Viewpoints

Autophagy in Myelinating Glia

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Autophagy is the cellular process involved in transportation and degradation of membrane, proteins, pathogens, and organelles. This fundamental cellular process is vital in development, plasticity, and response to disease and injury. Compared with neurons, little information is available on autophagy in glia, but it is paramount for glia to perform their critical responses to nervous system disease and injury, including active tissue remodeling and phagocytosis. In myelinating glia, autophagy has expanded roles, particularly in phagocytosis of mature myelin and in generating the vast amounts of membrane proteins and lipids that must be transported to form new myelin. Notably, autophagy plays important roles in removing excess cytoplasm to promote myelin compaction and development of oligodendrocytes, as well as in remyelination by Schwann cells after nerve trauma. This review summarizes the cell biology of autophagy, detailing the major pathways and proteins involved, as well as the roles of autophagy in Schwann cells and oligodendrocytes in development, plasticity, and diseases in which myelin is affected. This includes traumatic brain injury, Alexander's disease, Alzheimer's disease, hypoxia, multiple sclerosis, hereditary spastic paraplegia, and others. Promising areas for future research are highlighted.

Introduction

Autophagy, derived from the Greek words *phagos* meaning “eat” and *auto* meaning “self,” is an essential and conserved cellular process that drives the capture and recycling of proteins, pathogens, and organelles, allowing their removal from the cytosol and degradation in the lysosome (Levine and Klionsky, 2004; Eskelinen and Saftig, 2009; Dikic and Elazar, 2018). Well characterized in neurons (Lee, 2012; Wong and Holzbaur, 2015; Maday and Holzbaur, 2016; Menzies et al., 2017), autophagy in glial cells has been much less studied. This is surprising, considering the important involvement of autophagy in development, disease, and response to injury, and the essential functions of glia in these processes. Examples that will be discussed in this review include the new evidence of autophagy during oligodendrocyte development, in peripheral myelin compaction, and in facilitating myelin clearance after nerve injury. The functions of autophagy in myelinating glia (Schwann cells and oligodendrocytes) are even less well known than for astrocytes and microglia, yet the formation of myelin membrane and removal of myelin debris after injury involve robust transport and recycling of proteins, membrane lipids, organelles; cytoskeletal and membrane remodeling; and endocytosis and exocytosis: processes in which autophagy is critical in other cells. This review summarizes current information on autophagy in myelinating glia in association with a wide range of biological functions and in nervous system disorders, and it highlights promising areas for future research.

While other reviews have described autophagy signaling in detail (Glick et al., 2010; Dikic and Elazar, 2018), our goal is to synthesize mechanisms of autophagy in myelinating glia in the CNS and PNS during lifelong development, plasticity, injury, and disease. We first review the major components and interactions along the autophagy pathways. We then discuss studies that identify and manipulate autophagic processes in Schwann cells and oligodendrocytes across developmental and injury states. In doing so, we hope to underscore the crucial contribution that autophagy plays in myelinating glia and how these new insights may be targeted in therapies for neurological disease and injury.

Autophagy process and pathways

At least three forms of autophagy have been identified: (1) chaperone-mediated autophagy (Dice, 1990), which targets unfolded cytosolic proteins with chaperone proteins and translocates them through the lysosomal membrane; (2) microautophagy (Marzella et al., 1981), in which the lysosomal membrane undergoes local rearrangements to engulf portions of cytoplasm; and (3) macroautophagy (De Duve and Wattiaux, 1966), which, through double-membrane organelles called phagosomes, engulfs cellular material that is degraded and recycled. Microautophagy is induced by nitrogen starvation or rapamycin treatment (Li et al., 2012) and can be selective or bulk engulfment (Sahu et al., 2011). Microautophagy happens locally on the surface of lysosomes and only involves a small portion of these organelles. This review focuses on macroautophagy, henceforth referred to as autophagy.

Macroautophagy (De Duve and Wattiaux, 1966) can be induced by a number of signals (e.g., hypoxia, nutrient depletion, cellular damage, production of oxygen reactive species). Following induction, the cell starts to protrude double-membrane organelles called phagosomes, to surround and engulf the material targeted for degradation. The origin of the cup-shaped double membrane is not well understood, and is still a matter of intense

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Figure 1. Schematic representation of autophagic pathway. Cellular stress, such as amino acid starvation, targets the ULK1 complex, which then phosphorylates the PI3KC3. This triggers local phosphatidylinositol-3-phosphate (PI3P) production and nucleation of the omegasome. PI3P effectors, such as WIPI2, are then recruited, and interact with the ATG-7-ATG12-ATG5-ATG16L1-ATG3 LC3B-conjugation system. This complex, through ATG3, mediates phosphatidylethanolamine (PE) lipidation of ATG8 family proteins (e.g., LC3B), enabling their recruitment to the phagophore membrane. Membranes contributing the phagophore elongation can have different cellular origins, including recycling endosomes, mitochondria, and ATG9A-containing vesicles exported through AP-4. In selective autophagy, lipidated LC3B-II is critical for sequestration of cytosolic poly-ubiquitylated (Ub) aggregates through receptors, such as p62 and NBR1. The fusion of the mature, sealed, double-membrane autophagosome with lysosomes is mediated by SNARE and HOPS complex. Lysosomal hydrolases and proteases can then degrade the autophagic cargo and nutrients, making lipids and amino acids available for reuse in the cell.

debate. Some recent *in silico* work proposes that the double membrane is the result of progressive fusion of vesicles, followed by protein-mediated remodeling (Bahrami et al., 2017). However, macroautophagy does not always involve double-membraned structures (Mijaljica and Devenish, 2013).

