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Review

The role of autophagy in acute brain injury: A state of flux?☆

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ABSTRACT

It is established that increased autophagy is readily detectable after various types of acute brain injury, including trauma, focal and global cerebral ischemia. What remains controversial, however, is whether this heightened detection of autophagy in brain represents a homeostatic or pathologic process, or an epiphenomenon. The ultimate role of autophagy after acute brain injury likely depends upon: 1) the degree of brain injury and the overall autophagic burden; 2) the capacity of individual cell types to ramp up autophagic flux; 3) the local redox state and signaling of parallel cell death pathways; 4) the capacity to eliminate damage associated molecular patterns and toxic proteins and metabolites both intra- and extracellularly; and 5) the timing of the pro- or anti-autophagic intervention. In this review, we attempt to reconcile conflicting studies that support both a beneficial and detrimental role for autophagy in models of acute brain injury.

1. Introduction

Autophagy is vital to cellular homeostasis in brain and is tightly regulated under normal conditions. In conditions of stress (e.g. nutrient deprivation, starvation) or acute cellular injury (e.g. trauma, ischemia-reperfusion), autophagy is increased and appears to be proportional to the degree of stress/injury. Increased detection of autophagy has been reported after various types of acute brain injury, including traumatic brain injury (TBI), stroke, global cerebral ischemia, and seizures. However, whether this heightened detection of autophagy in brain represents a protective response, pathologic process, or an epiphenomenon remains controversial, with reports in experimental models showing beneficial effects of both promoting and inhibiting autophagy (Galluzzi et al., 2016). Indeed, since the review by Smith et al. (2011) there have been over 400 new citations listed on PubMed related to autophagy after acute brain injury promulgating mechanistic insights, but without further clarifying the role of autophagy in brain after injury. Our objective is to review recent studies and novel mechanistic discoveries, and attempt to provide a speculative synthesis as to the role of autophagy after acute brain injury.

2. Autophagy after brain injury

The presence of autophagosomes in the mature brain normally and during starvation is lower compared with other organs (heart, liver, pancreas, kidney, skeletal muscle) (Mizushima et al., 2004), a finding that could be related to reduced autophagy and/or enhanced turnover (Boland and Nixon, 2006). However, after acute brain injury, there is compelling evidence that autophagy is induced, observed in multiple experimental models by ultrastructural identification of autophagosomes and autolysosomes, and/or the presence of autophagy-related microtubule-associated protein 1 light chain 3-II (LC3-II) detected using western blot or immunohistochemistry (Galluzzi et al., 2016). Increased detection of autophagosomes may be a consequence of increased autophagy itself, or impaired autophagic flux—the process of fusion with lysosomes to form autolysosomes, and subsequent degradation (Fig. 1). During starvation-induced autophagy triggered in response to nutrient deprivation, or homeostatic autophagy recycling aging organelles and dysfunctional proteins, autophagic flux is balanced, i.e. can keep up with demand. Under conditions of injury or disease autophagic flux may be impaired and/or overwhelmed and unable to keep up with generation of autophagosomes. Impaired autophagic flux and lysosomal function have been suggested to contribute to Parkinson's disease (Su et al., 2017), Alzheimer's disease (Bordi et al., 2016), and TBI (Sarkar

