–kinase. J Biol Chem. 2010 Apr 2;285(14):10850–10861.
[99] Sung K, Jimenez–Sanchez M. Autophagy in astrocytes and its implications in neurodegeneration. J Mol Biol. 2020 Apr 3;432(8):2605–2621.

[100] Mo Y, Sun YY, Liu KY. Autophagy and inflammation in ischemic stroke. Neural Regen Res. 2020 Aug;15(8):1388–1396.
[101] Zheng Y, Zhou Z, Han F, et al. Autophagic regulation of neuroninflammation in ischemic stroke. Neurochem Int. 2021 Jun 27;148:105114.
[102] Peng L, Hu G, Yao Q, et al. Microglia autophagy in ischemic stroke: a double–edged sword. Front Immunol. 2022 Nov 16;13:1013311.
[103] Kirino T, Tamura A, Sano K. Delayed neuronal death in the rat hippocampus following transient forebrain ischemia. Acta Neuropathol. 1984;64(2):139–147.
[104] Petito CK, Lapinski RL. Postischemic alterations in ultrastructural cytochemistry of neuronal golgi apparatus. Lab Invest. 1986 Dec;55(6):696–702.
[105] Rafols JA, Daya AM, O'Neil BJ, et al. Global brain ischemia and reperfusion: golgi apparatus ultrastructure in neurons selectively vulnerable to death. Acta Neuropathol. 1995;90(1):17–30.
[106] Hu BR, Martone ME, Jones YZ, et al. Protein aggregation after transient cerebral ischemia. J Neurosci. 2000 May 01;20(9):3191–3199.
[107] Hu BR, Janelidze S, Ginsberg MD, et al. Protein aggregation after focal brain ischemia and reperfusion. J Cereb Blood Flow Metab. 2001 Jul;21(7):865–875.
[108] Liu CL, Martone ME, Hu BR. Protein ubiquitination in postsynaptic densities after transient cerebral ischemia. J Cereb Blood Flow Metab. 2004 Nov;24 (11):1219–1225.
[109] Liu C, Hu B. Alterations of N–ethylmaleimide–sensitive ATPase following transient cerebral ischemia. Neuroscience. 2004;128(4):767–774.
[110] Liu CL, Ge P, Zhang F, et al. Co–translational protein aggregation after transient cerebral ischemia. Neuroscience. 2005;134(4):1273–1284.
[111] Liu C, Chen S, Kamme F, et al. Ischemic preconditioning prevents protein aggregation after transient cerebral ischemia. Neuroscience. 2005;134 (1):69–80.
[112] Komatsu M, Waguri S, Ueno T, et al. Impairment of starvation–induced and constitutive autophagy in Atg7–deficient mice. J Cell Biol. 2005 May 9;169 (3):425–434.
[113] Hara T, Nakamura K, Matsui M, et al. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. Nature. 2006 Jun 15;441 (7095):885–889.
[114] Mizushima N, Hara T. Intracellular quality control by autophagy: how does autophagy prevent neurodegeneration? Autophagy. 2006 Oct–Dec;2 (4):302–304.
[115] Yan L, Vatner DE, Kim SJ, et al. Autophagy in chronically ischemic myocardium. Proc Natl Acad Sci USA. 2005 Sep 27;102(39):13807–13812.
[116] Carloni S, Buonocore G, Balduini W. Protective role of autophagy in neonatal hypoxia–ischemia induced brain injury. Neurobiol Dis. 2008 Dec;32(3):329–339.
[117] Carloni S, Girelli S, Scopa C, et al. Activation of autophagy and Akt/CREB signaling play an equivalent role in the neuroprotective effect of rapamycin in neonatal hypoxia–ischemia. Autophagy. 2010 Apr;6(3):366–377.
[118] Carloni S, Buonocore G, Longini M, et al. Inhibition of rapamycin–induced autophagy causes necrotic cell death associated with Bax/Bad mitochondrial translocation. Neuroscience. 2012 Feb 17;203:160–169. DOI:10.1016/j.neuroscience.2011.12.021.
[119] Zhang X, Yan H, Yuan Y, et al. Cerebral ischemia–reperfusion–induced autophagy protects against neuronal injury by mitochondrial clearance. Autophagy. 2013 Sep;9(9):1321–1333.

[120] Koike M, Shibata M, Tadakoshi M, et al. Inhibition of autophagy prevents hippocampal pyramidal neuron death after hypoxic–ischemic injury. Am J Pathol. 2008 Feb;172(2):454–469.