In most cells, basal levels of autophagy help maintain the integrity of intracellular organelles. However, autophagy is strongly induced under starvation (Hosokawa et al., 2009; Kim et al., 2011), hypoxia (Semenza, 2010), aging (Mizushima and Komatsu, 2011), cancer (Galluzzi et al., 2015), and infection (Gomes and Dikic, 2014), reflecting a critical role of recycling membrane, organelles, and macromolecules during these processes. Macroautophagy consists of five main steps: (1) initiation and nucleation, (2) elongation of the nascent double membrane, (3) cargo sequestration, (4) fusion of the mature autophagosome with lysosomes, and (5) recycling of nutrients (Dikic and Elazar, 2018). The autophagy-related (ATG) proteins, several of which have been identified and are encoded by different *Atg* genes (Dikic and Elazar, 2018), are among the most important proteins for this process. The fundamentals of the autophagy pathway are illustrated in Figure 1.

Different cellular compartments have been described as the origin for the autophagy membrane: the first organelle proposed as source for autophagosomal membrane is the endoplasmic reticulum (ER) (Axe et al., 2008). More specifically, it has been suggested that the ER-mitochondrion interface domains donate lipids to forming phagosomes (Hailey et al., 2010). In particular,

work done in eukaryotic cells shows that the outer membrane of the mitochondria must be physically connected with the ER during starvation to share phosphatidylethanolamine lipids with the nascent autophagosome and promote the lipidation of microtubule-associated protein light chain 3B I (LC3B-I) to LC3B-II (Hailey et al., 2010). The plasma membrane has also been hypothesized to contribute to the formation of autophagosomes, involving the heavy chain of clathrin and ATG16L1 protein (Ravikumar et al., 2010). The Golgi has also been suggested as a possible independent donor of membrane forming autophagosomes (Geng et al., 2010; Ohashi and Munro, 2010). There is strong scientific evidence for each of these processes, and therefore it is possible that each of these compartments participates in the formation of the autophagic membrane, depending on the condition or cell type.

Following amino acid starvation, the unc-51 like autophagy-activating kinase 1 (ULK1) forms a complex with ATG13, FIP200, and ATG101 (Hara et al., 2008; Ganley et al., 2009), and this promotes activation of the Class III phosphoinositide 3-kinase (PI3KC3) complex, consisting of VPS34, VPS15, Beclin1, AMBRA1, and ATG14L proteins (Fimia et al., 2007; Jean and Kiger, 2014). Activation of the PI3KC3 complex produces phosphatidylinositol 3-phosphate and catalyzes the nucleation and elongation of the preautophagosomal structure (Russell et al., 2013) with the consequent recruitment of additional ATG proteins and autophagy-specific phosphatidylinositol-3-phosph-

hate effectors. These nucleation sites are known as omegasomes because of their ω -shaped profile (Lamb et al., 2013).

Addition of membrane during this process depends on further mobilization of the transmembrane protein ATG9A (Mari et al., 2010; Orsi et al., 2012; Zhou et al., 2017). During expansion of the preautophagosomal structure, the ATG12-ATG5-ATG16L1 complex is recruited and acts as an E3-like ligase to mediate the conjugation of the LC3B and/or its family members GATE16 and GABA receptor-associated protein (GABARAP) to phosphatidylethanolamine. This enables the protein complex to associate with the autophagosomal membrane (Fig. 1). After closure of the phagophore, LC3B-II remains on what will become the inner surface of the autophagosome. Therefore, LC3B protein is a widely used marker for identifying autophagosomes and autophagy flux in cells. The association of lipidated LC3B (also called LC3B-II) and GABARAP proteins with the autophagic membrane is reversible and is mediated by ATG4 (Pengo et al., 2017).

Basal autophagy is capable of extraordinary cargo selectivity. Selective autophagy pathways use cytosolic cargo receptors and adaptors, such as SQSTM1/p62 (Shaïd et al., 2013) and neighbor of BRCA1 gene 1 (NBR1) (Stolz et al., 2014). These receptor proteins bind ubiquitinated protein aggregates to ATG8 family members, including LC3B-II (Pankiv et al., 2007; Birgisdottir et al., 2013), and sequester protein aggregates within the lumen of the forming autophagosome.

Fusion of the mature autophagosome with lysosomes is mediated by the STX17-VAMP8-SNAP29 trans-SNARE complex, and an ARL8B-dependent recruitment of the homotypic fusion and protein sorting complex to lysosomes (Itakura et al., 2012; Jia et al., 2017). Inside the fused autophagolysosome, the cytosolic cargo is finally hydrolyzed. This digestion and recycling allow lipids and amino acids to be available for reuse in the cell.

