

Autophagic activity in neuronal cell death

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As post-mitotic cells with great energy demands, neurons depend upon the homeostatic and waste-recycling functions provided by autophagy. In addition, autophagy also promotes survival during periods of harsh stress and targets aggregate-prone proteins associated with neurodegeneration for degradation. Despite this, autophagy has also been controversially described as a mechanism of programmed cell death. Instances of autophagic cell death are typically associated with elevated numbers of cytoplasmic autophagosomes, which have been assumed to lead to excessive degradation of cellular components. Due to the high activity and reliance on autophagy in neurons, these cells may be particularly susceptible to autophagic death. In this review, we summarize and assess current evidence in support of autophagic cell death in neurons, as well as how the dysregulation of autophagy commonly seen in neurodegeneration can contribute to neuron loss. From here, we discuss potential treatment strategies relevant to such cell-death pathways.

Keywords: autophagy; autophagic cell death; programmed cell death; apoptosis; necrosis; autosis; neurodegeneration

Introduction

Autophagy is an intracellular process of ‘self-eating’, which provides homeostatic maintenance through the capture and degradation of aggregate-prone proteins and dysfunctional organelles. More specifically, autophagy encompasses three separate mechanisms – microautophagy, chaperone-mediated autophagy, and macroautophagy – but only the latter is discussed in this review, and is referred to simply as ‘autophagy’^[1].

Briefly, autophagy proceeds through the capture of portions of cytoplasm containing target material inside expanding membranes, which finally enclose to form double-membrane vesicles called autophagosomes. Fully-formed autophagosomes are shuttled along microtubules to lysosomes, whereupon fusion and degradation occur^[2, 3]. This removal and recycling serves as an emergency energy supply during starvation, but autophagy has also

been linked to a diverse range of other protective roles^[4–7]. These include the capture of invading pathogens^[8], context-dependent tumor suppressive and tumorigenic qualities^[9–12], and the removal of toxic aggregate-prone proteins often linked to neurodegeneration^[13–15]. From these findings, interest in autophagy research has surged over the past decade or so^[16].

Despite these pro-survival roles, autophagy has also been implicated as a mechanism of programmed cell death (PCD)^[17–19]. Numerous studies have reported instances of dying cells displaying accumulated autophagosomes, which engulf large portions of the cell’s cytoplasm and which have been presumed to lead to excessive destruction of vital components^[20, 21]. However, this notion of ‘autophagic cell death’ (ACD) has met with some skepticism, with critics arguing that these accumulations of autophagosomes may represent a failed rescue response to a lethal stress, as

opposed to a direct lethal mechanism in its own right^[22-24].

Neurons have high energy demands, and as post-mitotic cells, quality control and homeostasis maintenance are vital^[25]. From these traits, one would assume neurons to rely heavily on autophagy, yet these cells typically contain very few autophagosomes, suggesting little autophagic activity. However, impairing lysosomal function leads to the accumulation of autophagosomes, revealing that autophagy is highly efficient in neurons, with a quick progression from vesicle formation to degradation^[26, 27]. Due to this high autophagic flux, it is possible that neurons are particularly sensitive to ACD. As pathologies like neurodegeneration are associated with a progressive loss of neurons, an appreciation of the diverse mechanisms of death in these cells may aid the design of future protective and preventative treatments for disease. In this review, after briefly covering the machinery of autophagy, we compare the seemingly paradoxical roles autophagy plays in both promoting survival and death in neurons. Then, we address the implications these findings have for our understanding of ACD, as well as potential applications for neuron therapy.

Regulation of the Autophagy Machinery

The autophagy machinery is highly conserved. Autophagy-related (Atg) genes were first reported in yeast^[28, 29], and many of the 30+ members of this group have mammalian homologues^[30]. The different Atg genes regulate each stage of autophagosome formation - from initiation of the process, to nucleation of the target membrane, and finally its subsequent elongation and fusion, forming the complete vesicle^[31]. Various origins of the membrane have been suggested, with sites including the endoplasmic reticulum^[32, 33], plasma membrane^[34-36], mitochondrial membrane^[37] and Golgi apparatus^[38], all receiving support.