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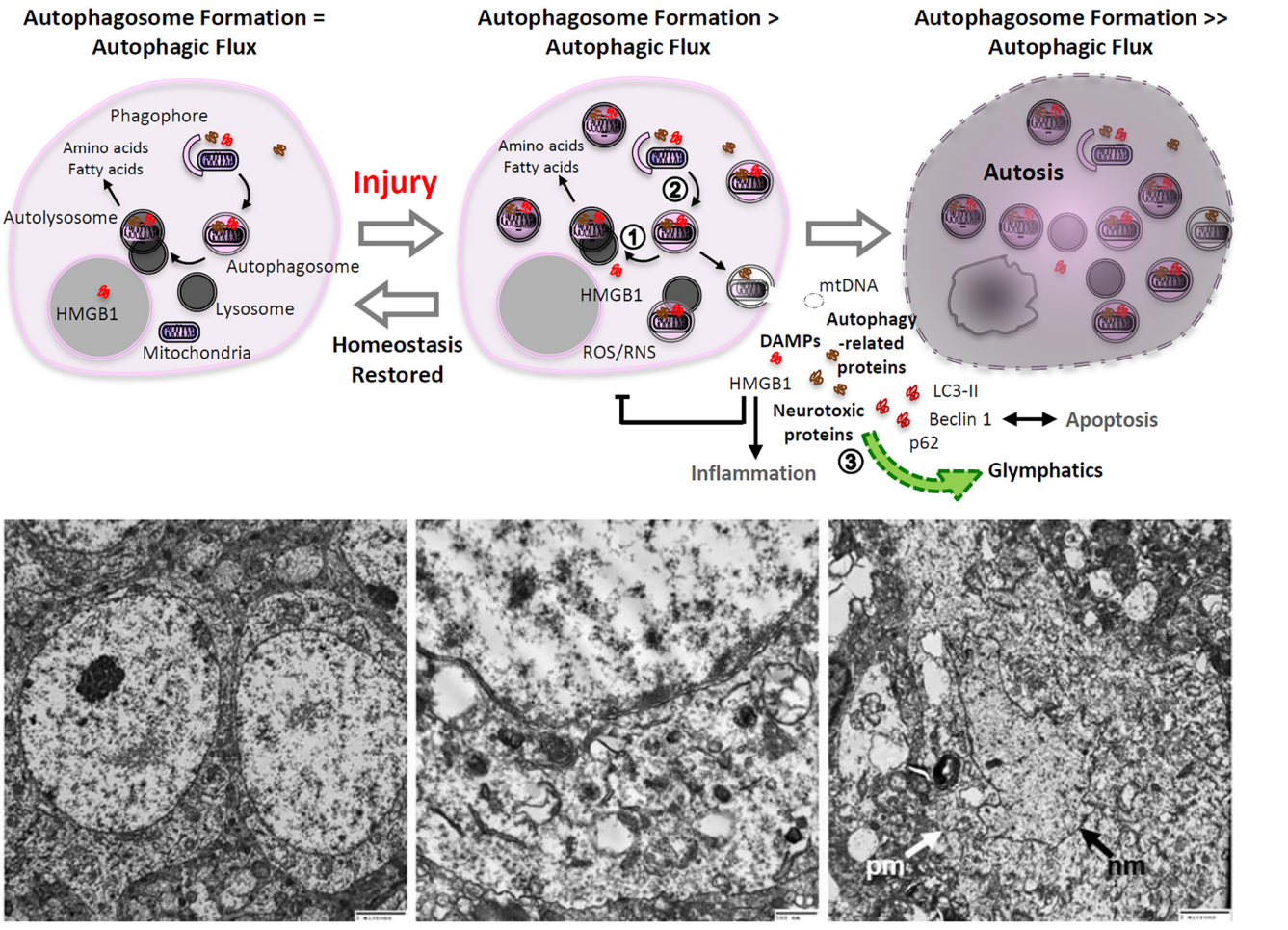

Fig. 1. A hypothetical view of the continuum from homeostatic autophagy to autosis after brain injury. In homeostasis autophagosome formation and autophagic flux are in equilibrium. After injury the cell's capacity to turn over autophagosomes may be exceeded, perhaps leading to secretory autophagy. Exhaustion of the cell's autophagic capacity may lead to autosis. Both secretory autophagy and cell disruption during autosis may lead to release of extracellular signals (DAMPs) and autophagic proteins that may regulate inflammation, apoptosis, and/or autophagy itself. These extracellular signals, neurotoxic proteins such as Aβ and tau, and other macromolecules can be eliminated via the glymphatic pathway, potentially reducing their impact. Therapies and/or interventions that 1) stimulate autophagic flux, 2) reduce autophagic burden, and/or 3) enhance clearance of DAMPs and neurotoxic proteins, may help restore cellular homeostasis. Representative electron micrographs from ultrathin sections taken from the CA1 region of the hippocampus. (Left to right: naïve 20 day old female rat, bar = 2 μm; 20 day old male rat 72 h after a 9 min asphyxia cardiac arrest, bar = 500 nm; 20 day old female rat 72 h after a 9 min asphyxial cardiac arrest, bar = 2 μm (nm = nuclear membrane; pm = plasma membrane). Images courtesy of Mara Sullivan, Center for Biologic Imaging, University of Pittsburgh.

et al., 2014). Enhancement of autophagic flux by activating transcription factor EB is protective in experimental brain injury produced by cadmium (Pi et al., 2017).