Methods used to measure and interpret dynamics along the autophagy pathway have long been discussed and debated (Klionsky et al., 2012). Manipulation of autophagy is closely linked with alterations in lysosomes and the ubiquitin proteasome. For that reason, pharmacologically manipulating both proteasome and lysosomal activity reveals mechanisms about autophagy and the basic contributions that protein and lipid recycling play in the cell. Importantly, autophagy can be measured at one time point, termed steady-state autophagy, or as a dynamic activity over time, termed autophagy flux. There are a number of methods currently used to assess both the steady state and autophagic flux. We have summarized some of these methods, including their interpretation and limitations, in Table 1.

Autophagy in myelinating glia

Autophagy in the CNS has been studied predominantly in neurons; it has been largely unexplored in glial cells. This is of interest if we consider that regulation of autophagy seems to differ in neuronal and non-neuronal cells, since few autophagosomes are found in healthy neurons (Mizushima et al., 2004; Boland et al., 2008). This suggests that neurons have a low basal level or a quick turnover of autophagy. Moreover, known inducers of autophagy in non-neuronal cells (e.g., rapamycin, nutrient starvation, and lithium chloride) can fail to increase autophagosome formation in primary neurons (Mizushima et al., 2004; Komatsu et al., 2007; Mitra et al., 2009). Rapamycin promotes autophagy by inhibiting the mammalian target of rapamycin (mTOR), a protein that balances energy input versus energy expenditure of cells through accessing nutrient availability and stress signals (Jung et al., 2010; Dunlop and Tee, 2014). The lack of increased autophagosome

formation with rapamycin or known inducers of autophagy in primary neurons indicates that neurons differ from non-neuronal cells in both mTOR-dependent and -independent autophagy-inducing pathways because mTOR inhibition is considered a classical autophagy inducer. Evidence of active glial cell autophagy under the same conditions when neuronal autophagy was found to be absent highlights the importance of studying the autophagic process in glial cells, especially when considering neurodegenerative diseases.

Myelinating glia undergo robust morphological and functional changes, including vast lipid and protein production and membrane compaction; and for that reason, autophagic processes are emerging as an essential component to Schwann cell and oligodendrocyte function in development, injury, and disease. When considering autophagy in myelinating glia, it is important to first understand how *Atg* transcripts vary in expression in these cells across development. Each ATG-protein type has a unique function, and the specific functions of each are reviewed extensively by Dikic and Elazar (2018). The literature to date does not have a centralized list of *Atg* expression across oligodendrocyte development, which could be used to identify ideal autophagy-related therapeutic candidates in oligodendrocytes. To address this need, a table of the relative expression of *Atgs* transcripts across oligodendrocyte development is presented in Table 2. Protein expression of various ATG subtypes, most frequently ATG5, has also been reported in oligodendrocytes' cytoplasm (Ohri et al., 2018; Bankston et al., 2019). The subcellular distribution of autophagy proteins in morphologically complex cells of the oligodendrocyte lineage is illustrated in Figure 2, which shows representative images of adaptor protein 4 (AP-4) expression and its cargo, ATG9A, in premyelinating oligodendrocytes. There is robust expression of AP-4 and ATG9A across the oligodendrocyte processes in the unstressed *in vitro* oligodendrocyte lineage cell.

Based on RNA-seq data (Zhang et al., 2014) (Table 2), the most highly expressed *Atgs* in oligodendrocytes are *Atg9a*, *Atg12*, and *Atg3*. Interestingly, *Atg* expression varies across oligodendrocyte development, with *Atg* expression usually decreasing as the cell matures. While extensive RNA-seq data reporting *Atgs* for Schwann cells are not available at this time, multiple studies have demonstrated expression of *Atgs* in Schwann cells (Gomez-Sanchez et al., 2015; Jang et al., 2015, 2016, 2017).

Autophagy in development of myelinating glia

Autophagy plays a key role in the maturation and structural plasticity of Schwann cells (Jang et al., 2015) and oligodendrocytes (Bankston et al., 2019). Autophagy is active in Schwann cells during organelle biogenesis and is used to promote myelin compaction through removing excess cytoplasm (Jang et al., 2015). The ability of autophagy to regulate appropriate cytoplasm levels has been suggested by studies in oligodendrocytes as well (Bankston et al., 2019). Indeed, axons myelinated by *Atg5*^{−/−} oligodendrocytes have larger G-ratio measurements (thicker myelin sheaths) compared with WT oligodendrocytes (Bankston et al., 2019) because the myelin formed by *Atg5*^{−/−} oligodendrocytes was loosely compacted with pockets of cytoplasm remaining. The role of autophagy in cytoplasm decompaction has interesting implications in the field of myelin plasticity, for example, in adults, where the number of myelin wraps can change over the course of weeks (Dutta et al., 2018). The ability of oligodendrocytes to quickly either compact additional wraps or remove wraps