Under normal conditions, autophagy proceeds at a relatively low basal rate. Multiple regulators of autophagy have been identified, but in mammals the best characterised is the mammalian target of rapamycin (mTOR), which inhibits the initiation of autophagosome formation. However, upon certain stimuli such as starvation, mTOR is inactivated, allowing autophagy to proceed. The initial stages of the process are mediated by the uncoordinated 51-like kinase 1 (ULK1) complex, which

activates the downstream phosphatidylinositol 3-kinase (PI3K) Class III complex^[39]. Vps34, the only mammalian Class III PI3K, catalyzes PI(3)P generation, allowing for recruitment of additional facilitators of autophagosome nucleation. Recently, PI(5)P has been shown to be able to substitute for PI(3)P in this regard, and this lipid is particularly important in responses to glucose starvation^[40]. Membrane elongation is completed through the action of two ubiquitin-like conjugation complexes: ATG12-5 and LC3-phosphatidylethanolamine (PE)^[41-43]. Although the ATG12-5-16L1 complex dissociates from completed vesicles, the LC3-PE conjugate (LC3-II) remains, making it a commonly-used marker of autophagosomes^[44]. At the end of the process, autophagosomes are shuttled to lysosomes and autophagosome-lysosome fusion occurs (Fig. 1). Lysosomal enzymes like cathepsins degrade the vesicles and their cargo, and permeases release amino-acids for recycling^[45]. The successful progression from autophagosome formation to degradation is referred to as 'autophagy flux'.

Autophagy in Neuronal Survival

Perhaps the clearest demonstration of the importance of autophagy in survival is that complete knockout of several of the Atg genes (such as Atg3, 5, 7, 9, and 16L1) results in neonatal lethality in mice^[30]. Neuron-specific Atg gene knockouts specifically reveal that basal rates of autophagy are required for normal neuronal survival^[46, 47]. Autophagy is a protective mechanism in response to numerous stresses. As well as during harsh environmental states such as starvation^[48, 49] or hypoxia^[50], autophagic activity can also promote survival through the clearance of faulty intracellular material. For instance, the specialized subdivision of autophagy that targets mitochondria, mitophagy, serves as a form of quality-control for these organelles. Defective mitochondria are targeted by machinery including PTEN-induced kinase 1 and the ubiquitin ligase parkin, which ubiquitinates proteins on their outer membrane, allowing for their selective engulfment in autophagosomes. The removal of damaged mitochondria limits the risk of further damage from the generation of reactive oxygen species (ROS)^[51-53]. In the event of ROS production, autophagy is triggered by upstream activators like AMPK, or by increased activity of ATG proteins, again affording protection to cells^[53]. In