After acute brain injury, several initiators of autophagy have been observed. These include classic pathways regulated by phosphatidylinositol-3-kinase (PI3K) and AMP-dependent protein kinase (AMPK), and recent discoveries focusing on selective clearance of mitochondria using autophagic components, termed *mitophagy* (Zimmermann and Reichert, 2017). Mitochondrial damage/dysfunction is a common denominator in acute brain injury and in aging mitochondria. Upstream redox-related pathways regulated by extracellular signal-regulated protein kinases (Zhu et al., 2007) and Nrf2/Keap1 (Hensley and Harris-White, 2015) link oxidative stress and mitophagy. In addition, other mitochondria-specific signaling pathways have been reported involving phosphatase and tensin homolog-induced putative kinase 1 (PINK1)/parkin RING-between-RING E3 ubiquitin protein ligase (PARK2) (Lazarou et al., 2015) and cardiolipin externalization to the outer mitochondrial membrane (Chu et al., 2013). Under conditions of overwhelming mitochondrial damage complete cellular failure accompanied by exhaustion of recyclable energy stores and plasma membrane rupture can be observed, culminating in a form of regulated cell death termed "autosis" (Liu et al., 2013), which may be thought of as "terminal autophagy".

3. Autophagy after TBI

The presence of autophagosomes and/or an increase in LC3-II in injured brain have been observed in several experimental models of TBI including fluid-percussion injury (FPI) (Liu et al., 2008), controlled cortical impact (CCI) (Lai et al., 2008), and weight drop injury (Sun et al., 2014). Autophagosomes and LC3-II have also been reported in human brain specimens from adult patients after TBI (Clark et al., 2008). In children after severe TBI, p62/sequestosome 1 (SQSTM1) and Beclin 1 are increased in cerebrospinal fluid (CSF) compared with CSF from control patients, with p62 concentration associated with unfavorable outcome, consistent with increased, overwhelmed, and/or impaired autophagic flux (Au et al., 2017).

Using a weight-drop model which produces diffuse brain injury, Y. Liu et al. (2017) recently showed that caloric restriction after mild TBI results in increased autophagy markers in the CA3 region of the hippocampus, with a twofold increase in Beclin 1, a threefold increase in LC3, as well as decreased mammalian target of rapamycin (mTOR)

compared to injured animals with normal diets. Calorie-restricted mice also demonstrated better cognitive performance, measured by escape latency on Morris-water maze testing. C. Wang et al. (2017) more directly evaluated the mTOR pathway's involvement in TBI-related autophagy in a weight drop model. After repetitive mild TBI, rats given rapamycin via intraperitoneal injection 4 h after injury showed decreased mTOR, increased Beclin 1, as well as increased mitophagy-related protein PINK1. Rapamycin-treated rats also had decreased hippocampal neuron apoptosis and improved performance in beam walking and open field tasks.

After CCI, a contusion model of TBI, Ma et al. (2015) tested the experimental cancer drug 17-allyamino-demethoxygeldanamycin (17-AAG). The drug showed beneficial effects, including reduced brain water content, improved functional assessment, and improved cortical neuron survival compared to injured animals treated with vehicle only. 17-AAG also resulted in a twofold increase in both LC3-II and Beclin 1. The effects of 17-AAG were amplified by inducing autophagy with the mTOR inhibitor rapamycin, and diminished by inhibiting autophagy with the PI3K inhibitor 3-methyladenine (3-MA) (Ma et al., 2015). An increase in the presence of autophagosomes, reduced neuronal death, and better behavioral outcome have also been reported in animals treated with methylene blue (Zhao et al., 2016) and hydrogen sulfide after CCI (Zhang et al., 2014). Mice treated with hydrogen sulfide after CCI had near-complete reversal of a TBI-induced decrease in cortical and hippocampal p62/SQSTM1 (Zhang et al., 2014). Taken together, these studies are consistent with the possibility that enhancing autophagy improves neurological outcome after TBI.