[121] Wen YD, Sheng R, Zhang LS, et al. Neuronal injury in rat model of permanent focal cerebral ischemia is associated with activation of autophagic and lysosomal pathways. Autophagy. 2008 Aug;4(6):762–769.
[122] Puyal J, Vaslin A, Mottier V, et al. Postischemic treatment of neonatal cerebral ischemia should target autophagy. Ann Neurol. 2009 Sep;66(3):378–389.
[123] Wang JY, Xia Q, Chu KT, et al. Severe global cerebral ischemia–induced programmed necrosis of hippocampal CA1 neurons in rat is prevented by 3–methyladenine: a widely used inhibitor of autophagy. J Neuropathol Exp Neurol. 2011 Apr;70(4):314–322.
[124] Ginet V, Puyal J, Clarke PG, et al. Enhancement of autophagic flux after neonatal cerebral hypoxia–ischemia and its region–specific relationship to apoptotic mechanisms. Am J Pathol. 2009 Nov;175(5):1962–1974.
[125] Gabryel B, Kost A, Kasprowska D. Neuronal autophagy in cerebral ischemia–a potential target for neuroprotective strategies? Pharmacol Rep. 2012;64 (1):1–15.
[126] Chen W, Sun Y, Liu K, et al. Autophagy: a double–edged sword for neuronal survival after cerebral ischemia. Neural Regen Res. 2014 Jun 15;9(12):1210–1216.
[127] Sheng R, Qin ZH. The divergent roles of autophagy in ischemia and preconditioning. Acta Pharmacol Sin. 2015 Apr;36(4):411–420.
[128] Wang M, Lee H, Elkin K, et al. Detrimental and beneficial effect of autophagy and a potential therapeutic target after ischemic stroke. Evid Based Complement Alternat Med. 2020 Sep 23;2020:8372647.
[129] Shi Q, Cheng Q, Chen C. The role of autophagy in the pathogenesis of ischemic stroke. Curr Neuropharmacol. 2021;19(5):629–640.
[130] Yoshii SR, Mizushima N. Monitoring and measuring autophagy. Int J Mol Sci. 2017 Aug 28;18(9):1865.
[131] Stanzione R, Pietrangelo D, Cotugno M, et al. Role of autophagy in ischemic stroke: insights from animal models and preliminary evidence in the human disease. Front Cell Dev Biol. 2024;12:1360014.
[132] Corti O, Blomgren K, Poletti A, et al. Autophagy in neurodegeneration: new insights underpinning therapy for neurological diseases. J Neurochem. 2020 Aug;154(4):354–371.
[133] Cappelletti P, Filareti M, Masuelli L, et al. Syntaxin–1a and SNAP–25 expression level is increased in the blood samples of ischemic stroke patients. Sci Rep. 2022 Aug 25;12(1):14483.
[134] Lee JH, Yang DS, Goulbourne CN, et al. Faulty autolysosome acidification in Alzheimer's disease mouse models induces autophagic build–up of Aβ in neurons, yielding senile plaques. Nat Neurosci. 2022 Jun;25(6):688–701.
[135] Kotoda M, Furukawa H, Miyamoto T, et al. Role of myeloid lineage cell autophagy in ischemic brain injury. Stroke. 2018 Jun;49(6):1488–1495.
[136] Pluta R, Miziak B, Czuczwar SJ. Post–ischemic permeability of the blood–brain barrier to amyloid and platelets as a factor in the maturation of Alzheimer's Disease–type brain neurodegeneration. Int J Mol Sci. 2023 Jun 27;24(13):10739.
[137] Park S–K, Cho JM, Mookherjee S, et al. Brain endothelial cell barrier function is compromised by autophagy depletion. FASEB J. 2022 May 13;36(S1). https://doi.org/10.1096/fasebj.2022.36.S1.R5826

[138] Kim KA, Shin D, Kim JH, et al. Role of autophagy in endothelial damage and blood–brain barrier disruption in ischemic stroke. Stroke. 2018 Jun;49(6):1571–1579.
[139] Wang Y, Liu G, Hong D, et al. White matter injury in ischemic stroke. Prog Neurobiol. 2016 Jun;141:45–60.
[140] Uniken Venema SM, Postma AA, van den Wijngaard IR, et al. MR CLEAN registry investigators*. white matter lesions and outcomes after endovascular treatment for acute ischemic stroke: MR CLEAN registry results. Stroke. 2021 Aug;52 (9):2849–2857.