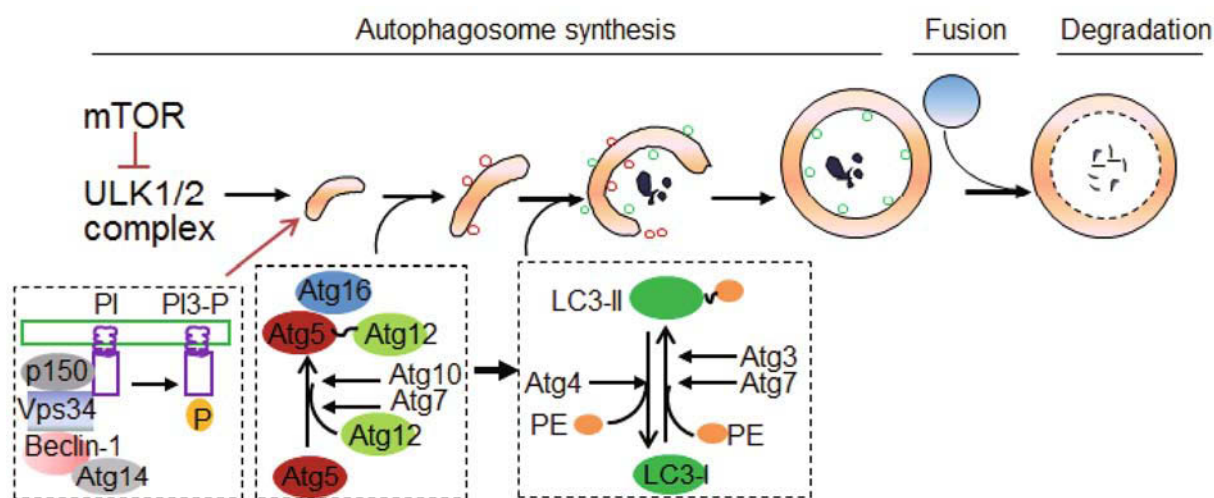


Fig. 1. Schematic diagram of the autophagy process. The autophagy process includes autophagosome biogenesis, autophagosome-lysosome fusion, and cargo degradation. The ULK1/2-Atg13-FIP200 complex, negatively regulated by mTOR, senses the signals for autophagosome initiation. The class III PI3-kinase complex, containing Vps34, beclin-1/Atg6, p150/Vps15, and Atg14, generates PI3P required for autophagosome nucleation. Autophagosome elongation involves two ubiquitin-like (UBL) conjugations: conjugation of the UBL protein Atg12 to Atg5, and conjugation of the UBL protein LC3 to PE. Atg5-Atg12 conjugation is catalyzed sequentially by the E1-like enzyme Atg7 and the E2-like enzyme Atg10. The Atg5-Atg12 complex then forms a larger complex with Atg16 and the site of Atg16 recruitment is enabled by its binding to WIPI2, which, in turn, is recruited to membranes enriched in PI3P or PI5P. The Atg5-12 conjugate is an E3 ligase that catalyzes LC3-PE (LC3-II) conjugation that also requires E1-like Atg7 and E2-like Atg3. LC3-II, the lipidated form of LC3, is required for the expansion and completion of pre-autophagosomal membranes.

some circumstances, autophagy suppresses apoptosis and necrosis^[54–57]. Given these roles, it is of little surprise that autophagy has emerged as one of the central targets in anti-ageing studies. Regimens that enhance the process have led to reductions in pathologies that manifest with age across several models^[58].

One of the branches of age-related disease that autophagy has been shown to influence is neurodegeneration. A common feature shared across these pathologies is the progressive accumulation of toxic aggregate-prone proteins. The identity of the aggregates varies between diseases: Alzheimer's disease (AD) features amyloid- β (A β) plaques and intracellular neurofibrillary tangles containing tau aggregates^[59, 60]; Parkinson's disease (PD) is characterised by Lewy body inclusions that have α -synuclein as a major constituent^[61, 62]; and Huntington's disease (HD) is the result of polyglutamine expansions of the huntingtin (Htt) protein^[13, 63]. As neurons are post-mitotic, 'in-house' modes of waste clearance are imperative to prevent the formation of these aggregates^[25]. Autophagy seems vital in this regard, as the narrow entry to the

proteasome precludes it handling oligomeric assemblies^[15]. Therefore, dysfunction in autophagy is likely to be a major contributor to the onset of neurodegeneration. Indeed, autophagic activity has been suggested to decrease with age in human tissues, including the brain^[15, 64, 65]. In some cases, degenerating neurons show accumulations of non-degraded autophagosomes in addition to the aggregates, implicating a failure of the lysosomal clearance stage in these diseases^[66–68]. An interesting exception is in the case of HD, where mutant Htt appears to reduce the recognition and capture of certain cargoes, as revealed by the recently-discovered roles that the protein plays in autophagosome-substrate interactions^[69, 70].

Consistent with these observations, knockout of the autophagy regulators Atg5 or Atg7 in the mouse CNS results in pathologies comparable to the effects of neurodegeneration, including the presence of protein aggregates coupled with neuronal damage and loss^[46, 47]. Beclin-1 (mammalian Atg6 homologue) activity decreases in ageing and neurodegenerative brains, and similarly, its loss enhances aggregate formation in models of AD, PD, and

HD^[64, 71]. Strategies of beclin-1 overexpression complement these findings, with enhanced clearance of toxins and reduced neuronal damage^[66, 72]. Mutations commonly associated with neurodegenerative pathologies have also been shown to affect autophagy. As examples, mutated presenilin-1 in AD alters the acidification of lysosomes, causing a blockade of autophagosome degradation^[73]. The AD PICALM locus is a well-validated 'hit' from genome-wide association studies and loss of this protein impairs autophagy as well as tau clearance^[74]. In PD, defects in parkin and PINK-1 result in insufficient labelling of damaged mitochondria for mitophagy, increasing the risk of ROS generation and further neuron damage^[59]. Furthermore, the α -synuclein accumulation that characterizes this disease impairs autophagosome formation^[75, 76], and the VPS35 D620N PD mutation has a similar impact on the pathway, impairing autophagy substrate clearance^[77].