Autophagy has also been linked to reduced neuroinflammation after TBI. After FPI in rats, Jin et al. (2017) observed an increase in microglial activation, with increased expression of ionized calcium-binding adapter molecule 1 (Iba1) suggestive of microglial activation in the hippocampus ipsilateral to the injury site. Tumor necrosis factor α (TNFα) and interleukin-1β (IL-1β) expression also increased following injury. Inhibition of autophagy with 3-MA 24 h after injury amplified the injury-induced increases in all three proteins, indicating a possible relationship between autophagy, microglial activation, and inflammation. Post-treatment with fucoxanthin, an antioxidant carotenoid compound, was associated with reduced lesion volume, edema, and less severe neurologic deficits after weight drop TBI in mice (L. Zhang et al., 2017). Fucoxanthin was also associated with greater glutathione peroxidase (GPx) activity, an amplified TBI-associated increase in Beclin 1 and LC3-II and decrease in p62/SQSTM1, and attenuated cleavage of inflammasome-related caspase-1. Caution is in order, however, as neuroinflammation after TBI is highly complex and likely has multifaceted and temporally-dependent roles, and reduced inflammation in these studies may simply represent a proportional response to reduced cell death (Simon et al., 2017).

Since autophagy and apoptosis are interrelated, complex modeling is required to separate crosstalk and overlap versus distinctions between the two pathways (B. Liu et al., 2017). Using an open-skull weight-drop model of TBI in rats, the non-selective antioxidant tetrahydrocurcumin reduced apoptosis and neurologic severity, while increasing Beclin 1 and LC3-II abundance as well as GPx activity compared with vehicle-treated rats (Gao et al., 2017). Neuroprotection was partially reversed with 3-MA, suggesting that increased autophagy produced by tetrahydrocurcumin leads to a concomitant reduction in apoptosis after TBI; however, it is also possible that inhibition of apoptosis using non-selective antioxidant treatment reduces apoptotic cell death at the expense of increased autophagy (Maiuri et al., 2007).

In contrast to the studies discussed above, divergent studies have suggested potentially detrimental effects of autophagy after TBI. After CCI in mice, Zhang et al. recently reported reduced lesion volume, neuronal apoptosis, and behavioral deficits in mice treated with the signaling protein Wnt3a (J. Y. Zhang et al., 2018). An observed decrease in TBI-induced Beclin 1 and LC3-II abundance led to the authors to conclude that suppressing autophagy is beneficial after TBI. Multiple antioxidant compounds have been evaluated in experimental TBI models, with improved histologic and behavioral outcomes observed concomitantly with a reduction in biomarkers of autophagy (Cordaro et al., 2016; Feng et al., 2017; Feng et al., 2016). Given that antioxidants have the potential to attenuate many cellular processes associated with multiple cell death pathways, these findings may simply reflect a reduction in cell stress and therefore less provocation of autophagy. Alternatively, antioxidants may increase autophagic flux or capacity after TBI. H. Liu et al. (2017) induced expression of ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) in rats subjected to CCI. UCH-L1 overexpression improved survival of hippocampal neurons, reduced accumulation of amyloid-precursor protein, and decreased Beclin 1 and LC3-II in injured rats. Given a role for UCH-L1 in proteasomal clearance of misfolded proteins, these results may reflect increased autophagic flux and/or decreased intracellular demand for autophagy. Similarly, neuroprotection and decreased abundance of Beclin 1 and LC3-II observed when rats are treated with rosiglitazone after CCI may reflect anti-oxidant and other salutary effects of the drug and may not represent cause and effect (Yao et al., 2015). However, these studies are consistent with effective autophagic flux corresponding with improved neurological outcome after TBI.

Clinically used sedative medications have also been shown to exert neuroprotective effects in experimental TBI. A recent study using a weight-drop model demonstrated improved behavioral outcome and reduced Beclin 1 and LC3-II abundance following treatment with the NMDA receptor antagonist ketamine (C. Q. Wang et al., 2017). Ketamine treatment resulted in partial preservation of cellular ATP stores in addition to attenuation of TNFα and interleukin-6 (IL-6) compared with vehicle. One could speculate that ketamine reduces neuronal excitation and therefore metabolic demands in brain, thereby reducing autophagic pressure.

4. Autophagic flux after TBI

Interpretation of Beclin 1, p62/SQSTM1, and LC3-II abundance as a reflection of autophagy as a whole is likely an oversimplification, as measuring snapshots of autophagy biomarkers do not reflect the dynamic process of initiation, nucleation, elongation, autophagosome formation, fusion with lysosomes to form autolysosomes, and degradation. Yin et al. recently reported TBI-induced increases in autophagy-related protein expression in the hippocampus, as well as the lysosome-associated membrane protein 1 (LAMP-1) and the lysosomal protease cathepsin D (Yin et al., 2018). TBI impaired cleavage of cathepsin D to its active form, indicating impaired lysosomal function. Treatment with docosahexanoic acid restored lysosomal function, attenuated hippocampal and white matter damage, and improved performance in the Morris-water maze. Cui et al. recently evaluated calcitriol treatment after CCI, finding evidence of enhanced autophagic flux determined by LC3-II/LC3-I ratio and improved neurological function with treatment (Cui et al., 2017). Inhibiting lysosomal function and endosomal acidification with chloroquine reversed the effects of calcitriol. Similar findings were reported when chloroquine was administered with lanthionine-ketamine ethyl ester after FPI in mice (Hensley et al., 2016). Taken together, these studies suggest that enhancing autophagic flux in brain may be beneficial after TBI.