Table 1. Common methods used to detect and analyze the autophagic process

Method	Interpretation	Method limitations	Select references
FluoroMyelin ^a and pHrodo ^b	Demonstrates lysosomal storage accumulation and pH level	Not clearly indicative of which step of the autophagy pathway is affected, but only of the general physiological state of lysosomes	Brosius Lutz et al. (2017)
LC3B-I and LC3B-II ratio and protein expression ^{a,b}	LC3B-II is degraded during autophagy flux; therefore, altered LC3B levels can indicate the following: (1) Higher production of the LC3B-I protein (2) Activation of autophagy (increased LC3B-I lipidation into LC3B-II) (3) Defective autophagy (if autophagy is impaired and LC3B-II degradation does not occur, LC3B-II accumulates)	A Western blot of the LC3B-II/I ratio cannot be used to assay autophagy flux unless perturbation of autophagy in time is analyzed	Gomez-Sanchez et al. (2015) Marinelli et al. (2014) Huang et al. (2016) Shi et al. (2013)
Expression of autophagic proteins ^b	Commonly used to increase autophagy activity	Overexpression can lead to protein aggregation independent of autophagy (Kuma et al., 2007)	Zhang et al. (2018) Pyo et al. (2013) Oz-Levi et al. (2012) Muñoz-Galdeano et al. (2018) Shi et al. (2013) Chen et al. (2016)
Transmission electron microscopy (TEM) ^a	Microscopy used to identify the presence of autophagosomes directly and quantitatively	Unable to analyze dynamic turnover	Marinelli et al. (2014) Huang et al. (2016) Kimura et al. (2007)
RFP-EGFP-LC3B ^b	Indicates dynamic LC3B maturation	Overexpression can lead to formation of LC3B aggregates, independent of autophagy. Due to the high molecular weight of RFP and GFP, this construct cannot be used to distinguish LC3B-I and -II in Western blotting.	Bankston et al. (2019)
Global or cell-specific deletion of autophagy-related genes ^{a,b}	Commonly used method to interrupt normal autophagy function, also used to understand the physiological role of a protein of interest in the autophagy process	Some gene deletion could cause lethality in early stages. Method facilitates a steady-state analysis rather than autophagy flux.	Jang et al. (2015) Ohri et al. (2018) Kuma et al. (2017) Mizushima et al. (2004)
Transgenic GFP-LC3B mice ^b	Widely used to monitor autophagy <i>in vivo</i>	Autophagy flux in the animal would vary by tissue type and organ system. Therefore, more variables to consider compared with cell culture.	Kuma et al. (2017)

^aMethods used to monitor steady-state autophagy.^bMethods that allow measurement of autophagic flux.**Table 2. Expression of autophagy-related (*Atg*) transcripts across oligodendrocyte development^a**

Autophagy-related (<i>Atg</i>) gene name	Oligodendrocyte progenitor cell	Newly formed oligodendrocyte	Myelinating oligodendrocyte
<i>Atg3</i>	+++	+++	++
<i>Atg4a</i>	-	-	-
<i>Atg4b</i>	++	++	++
<i>Atg4c</i>	+	++	++
<i>Atg4d</i>	+	+	+
<i>Atg5</i>	++	+	+
<i>Atg7</i>	+	+	+
<i>Atg9a</i>	++++	++++	++++
<i>Atg10</i>	+	-	-
<i>Atg12</i>	+++	++	+
<i>At13</i>	++	++	++
<i>Atg14</i>	+	+	-

^a—, 0–2 FPKM; +, 2–10 FPKM; ++, 10–20 KPKM; +++, 20–30 FPKM; +++++, 30+ FPKM. Table generated from RNA-seq data collected and made available by Zhang et al. (2014).

under nondisease states indicates that there must be ongoing physiological control of autophagy in mature oligodendrocytes throughout life. Given the role that autophagy plays in promoting structural plasticity of Schwann cells, oligodendrocyte autophagy during development and across adulthood presents an intriguing path of study.

Bankston et al. (2019) demonstrate that autophagy protein ATG5 is also necessary for oligodendrocyte development. The researchers present evidence of increased autophagic flux, measured by LC3B-II/I levels and autophagosome puncta, in the distal processes of oligodendrocytes during cell differentiation. The authors also show that animals in which *Atg5* was knocked out selectively in cells of the oligodendrocyte lineage had reduced levels of MBP in the corpus callosum, a lower percentage of myelinated axons, and abnormal myelin compaction. Further evidence for the importance of autophagy in promoting oligodendrocyte differentiation is that activation of autophagy with the autophagy induction peptide Tat-beclin1 significantly increased the length of myelin segments in an oligodendrocyte-neuron coculture. Conversely, inhibition of autophagy by disrupting autophagosome closure with the drug KU55933 or

Figure 2. Autophagy protein expression in oligodendrocyte lineage cells. Cells are premyelinating oligodendrocyte lineage cells cultured from P4 WT rats and plated in growth medium. **A**, Transcription factor Olig2 (green) for oligodendroglia, adaptor protein 4 (red) and DAPI (blue) for nuclei. **B**, Transcription factor Olig2 (red), autophagy protein ATG9A (green), and DAPI (blue).

verteporfin significantly reduced the number and length of myelin segments on neurons.