Autophagy in Cell Death

Are increases in autophagic activity always beneficial for neuronal health? The concept of ACD has persisted from the early days of autophagy research, representing Class II death in the recently-abandoned morphological classifications of PCD, alongside apoptosis (Class I), and necrosis (Class III)^[23, 78]. Cells undergoing ACD are characterised by enhanced numbers of autophagosomes, resulting in extensive cytoplasmic vacuolization^[17, 20]. This has largely been attributed to increases in autophagosome synthesis and flux, causing excessive degradation of important cell components^[78]. However, this concept has courted controversy across the literature, with some groups proposing these increases in autophagic vacuoles are representative of roles more in keeping with autophagy as a pro-survival system^[22, 24]. Suggestions include autophagy up-regulation as a failing salvage effort against lethal stresses, or a clearance system of dying cells, rather than a direct route of cell death in and of itself. To try and provide more clarity on this issue, various guidelines have been suggested that themselves have attracted criticism for being overly stringent^[19, 23]. It has been proposed that for an instance of cell death to be truly mediated by autophagy, then autophagy ablation by pharmacological or genetic inhibitors should provide some protection from lethality. In addition, suppression of apoptotic or necrotic

processes should provide no such alleviation. Several cases of ACD have been strongly supported, such as large-scale clearance during development^[79-81], and the actions of some chemotherapeutic agents, at least *in vitro*^[21, 82-85]. While a number of studies have supported the concept of autophagic cell death, for instance by showing that such death is attenuated by the loss of autophagic genes^[21, 79, 85, 86], the interpretations of such studies are not always straightforward. It is possible that some autophagy is required to enable the execution of cell death after certain insults, and such experimental paradigms using autophagy-null states certainly support the concept that autophagy may be permissive in these scenarios. However, in order to test if the increased autophagy associated with certain forms of cell death is causal, one needs to ideally manipulate autophagy back to normality and not to the null state. As this type of manipulation is very challenging, most studies have not excluded the possibility that the increased autophagy they found does not cause the cell death but rather that some autophagy is required to execute cell death in a manner analogous to ATP being required for apoptosis. The extent of autophagic modulation of cell death may depend on cellular contexts and on the duration and strength of autophagy induction. Berry and Baehrecke^[79] initially reported that both autophagy and caspase activity are required for the cell death in salivary glands during *Drosophila* development, and autophagy selectively degrades the caspase inhibitor dBruce to induce *Drosophila* ovary cell death^[87]. These studies suggest that multiple possible mechanisms may be involved in ACD. In mammalian cells, the role of cellular contexts in ACD remains more elusive, while autophagy-relevant proteins such as beclin-1, Atg7, or DRAM have been reported to play a role in cell death in a variety of tumor cell lines^[88].

ACD in Excitotoxic and Ischemic Neuronal Stress

Some of the strongest support for ACD as a pathological process has been found under conditions of excitotoxicity and hypoxia-ischemia, stresses that may result from trauma or stroke^[89, 90]. Both conditions are potent inducers of autophagy, a response presumably associated with damage-limitation and the promotion of survival^[91]. Some have reported that the pharmacological induction of autophagy with rapamycin reduces apoptotic and necrotic

death during hypoxia, while inhibition with 3-methyladenine (3-MA) and wortmannin enhances this loss^[60, 91, 92]. Contrary to this, many groups have reported that this increase in autophagy can contribute to lethality. Using the glutamate receptor activator kainate as a model of excitotoxicity results in death in rat cortical neurons that is largely independent of apoptotic caspase activation. However, this cell death is reduced by inhibition of autophagosome formation using the PI3K inhibitor 3-MA, or *via* genetic knockdown of Atg7 and beclin-1^[93]. These findings have been supported in other excitotoxic models^[94, 95]. Similar approaches during hypoxia-ischemia have also aided in alleviating neuron loss both *in vitro* and *in vivo*. The administration of 3-MA has proven neuroprotective in several rodent hypoxia-ischemia models^[96, 97], although it is worth bearing in mind that this agent inhibits many PI3 kinases and thus has many autophagy-independent effects. Interestingly, Atg7 loss in pyramidal neurons appears to suppress both caspase-dependent and -independent death, suggesting that apoptosis and ACD may both occur in neurons under hypoxia, with autophagy serving as a positive mediator of both processes^[94]. The distinctive morphological changes and increases in autophagic vesicles following hypoxia in rat hippocampal neurons have even aided in the coining of a new subtype of ACD, autosis. Autosis has been characterised by an increase in both autophagosomes and autolysosomes, and displays other unique morphological characteristics, such as mild chromatin condensation and focal swelling of the perinuclear space^[86, 98]. Notably, autotic death shows an independence from apoptosis and necroptosis, instead requiring the activity of the Na⁺, K⁺-ATPase. This pump can be blocked by cardiac glycosides. Neriifolin belongs to this class of compounds, and reduces cerebral infarct size in rodent ischemic models^[99]. Importantly, these improvements in neuron survival are coupled with a decline in the number of autophagic vesicles, as well as the absence of other autotic features^[86]. Therefore, autosis seems to be a distinct form of canonical ACD that occurs in neurons. The dependence of this phenomenon on the Na⁺, K⁺-ATPase may be relevant for the treatment of hypoxia-ischemia, as many cardiac glycosides have well-characterised safety profiles and are widely used in clinical medicine^[99].