Zeng et al. recently examined induction of autophagy and autophagic flux after CCI of varying injury severity (Zeng et al., 2018). LC3-II abundance was increased in ipsilateral cortical tissue at 1 and 3 days in an injury severity-dependent manner. After mild CCI increased induction of autophagy-related proteins Beclin 1, Atg5 and Atg12 concomitant with a reduction in p62/SQSTM1 and autophagosomes, and an increase in autolysosomes was seen, consistent with enhanced autophagic flux. Treatment with chloroquine after mild CCI resulted in sustained LC3-II abundance (vs. sham), increased apoptosis, and worse neurological outcome (vs. vehicle treatment). After moderate or severe CCI a lack of conversion from autophagosomes to autolysosomes was

seen, consistent with an impairment in autophagic flux. These data suggest that the autophagic flux capacity may be injury-severity dependent.

5. Autophagy after ischemia-reperfusion

As with TBI, divergent outcomes in in vivo and in vitro studies have led to conflicting insights regarding the role of autophagy after ischemia-reperfusion in brain. These divergent outcomes may in part be explained by cell-type and brain region-specific differences in response to ischemia-reperfusion. In astrocyte and neuron co-cultures subjected to oxygen-glucose deprivation (OGD), enhancement of autophagy in astrocytes with rapamycin reduced apoptotic neuronal death, whereas inhibiting autophagy in astrocytes with 3-MA or small interfering RNA (siRNA) against Atg5 increased apoptotic neuronal death (X. Liu et al., 2017). In astrocyte monocultures, Kasprowska et al. observed that inhibiting autophagy with 3-MA increased apoptosis at 1 and 4 h, and necrosis at 8 and 24 h after OGD (Kasprowska et al., 2017). In contrast, other studies have shown that 3-MA reduces neuronal death after OGD (Shi et al., 2012), and that inhibiting induction of autophagy using siRNA targeting Atg7 reduces neuronal death after nutrient deprivation (Du et al., 2009). Interestingly, in the study by Du et al., while Atg7 siRNA protected primary neurons it exacerbated cell death in fibroblasts, suggesting that the role of autophagy in response to nutrient deprivation and/or ischemia may differ in non-dividing (primary neurons) versus dividing (fibroblasts, astrocytes) cells (Du et al., 2009).

Multiple studies in experimental models of ischemia-reperfusion injury suggest that augmenting autophagy using rapamycin is neuroprotective, including studies by Carloni et al. using a neonatal model of hypoxia-ischemia (Carloni et al., 2008) and by Buckley et al. using both transient and permanent middle cerebral artery occlusion (MCAO) (Buckley et al., 2014). In a more recent study in 10 day old rats after unilateral carotid artery ligation and hypoxemia, preventing microRNA (mi*R*)-30d-5p associated Beclin 1 suppression using antagomir enhanced autophagy, inhibited apoptosis, reduced infarct volume, and improved neurological outcome (Zhao et al., 2017). In contrast to studies showing that pharmacologically augmenting autophagy is protective after cerebral ischemia-reperfusion in vivo, are reports such as those by Puyal et al. showing that 3-MA reduces lesion volume in 12 day old rats after MCAO (Puyal et al., 2009). More recently, Dong et al. observed that rapamycin exacerbates neuronal death in rats after forebrain ischemia (Dong et al., 2017) and Wang et al. observed that 3-MA reduced infarct volume after ischemia-reperfusion (Wang et al., 2018). In a model of chronic cerebral ischemia, the fatty acid amide hydrolase URB597 reduced Beclin 1 and LC3-II abundance and attenuated cognitive impairment, an effect was reversed with co-administration of 3-MA (D. Wang et al., 2017).