As mentioned previously, mTOR activity is closely associated with autophagy induction. Interestingly, mTOR activity is also associated with oligodendrocyte myelination in multiple contexts (for review, see Dello Russo et al., 2013; Figlia et al., 2018); and as reported by Dello Russo et al. (2013), maximal white matter mTOR activity has been identified in the postnatal rat brain during PFC myelination (Tyler et al., 2011). Similarly, these authors discovered that mTOR activity was necessary for oligodendrocyte differentiation in OPC-DRG cocultures (Tyler et al., 2009). Additional studies have found that tight regulation of the mTOR complex mTORC1 is necessary for oligodendrocyte myelination (Lebrun-Julien et al., 2014). mTOR signaling complexes were shown to modulate myelin genes at the mRNA level; therefore, the effects of mTOR signaling on oligodendrocyte development could occur through changes in autophagy or changes to myelin gene expression. The exact mechanisms underlying these relationships remain to be elucidated. The autophagy activator rapamycin and its cellular target mTOR have emerged as a potential therapeutic for experimental autoimmune encephalomyelitis and multiple sclerosis (Dello Russo et al., 2013). Given that mTOR is involved in multiple cell processes beyond autophagy, however, it is important when manipulating this pathway to distinguish direct effects on autophagy from an autophagic response to the manipulation of other signaling pathways.

In summary, the work discussed in this section shows a clear and well-regulated function of autophagy across oligodendrocyte development and maturation. The role of autophagy in oligoden-

drocyte development is also suggested by the pathologies of various developmental white matter disorders. Clinical evidence suggests that autophagy plays an important role in childhood white matter development (Tyler et al., 2011; Ebrahimi-Fakhari et al., 2014, 2018), but more studies are needed to better understand this process and how it may be modulated or targeted as a therapeutic for developmental disorders.

Traumatic injury and regeneration

Schwann cell autophagy: mechanisms of myelin clearance following nerve lesion

During injury, a major role that Schwann cells perform is the phagocytosis of myelin debris. Recently, Schwann cell autophagy-mediated myelin clearance has been widely demonstrated (Gomez-Sanchez et al., 2015; Jang et al., 2015; Weiss et al., 2016; Brosius Lutz et al., 2017). In these studies, a selective autophagy termed “myelinophagy” was shown to drive myelin debris clearance following nerve injury (Gomez-Sanchez et al., 2015). Schwann cell-mediated myelin clearance was demonstrated with the findings of myelin debris inside Schwann cell autophagosomes and through elimination experiments that used pharmacological inhibition of autophagy and Atg7-KO animals to show that inhibition of autophagy significantly delays myelin breakdown after injury (Gomez-Sanchez et al., 2015). Further, Schwann cell specificity was demonstrated in animals with Schwann cell-specific Atg7 KO, which exhibited delayed myelin clearance after Wallerian degeneration (Jang et al., 2016). Surprisingly, further investigations revealed that myelin clearance by macrophage cells is also decreased in these animals (Jang et al., 2017). Jang et al. (2017) proposed that Schwann cell autophagolysosome activity was required to provide the membrane remodeling necessary to allow macrophage cells access to myelin for digestion.

In addition to classical autophagic markers, autophagy is implicated in Schwann cell function during injury through studies of miRNA changes. Spinal nerve ligation increases total RNA levels of miR-195 in dorsal horn spinal cord sections from 2 d until 14 d after injury (Shi et al., 2013). miR-195 inhibition increases the LC3B-II:LC3B-I ratio, indicating increased autophagy, and the downstream target of miR-195 was determined to be ATG14 (Shi et al., 2013). Therefore, miR-195 was determined to be a negative regulator of autophagy in the spinal cord, and in cultured primary microglia (Shi et al., 2013). miR-195 expression was not analyzed in the white matter in this study; interestingly, however, miR-195 is upregulated in Schwann cells during myelination and significantly decreased in Schwann cells with defective and thin myelin (Bremer et al., 2010). Together, these data suggest that miR-195 acts in separate contexts to inhibit autophagy and promote maturation of Schwann cells. This relationship provides further evidence that autophagy is directly involved in Schwann cell development and consequent myelin architecture. Moreover, miR-195 may be a potential therapeutic target in spinal cord injury given its ability to affect both autophagy and myelin levels.

In addition to autophagy-mediated pathways, Schwann cells can also clear myelin using Axl- and Merck-receptor-mediated phagocytosis (Brosius Lutz et al., 2017). Axl and Merck are expressed by CNS astrocytes; therefore, it has been proposed that these receptors may present therapeutics and pharmacological targets to promote myelin clearance in the CNS (Brosius Lutz et al., 2017). Together, the studies reported here suggest that autophagy is a major physiological process that allows myelinating glia to mediate myelin debris clearance.