So, how can we explain these dramatically opposing results of autophagy activation on the susceptibility to

cell death? One possibility is that the extent of autophagy induction dictates the outcome. Physiological levels likely still serve a protective role, providing an energy source and relief from oxidative stress. However, over-activation may lead to the destruction of cellular components, as well as exerting additional strain on the neuron through continued autophagosome formation^[89, 100] (Fig. 2). This form of ACD by excessive autophagy stimulation has also been implicated in the neurotoxicity caused by drugs such as MDMA^[101]. The involvement of other forms of PCD appears to vary under ischemic stresses, with both caspase-dependent and -independent cases documented^[94, 97]. While neuron type seems an unlikely determinant of which scenario occurs (as both have been reported in the same population of pyramidal neurons^[94]), the neuronal region or nature of the stress may shape the outcome^[89]. It is important to consider that to date there is no evidence that the specific activation of autophagy induces cell death, and thus it is possible that other signaling pathways induced by different neuronal stressors may determine the impact of autophagic activity on cell survival. It has even been postulated that under certain circumstances, autophagy may promote apoptosis as a form of damage-limitation against inflammatory necrosis^[91].

Autophagy-Lysosome Dysfunction in Neurodegenerative Disease

Rather than over-activation of autophagosome synthesis, the combination of autophagosome and aggregate accumulations seen in neurons in neurodegenerative diseases are frequently the result of impaired lysosomal degradation^[63, 102]. Therefore, in such instances it seems unlikely that ACD occurs *via* excessive degradation of cellular components, as the autophagic flux is impaired. However, these accumulations may still have detrimental effects on cell survival. Without a means of waste removal, the hallmark toxic aggregates associated with conditions like AD, PD, and HD can accumulate^[59]. Other important homeostatic processes, such as faulty organelle removal, are not fulfilled either in these conditions, which may exacerbate the damage. Neurotoxins like rotenone and MPTP mimic PD pathology by inhibiting complex I of the mitochondrial electron transport chain and causing ROS generation^[103–105]. Defective mitochondria may disrupt the