Caveats related to pharmacologic modulation of autophagy include off-target effects of the drug(s). However, more selective approaches targeting autophagy have also generated conflicting results. In *Atg7* deficient mice, unilateral carotid artery ligation followed by hypoxemia in 7 or 9 day old mice results in reduced autophagy and hippocampal neuronal death (Koike et al., 2008; Xie et al., 2016). Studies in *Atg7* deficient mice are confounded by the fact that inhibition of autophagy throughout development leads to neurodegeneration (Komatsu et al., 2006). In a 9-min asphyxial cardiac arrest model in juvenile rats, targeted inhibition of Atg7 using siRNA in the cerebellum reduced LC3-II and improved motor function and Purkinje cell survival (Au et al., 2015). In contrast, targeted overexpression of Atg7 in astrocytes using adeno-associated virus (AAV)-GFAP-Atg7 reduced infarct volume in mice after MCAO (D. Liu et al., 2017). It is interesting to note that inhibiting neuronal *Atg7* using $Atg7^{flox/+;Nes\text{-}Cre}$ (Xie et al., 2016), and over-expressing astrocyte Atg7 using AAV-GFAP-Atg7 both appear neuroprotective (X. Liu et al., 2017).

6. Autophagic flux after ischemia-reperfusion

Evaluating the protective effects of sodium hydrosulfide after transient MCAO in mice, Zhu et al. co-administered bafilomycin A1 to inhibit formation of autolysosomes (Zhu et al., 2017). Co-administration of bafilomycin A1 reversed the pro-autophagic and neuroprotective effects of sodium hydrosulfide. Following MCAO in diabetic rats, administration of chloroquine exacerbates brain microvascular endothelial cell injury presumably via inhibiting lysosomal function (Fang et al., 2015). In a model of transient forebrain ischemia, Zhan et al. observed ischemia-induced increases in LC3-II and p62 in CA1 hippocampal neurons (Zhan et al., 2017). LAMP2 was decreased in neurons but increased in glial cells. Inhibiting autophagic flux with chloroquine decreased cell survival and increased LC3 and p62, and hypoxic preconditioning partially reversed injury these effects. These findings indicate that preventing lysosomal fusion and subsequent formation of autolysosomes and clearance of autophagosomes exacerbates brain injury. However, in contrast, after asphyxia cardiac arrest in rats treatment with bafilomycin A1 (or 3-MA) reduces hippocampal neuronal death (Cui et al., 2016).

Recently, Zhou et al. evaluated autophagic flux in hippocampal neurons in vitro (Zhou et al., 2017). OGD resulted in decreased lysosomal number and acidity, and decreased LAMP2. Using a GFP-RFP-tf-LC3 assay, the investigators quantified autolysosomes and autophagosomes. OGD decreased the ratio of autolysosomes to autophagosomes, indicating impaired flux. Notably, mild hypothermia in this study enhanced autophagic flux after OGD in neurons. A reduction in LAMP2 and an increase in LC3-II is also reported after permanent MCAO in rats (Y.Y. Liu et al., 2017). The experimental compound pseudoginsenoside-F11, which has been reported to have wide-ranging effects, improved lysosomal function and reduced infarct volume, but not with co-administration of chloroquine.

7. Secretory autophagy and brain injury

Secretory autophagy refers to a specialized variation of autophagy, which begins with canonical initiation of autophagy and culminates in autophagosome fusion with the plasma membrane and expulsion of contents, rather than lysosomal fusion, formation of autolysosomes, and degradation (Davis et al., 2017; Farhan et al., 2017). In contrast to conventional secretory pathways, secretory autophagy allows cells to clear proteins lacking N-terminal signal peptides, and aggregation-prone proteins including amyloid β (Aβ) (Ponpuak et al., 2015; Ventruti and Cuervo, 2007). When the degradative final steps of autophagic flux are inhibited, secretory autophagy can be induced to rid cells of dysfunctional proteins. For example, in an in vitro model of Parkinson's disease, Ejlerskov et al. observed that inhibiting autophagosome mobility hindered their fusion with lysosomes and increased extracellular secretion of α-synuclein (Ejlerskov et al., 2013). Neurodegenerative disorders including Alzheimer's disease and Parkinson's disease are characterized by impaired autophagy and both intracellular and extracellular accumulation of abnormal proteins (Ghavami et al., 2014; Nixon et al., 2005; Wong and Holzbaur, 2015). Extracellular accumulation of these same proteins are implicated in chronic traumatic encephalopathy (Asken et al., 2017; Ikonomovic et al., 2004).