Table 3. Pharmacological activators and inhibitors of autophagy

Activators	Mechanism	Uses in myelinating glia
Rapamycin	Binds to the FKBP12 protein, forming a gain-of-function complex that works as an allosteric inhibitor of mTORC1 (Sabatini et al., 1994) with consequent activation of autophagy	Ohri et al. (2018) Ko et al. (2018) Jang et al. (2015) Marinelli et al. (2014) Chen et al. (2016) Not yet in the myelinating glia literature
Ridaforolimus EN6	Analog of rapamycin, induces inhibition of mTORC1 by forming complex with cytosolic FKBP12 (Gore et al., 2013)	Bankston et al. (2019)
Tat-Beclin ^a Inhibitors	Targets one subunit of the vATPase, leading to the inhibition of mTORC1 (Chung et al., 2019)	
3-Methyladenine	Autophagy inducing peptide, lipidates LC3B and increases p62 protein levels (Shoji-Kawata et al., 2013)	
	Inhibits PI3KC3 by binding the ATP-binding site, with consequent blockage of autophagosome formation	Gomez-Sanchez et al. (2015) Chen et al. (2016) Ko et al. (2018) Marinelli et al. (2014) Ohri et al. (2018)
Spautin-1 ^a	Promotes the degradation of PI3KC3 kinase complex by inhibiting two ubiquitin specific peptidases, USP10 and USP13, that target the Beclin1 subunit of the complex (Liu et al., 2011)	
Bafilomycin A1	Inhibits autophagosomal/lysosomal membrane fusion, by targeting the vATPase	Gomez-Sanchez et al. (2015)
Wortmannin	Inhibits PI3KC3 and suppressing PI3P production (Petiot et al., 2000)	Chen et al. (2016)
KU55933 ^a	Selective inhibitor of the VPS34 complex (Farkas et al., 2011)	Bankston et al. (2019)
Verteporfin ^a	Stimulates oligomerization of p62, preventing autophagosome closure (Donohue et al., 2011)	Bankston et al. (2019)
Docosahexaenoic acid	Triggers p53 oxidative reduction, with consequent activation of AMPK/mTOR signaling (Jing et al., 2011)	Yin et al. (2018)

^aDirectly affects autophagy.*Schwann cell autophagy: pharmacological manipulation of myelin clearance following nerve lesion*

Pharmacological manipulation of autophagy has both enhanced and complicated understanding of Schwann cell autophagy following injury. The contributions of Schwann cell autophagy to recovery from PNS injury has been evaluated through a series of studies that activate autophagy. Autophagy is a major step in the injury process, as nerve cut was shown to increase autophagy-related mRNA transcripts and autophagy-related protein expression in injured nerve segments (Marinelli et al., 2014; Gomez-Sanchez et al., 2015; Huang et al., 2016; Jang et al., 2016). This involvement of autophagy in injury has been manipulated therapeutically. Promoting Schwann cell autophagy with rapamycin in the first week after mouse sciatic nerve injury produced anti-inflammatory effects and led to increased myelin compaction along the sciatic nerve (Marinelli et al., 2014). Rapamycin treatment after nerve injury also increased Schwann cell proliferation (Marinelli et al., 2014). It has also been reported that induction of autophagy in Schwann cells promotes nerve regeneration after injury (Huang et al., 2016). After Wallerian degeneration, inhibition of the proteasome limits Schwann cell expression of p75, a nerve growth factor receptor and marker of Schwann cell dedifferentiation. Dedifferentiation of Schwann cells is associated with peripheral axon demyelination (Kim et al., 2014).

Interestingly, inhibiting Schwann cell autophagy pharmacologically with 3-methyladenine or using a mouse haploinsufficient at the *Ambra1* gene resulted in increased and prolonged neuropathic pain behavior after nerve injury (Marinelli et al., 2014). Furthermore, inhibition of autophagy after nerve damage is accompanied by increased neurofilament accumulation (Ko et al., 2018), suggesting that Schwann cell autophagy slows and ameliorates scar formation after injury.

As noted above, multiple pharmacological agents have been used to activate or inhibit autophagy; in particular, activation of autophagy is often stimulated by inhibitors of mTOR (e.g., rapamycin, or activators of AMPK), which subsequently inhibits mTOR. Since both mTOR and AMPK act as regulators for several cellular pathways, it is only with more specific drugs that pharmacological data can be fully interpreted. Some of the drugs used also have more than one target. For example, Wortmannin tar-

gets PI3KC1/3, myosin light chain kinase, mTOR, and DNA-dependent protein kinase (Kong and Yamori, 2008). We have summarized the pharmacological drugs most often used to manipulate autophagy pathway in Table 3, indicating which are specific for autophagy.

It is important to note that the language surrounding Schwann cell autophagy in relation to myelin clearance and architecture tends to polarize the literature. Some researchers state that Schwann cell autophagy is beneficial and facilitates necessary myelin debris clearance, whereas others claim that Schwann cell autophagy is harmful and causes unnecessary myelin degradation, eliminating myelin architecture. Importantly, half of the literature suggests that autophagy inhibitors would promote recovery after nervous system injury, whereas the other half recommends autophagy activators to promote functional recovery. Given that factors regulating the context and extent of myelin clearance are areas of active investigation, more research is required before autophagy modulators can be used to maximum benefit as a therapeutic target in recovery from CNS or PNS injury.