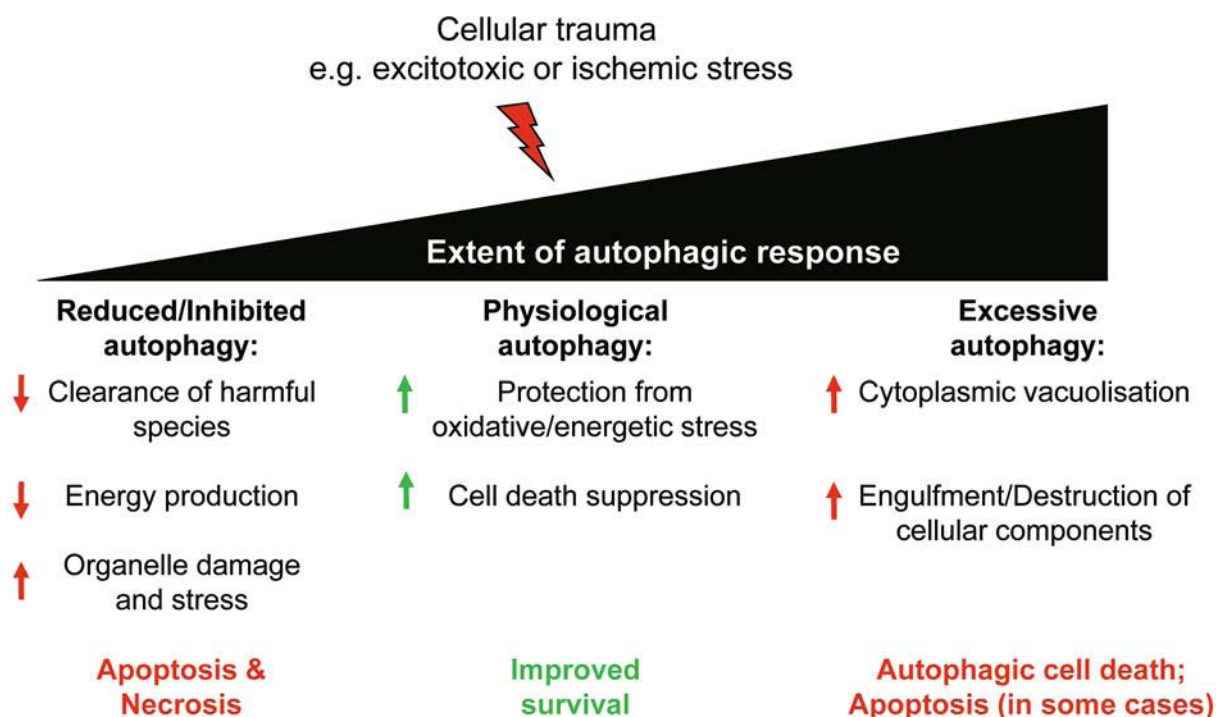


Fig. 2. One hypothetical model where the extent of autophagy dictates the fate of neurons under stress. When faced with a harsh stress, neurons rely on autophagy induction as a means of protection and damage limitation, suppressing cell death and promoting survival. This means an inefficient or inhibited level of autophagy can be detrimental to neuronal health. At the other end of the spectrum, an excessive autophagic response may result in the degradation of vital cellular components, culminating in ACD. Therefore, it seems that in order for autophagy to exert its protective effects, a balance needs to be maintained to avoid neuronal death.

microtubule-dependent trafficking of autophagosomes to lysosomes further, decreasing the clearance of aggregates and ROS even more^[106]. These conditions also favor the mitochondrial release of cytochrome *c* into the cytosol, promoting cell death by apoptosis^[107]. Elevated ROS have a negative impact on lysosomes: decreasing both their number and membrane integrity. Lysosomal membrane permeabilization can lead to leakage of proteases like cathepsins with damaging consequences^[104, 105]. While these events can hardly be classified as a bona fide case of ACD, they still highlight how impairment of the system can lead to neuronal death (Fig. 3). Death associated with autophagy-lysosome dysfunction is likely to be of particular relevance to conditions like AD and PD, which commonly display defective autophagic flux^[15, 59, 104].

In such cases, efforts should focus on salvaging lysosomal function and boosting autophagic flux. Pharmacological inducers of autophagy have been trialed across

a variety of animal models, and can aid the removal of aggregates associated with AD, PD, and HD^[59, 108, 109]. In some cases, these treatments provide a degree of cognitive restoration^[71, 110]. Notably, a number of these therapies have been achieved with approved drugs, such as rapamycins^[71, 111-114], rilmenidine^[110]. Furthermore, benefits have been seen in a number of animal models with the polyamine spermidine, which induces autophagy^[115].

A variety of strategies aimed at a number of targets have adopted this concept, with some encouraging findings. Agents like the small molecule GTM-1 and natural product arctigenin both enhance autophagic clearance, and are associated with reductions in toxic aggregates and improved cognition in AD mouse models^[116, 117]. Boosting cathepsin activity also provides similar benefits, although the incidence of lysosomal membrane permeabilization in some disease states may make this mechanism unfeasible^[118]. Glucosylceramide (GlcCer) has