[141] Krafft PR, Bailey EL, Lekic T, et al. Etiology of stroke and choice of models. Int J Stroke. 2012 Jul;7(5):398–406.
[142] Martín–Lopez G, Mallavibarrena PR, Villa–Gonzalez M, et al. The dynamics of oligodendrocyte populations following permanent ischemia promotes long–term spontaneous remyelination of damaged area. Biochim Biophys Acta Mol Basis Dis. 2024 May 31;1870(7):167270.
[143] Fernandez–Castaneda A, Gaultier A. Adult oligodendrocyte progenitor cells – Multifaceted regulators of the CNS in health and disease. Brain Behav Immun. 2016 Oct;57:1–7.
[144] Dawson MR, Polito A, Levine JM, et al. NG2–expressing glial progenitor cells: an abundant and widespread population of cycling cells in the adult rat CNS. Mol Cell Neurosci. 2003 Oct;24(2):476–488.
[145] Dutta DJ, Woo DH, Lee PR, et al. Regulation of myelin structure and conduction velocity by perinodal astrocytes. Proc Natl Acad Sci USA. 2018 Nov 13;115 (46):11832–11837.
[146] Saraswat Ohri S, Bankston AN, Mullins SA, et al. Blocking autophagy in oligodendrocytes limits functional recovery after spinal cord injury. J Neurosci. 2018 Jun 27;38(26):5900–5912.
[147] Belgrad J, De Pace R, Fields RD. Autophagy in Myelinating Glia. J Neurosci. 2020 Jan 08;40(2):256–266.
[148] Zhang SQ, Ding FF, Liu Q, et al. Autophagy inhibition exerts neuroprotection on white matter ischemic damage after chronic cerebral hypoperfusion in mice. Brain Res. 2019 Oct 15;1721:146337.
[149] Wang H, Liu Y, Guo Z, et al. Enhancement of oligodendrocyte autophagy alleviates white matter injury and cognitive impairment induced by chronic cerebral hypoperfusion in rats. Acta Pharm Sin B. 2023 May;13(5):2107–2123.
[150] Li CY, Li X, Liu SF, et al. Inhibition of mTOR pathway restrains astrocyte proliferation, migration and production of inflammatory mediators after oxygen–glucose deprivation and reoxygenation. Neurochem Int. 2015 Apr–May;83–84:9–18.
[151] Zhang Y, Miao JM. Ginkgolide K promotes astrocyte proliferation and migration after oxygen–glucose deprivation via inducing protective autophagy through the AMPK/mTOR/ULK1 signaling pathway. Eur J Pharmacol. 2018 Aug 5;832:96–103.
[152] Su PW, Zhai Z, Wang T, et al. Research progress on astrocyte autophagy in ischemic stroke. Front Neurol. 2022 Aug 30;13:951536.
[153] Ortiz–Rodriguez A, Arevalo MA. The contribution of astrocyte autophagy to systemic metabolism. Int J Mol Sci. 2020 Apr 3;21(7):2479.
[154] Pakkenberg B, Pelvig D, Marner L, et al. Aging and the human neocortex. Exp Gerontol. 2003 Jan–Feb;38(1–2):95–99.
[155] Tammineni P, Cai Q. Defective retrograde transport impairs autophagic clearance in Alzheimer disease neurons. Autophagy. 2017 May 04;13(5):982–984.

[156] Gowrishankar S, Yuan P, Wu Y, et al. Massive accumulation of luminal protease–deficient axonal lysosomes at Alzheimer's disease amyloid plaques. Proc Natl Acad Sci USA. 2015 Jul 14;112(28):E3699–708.
[157] Cai Q, Lu L, Tian JH, et al. Snapin–regulated late endosomal transport is critical for efficient autophagy–lysosomal function in neurons. Neuron. 2010 Oct 6;68 (1):73–86.
[158] Yap CC, Digilio L, McMahon LP, et al. Degradation of dendritic cargos requires Rab7–dependent transport to somatic lysosomes. J Cell Biol. 2018 Sep 3;217 (9):3141–3159.
[159] Cheng XT, Zhou B, Lin MY, et al. Axonal autophagosomes recruit dynein for retrograde transport through fusion with late endosomes. J Cell Biol. 2015 May 11;209(3):377–386.
[160] Giovedì S, Ravanelli MM, Parisi B, et al. Dysfunctional autophagy and endolysosomal system in neurodegenerative diseases: relevance and therapeutic options. Front Cell Neurosci. 2020;14:602116.