Oligodendrocyte autophagy: inflammation, stroke, brain and spinal cord injury

Although still incompletely understood, recent literature has made it clear that oligodendrocyte autophagy is involved in both CNS injury and recovery. Multiple studies have reported an increase in autophagy-related transcripts and proteins, including *Atg5* and LC3B, 1 week after spinal cord injury (Muñoz-Galdeano et al., 2018; Ohri et al., 2018). Furthermore, genetic deletion of *Atg5* in oligodendrocytes significantly impairs the extent of functional recovery, as measured by Basso Mouse Scale locomotor analysis 28–42 d after spinal cord injury (Ohri et al., 2018). In contrast, functional recovery was also more likely when ER homeostasis, as opposed to ER stress (an initiator of autophagy) (Yorimitsu et al., 2006; Ohri et al., 2018), was promoted (Ohri et al., 2011, 2013). Additionally, there was a reduction in the spared white matter of transverse histological section 6 weeks after spinal cord injury in animals in which *Atg5* was genetically deleted, which was interpreted to represent decreased function recovery. In past studies, it has been argued that spared white

matter is more predictive of functional recovery than neuronal loss, specifically for thoracic contusions (Ohri et al., 2018). Therefore, decreased spared white matter in the *Atg5*^{−/−} tissue suggests that active autophagy may contribute to the maintenance and protection of myelin after injury.

Altered oligodendrocyte autophagy has been also associated with additional types of injury, including inflammation, traumatic brain injury (TBI), and stroke (Zhang and Wang, 2018). Following white matter injury, pattern-recognition Toll-like receptors TLR3 and TLR4 has been shown to induce autophagy, and TLR3 was found to colocalize with the autophagosome (Vontell et al., 2015). This demonstrates that, within the brain parenchyma, white matter injury induces autophagosome formation. Autophagy inhibitor docosahexaenoic acid, when given immediately following a TBI, was shown to limit the extent of both white matter and gray matter injury in the hippocampus and cortex (Yin et al., 2018). The protection of gray and white matter was correlated with improved Morris water maze performance in docosahexaenoic acid-treated versus nontreated animals (Yin et al., 2018). These recent advances highlight the therapeutic potential that targeting autophagy may provide to improve TBI treatment.

Ischemic stroke is a sudden loss of glucose and oxygen, and this often causes extensive damage to white matter (Wang et al., 2016). Given that autophagy is induced by hypoxia, growth factor withdrawal, and glucose starvation (Dikic and Elazar, 2018), autophagic activity in oligodendrocytes after ischemic stroke would seem an important contributor to white matter injury and functional recovery. Given the recent success of autophagy inhibitors in treating TBI, autophagy seems a promising therapeutic target to treat damage from stroke.

Autophagy contributions to neurological disorders

CNS myelin disorders

Abnormal autophagy flux is associated with dozens of neurological disorders that have myelin abnormalities as a hallmark feature, presenting an important contributor and therapeutic target for these diseases (Eskelinen and Saftig, 2009; Jiang and Mizushima, 2014; Menzies et al., 2015; Levine and Kroemer, 2019). Diseases with additional myelin phenotypes and autophagic dysfunction include amyotrophic lateral sclerosis (Rubino et al., 2012; Hirano et al., 2013), Alzheimer's disease (Li et al., 2010; Zare-Shahabadi et al., 2015; Uddin et al., 2018), frontotemporal dementia/degeneration (Rubino et al., 2012), hereditary spastic paraplegias (Winner et al., 2004; Olmez et al., 2006; Al-Yahyaee et al., 2006; Oz-Levi et al., 2012), multiple sclerosis (Liang and Le, 2015; Igci et al., 2016; Patergnani et al., 2018), Nasu-Hakola disease (Satoh et al., 2014), and Parkinson's disease (Gan-Or et al., 2015). More research is needed to elucidate the relationship between autophagy and lifelong myelin development, plasticity, and maintenance in association with neurological disease.

Hereditary spastic paraplegia (HSP) is a rare genetic disorder, with dozens of genetic subtypes, that presents in childhood and leads to progressive lower-limb paralysis and spasticity (Gould and Brady, 2004; Fink, 2013). A large portion of the >70 genes linked to HSPs are involved in the autophagy pathway, axon transport, ER, or mitochondrial stress (Fink, 2013), and a large subset of HSP cases are known as a “disease of autophagy.” A thin corpus callosum is frequently reported in patients with HSP (Winner et al., 2004; Al-Yahyaee et al., 2006; Olmez et al., 2006; Ebrahimi-Fakhari et al., 2018) and in animal models of HSP (De Pace et al., 2018). Because HSP is both a developmental and degenerative disorder with symptoms that can present at any age

(Fink, 2013), HSP pathology suggests a crucial role of autophagy in CNS and PNS white matter development and maintenance.