Extracellular macromolecules, including misfolded proteins such as Aβ, are carried through the brain interstitial space via bulk flow of fluid, and removed from the brain via paravascular channels (termed the glymphatic system due to their delineation by astrocyte foot processes) that eventually drain into the lymphatic system (Iliff et al., 2012). Impaired flow through the glymphatic system preceded reduced Aβ clearance in a mouse model of Alzheimer's disease (Peng et al., 2016). Glymphatic flow depends on movement of water through aquaporin channels. Genetic knockout of aquaporin-4 in a mouse model of impairs glymphatic flow and promotes pathologic accumulation of phosphorylated tau (Iliff et al., 2014). These studies may highlight a

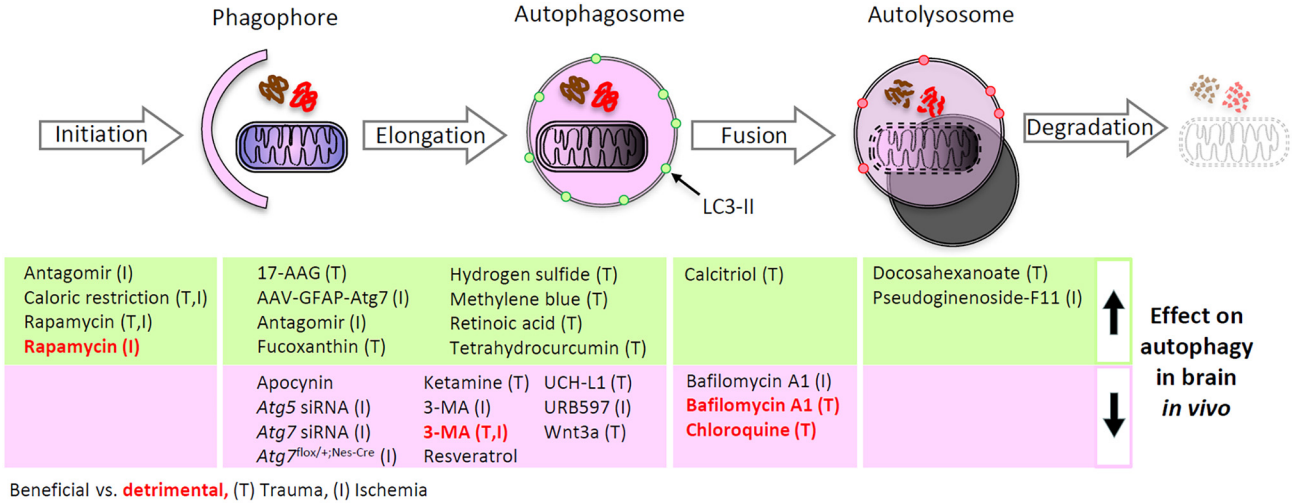

Fig. 2. Recently published interventions organized based on the stage of the autophagic process targeted. Interventions are separated based on whether they enhance (green box) or inhibit (pink box) autophagy after acute brain injury (T = TBI; I = cerebral ischemia) in vivo. Interventions that produce detrimental effects are typed in red font (note that some have been reported to have both beneficial and detrimental effects). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mechanistic link between a cell's autophagic capacity, overwhelmed autophagic flux, secretory autophagy, extracellular accumulation of neurotoxic proteins, and glymphatic elimination (Fig. 1). In a repetitive mild TBI model in mice, Saykally et al. reported decreased LC3-II, LAMP2, and Atg7 at 3 days, and a subsequent increased aggregation of transactivation response DNA protein 43, a protein associated with neurodegeneration (Saykally et al., 2018).