One of the proteins affected in HSP is adaptor protein 4 (AP-4), which exports ATG9A from the Golgi to preautophagosomes (Hirst et al., 2013; Mattera et al., 2017; Roubertie et al., 2018) (Fig. 1). The relationship between AP-4 and ATG9A is an interesting one because AP-4 is linked to abnormal myelination in HSP (Ebrahimi-Fakhari et al., 2018) and ATG9A mRNA levels are reported to be significantly reduced in blood samples from patients with the classical demyelinating disorder multiple sclerosis (Igci et al., 2016). LC3B, the marker of active autophagy, was not, however, identified in oligodendrocytes surviving multiple sclerosis lesions when analyzed in postmortem brains (Satoh et al., 2014). Autophagy activation has been reported in additional types of demyelinated lesions. Autophagy marker LC3B was found to be highly expressed in surviving oligodendrocytes located in demyelinated lesions of postmortem brains with Nasu-Hakola disease, a rare genetic disorder hallmarked by sclerosing demyelinating regions (Satoh et al., 2014). Together, these studies present intriguing but complex evidence for the involvement of autophagy in both developmental and neurodegenerative CNS dysmyelinating disorders.

Leukoencephalopathy with vanishing white matter is a genetically heterogeneous collection of white matter disorders that can vary in childhood-, adolescent-, or adult-onset stages (Bugiani et al., 2010). Canonically, leukoencephalopathy is associated with mutations to eukaryotic translation initiation factor 2 (eIF2B) subunits 1–5 (Bugiani et al., 2010; Matsukawa et al., 2011; Schiffmann et al., 2012). In oligodendrocytes with a mutant version of eIF2B, autophagic flux was slowed and tolerance to ER stress was significantly diminished (Chen et al., 2016). In a different genetic subtype of leukoencephalopathy, researchers found that mutations to the *Vps11* gene, which encodes the vacuolar protein sorting-associated protein 11 (VPS11), was present (Zhang et al., 2016). When expressed in zebrafish, the gene mutation led to progressive myelin loss and elevated levels of LC3B and p62, markers of elevated autophagy and autophagy dysfunction, respectively (Zhang et al., 2016). The link between the dysregulated autophagy and abnormal myelin phenotypes is not causally tested in this study, but the findings provide an important avenue for new research.

Disruption of autophagy in nonmyelinating cells can also affect white matter structure. For example, pathology of Alexander's disease has been linked with autophagic dysregulation, and accumulation of GFAP protein in white matter astrocytes (Brenner et al., 2001; Hagemann et al., 2006; Tanaka et al., 2007). High levels of oxidative stress in the corpus callosum have also been observed in a mouse model of Alexander's disease (Hagemann et al., 2006). Further, the pathology of Alexander's disease highlights that, despite often being used interchangeably, myelinating glia, myelin architecture, and white matter each have independent biologies and each may require different types of inquiry or therapeutic strategies.

PNS myelin disorders

In addition to the well-documented role that autophagy in myelinating glia plays in diseases of the CNS, emerging evidence suggests contributions of autophagy to the pathogenesis of several PNS demyelinating diseases. In Charcot-Marie-Tooth disease type 1A (CMT1A), a peripheral demyelinating disorder, multiple groups have found evidence of dysregulated autophagy in Schwann cells (Hutton et al., 2011; Hantke et al., 2014; Lee et al., 2018). For example, elevated c-Jun levels, an activator of au-

tophagy, were reported in Schwann cells of a CMT1A animal model (Hutton et al., 2011; Hantke et al., 2014). Additionally, increased LC3B-II levels and autophagosome counts have been reported in CMT1A-patient-derived Schwann cells (Lee et al., 2018). In a similar disorder, Charcot-Marie-Tooth disease Type 4J (CMT4J), a mutation in the protein FIG4, results in Schwann cell demyelination and decreased autophagy-mediated myelin clearance (Vaccari et al., 2015). The authors highlight the necessity of endosomal trafficking, which is a feature of autophagic flux, in Schwann cells for myelin maintenance.

In an animal model for the PNS disease chronic inflammatory demyelinating polyradiculoneuropathy, researchers have identified significantly decreased colocalization of MAP1LC3B- and S100-positive Schwann cells in the sciatic nerve of chronic inflammatory demyelinating polyradiculoneuropathy animals (Brun et al., 2017). In a different animal model of inflammatory demyelination, B7-2-deficient NOD mice, autophagy-associated proteins LC3B and LAMP1 were also identified in Schwann cells during Wallerian degeneration (Jang et al., 2015). The authors conclude that autophagy is active in Schwann cells during inflammatory peripheral demyelination disorders. Autophagy has been also implicated in the demyelinating PNS disorder Guillain-Barré syndrome (Muller et al., 2017; Brun et al., 2017); however, current studies have not determined whether the autophagy abnormalities are specific to the white matter. It is important to note that, similar to the literature on Schwann cell autophagy in injury and repair, it is unclear in the PNS disease literature whether Schwann cell autophagy is a sign of productive physiological recovery or pathological harm. This question surrounding the role of autophagy in myelin-based disease and repair remains an important and highly debated area for future research.

In conclusion, given the close association with myelin repair and recovery after injury, autophagy is a critical, and at present overlooked, component of myelinating glial function and plasticity. Targeting autophagy in myelinating glia presents a potential new direction for disease and injury treatments, and such research will ultimately unveil fundamental underlying biology regulating the development and plasticity of the nervous system.

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