Secretory autophagy and the efficiency of glymphatic elimination may also effect regulation of inflammation and cell death (Fig. 1). Contents of secreted autophagosomes are known to contain a number of damage-associated molecular patterns (DAMPs), including high-mobility group box 1 (HMGB1), ATP, IL-1β, and DNA (Zhang et al., 2013). HMGB1, IL-1β, and mitochondrial DNA have all been shown to increase in CSF from patients after severe TBI (Au et al., 2012; Satchell et al., 2005; Walko 3rd et al., 2014). In the case of HMGB1, its release has been shown to regulate Beclin 1-dependent autophagy in cancer cells (Tang et al., 2010). A recent study suggests that the protective effects of ischemic preconditioning may be associated with concomitant reduction in plasma HMGB1 and autophagy (Wang et al., 2016). Interestingly, reducible HMGB1 induces autophagy and promotes tumor resistance to chemotherapeutic agents, whereas oxidized HMGB1 increases apoptosis and sensitizes tumor cells. Oxidative stress is universally observed in all types of acute brain injury, and is thought to regulate autophagy (Hensley and Harris-White, 2015). Autosis, mediated by the Na^+, K^+-ATPase pump, displays numerous autophagosomes and autolysosomes without late stage secondary lysosomes, and plasma membrane rupture suggesting osmotic disequilibrium (Liu and Levine, 2015). Intriguingly, there is also a link between HMGB1 and the water channel aquaporin (Laird et al., 2014). It is tempting to speculate that secretory autophagy of DAMPs and autophagy-related proteins may regulate inflammation, autophagy, and/or apoptosis in neighboring cells, perhaps acting as “survival signals”, whereas overwhelming autophagy exceeding the capacity of autophagic flux may lead to toxic accumulation of autophagosomes and autosis, the consequence of which would be large-scale release of DAMPs and other “surrender signals”. Extracellular signaling of autophagy by DAMPs would also be affected by the rate of glymphatic flux.

8. Where does the field stand now?

Taken together, studies of autophagy after acute brain injury are consistent in the following: 1) autophagosomes and autophagy-related proteins are more readily detectable after acute brain injury and this may be due to increased autophagy itself and/or impaired autophagic flux; 2) just as autophagy is a vital homeostatic process, some degree of autophagy represents a homeostatic response to cellular insults; 3) feedback regulation of autophagy may be controlled by both intracellular and extracellular autophagic flux; and 4) glymphatic flow may work in concert with secretory autophagy to clear potentially toxic proteins and metabolic waste products from the brain.

What remains less clear is whether inhibition or augmentation of autophagy after acute TBI or cerebral ischemia produces detrimental or beneficial effects, since all combinations have been reported in various experimental models. Definitive studies have been hampered by the lack of highly selective pharmacological tools to manipulate autophagy. Case in point, the most commonly used drugs in studies of autophagy, rapamycin to stimulate autophagy and 3-MA to inhibit it, each have multiple off-target effects. Rapamycin is a clinically used immunosuppressive drug that has effects on inflammation, cell cycle arrest and cell proliferation. 3-MA also inhibits non-class III PI3-kinases and promotes glycogenolysis. Furthermore, knockout studies targeting autophagy-related genes to-date have not utilized inducible transgenic mice. Further study using more autophagy-selective pharmacological interventions, inducible, cell type-specific transgenic animals, and/or genome editing in models of acute brain injury would assist greatly in this regard.

In the meantime, we have organized recently published interventions where their effect on autophagy-related protein abundance, autophagosome formation, and/or autophagic flux was evaluated, along with at least one neurological outcome, after experimental acute brain injury in vivo (Fig. 2). Interventions were further organized based on the stage of the autophagic process targeted, i.e. initiation, elongation, fusion, or degradation. Most publications report benefit of the intervention whether the effect is to promote or inhibit autophagy. The most generalizable inference is that inhibiting autolysosome formation or lysosome function without reducing autophagosome formation appears detrimental after acute brain injury. Taken together, interventions that stimulate autophagic flux, reduce autophagosome abundance, and enhance autophagosome clearance may afford neuroprotection in experimental models. The clinical corollary is the observation that higher CSF p62/SQSTM1 is associated with poor neurological outcome after severe TBI in children (Au et al., 2017).

9. Conclusion

Recent studies of autophagy in acute brain injury have provided new mechanistic insights, while at the same time highlighting the complexity of the autophagic process from autophagosome formation to intracellular and extracellular clearance of macromolecules, related to interconnected signaling mechanisms and crosstalk between cell death pathways. The role of autophagy after acute brain injury likely depends upon: the degree of brain injury and the overall autophagic burden; the capacity of individual cell types to ramp up autophagic flux; the local redox state and signaling of parallel cell death pathways; the capacity to eliminate DAMPs and toxic proteins and metabolites both intra- and extracellularly; and the specific component of the autophagic process targeted. What appears consistent is that interventions that offload the intracellular burden of autophagosomes (reducing formation, stimulating autophagic flux, enhancing clearance) may afford neuroprotection after acute brain injury in experimental models. However, while much has been learned since the review by Smith et al. (2011), understanding of autophagy's role after acute brain injury remains in a state of flux.

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