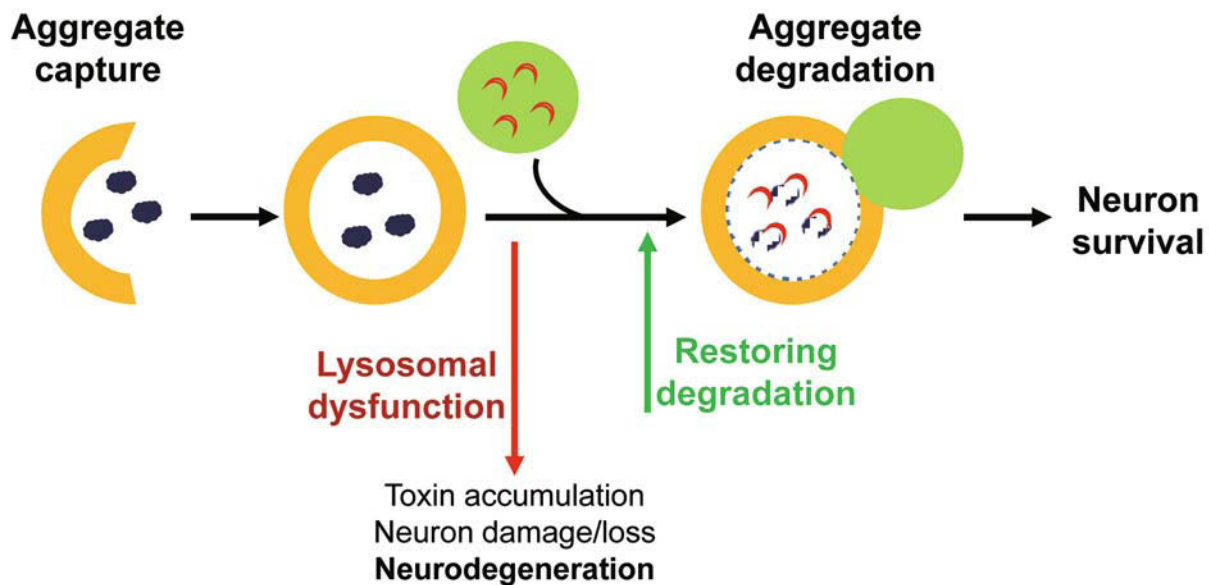


Fig. 3. Dysfunctional lysosomal clearance of autophagy promotes the accumulation of toxic aggregates associated with neurodegeneration. Efficient autophagic clearance is required to remove potentially toxic aggregate-prone proteins from neurons. In instances of lysosomal dysfunction, these accumulations can form, and, coupled with the loss of protective autophagy, cause further damage to neurons. Ultimately, these stresses can result in neuronal loss and neurodegeneration. Strategies for restoring lysosomal integrity and function allow autophagic degradation to resume, and may provide a means of prevention against these pathologies.

been associated with reductions in lysosomal activity, and inhibitors of GlcCer appear to have the desired restorative effects on organelles and improve PD pathology^[119, 120]. Niemann-Pick Type C (NPC) is another neurodegenerative disease that has a pathology similar to AD^[121]. In NPC mice, enhancing degradation aids neuron survival^[122]. An exciting candidate that has emerged in recent years is the transcription factor EB (TFEB). TFEB is a positive regulator of a number of lysosomal and autophagy-related genes; its expression is associated with enhanced lysosome biogenesis and substrate clearance^[123]. As the brains of mouse models of neurodegeneration have been reported to show reductions in TFEB, the effects of elevating its expression have been investigated^[25, 67]. Importantly, increased TFEB aids in the degradation of misfolded tau^[124], α -synuclein^[67], and mutant Htt^[25] both *in vitro* and *in vivo*. There has been less clear support for A β plaque removal^[124]. Pharmacological autophagy inducers like rapamycin and trehalose also activate TFEB, and aid in the clearance of protein aggregates in mouse models of neurodegeneration, as well as reducing the damage associated with neurotoxins like rotenone and MPTP^[67, 104, 105]. Selected pharmacological

agents/strategies used for autophagy flux restoration in neurodegenerative models are listed in Table 1.

Concluding Remarks

The role of autophagy in neuronal survival appears complex. While its homeostatic functions seem vital for survival through protection against stress and the removal of toxins, imbalances in the pathway can promote lethality. Interestingly, it appears that both over-activation and inactivation of autophagy can lead to neuronal death. Support for ACD by over-activation has come from observations made during periods of harsh stress like excitotoxicity and hypoxia. In these situations, it is possible that autophagy acts as an out-of-control protective response, and induces death by excessive capture and destruction of intracellular components. Genetic ablation of the autophagy machinery can alleviate this phenomenon. However, it is important to consider that the experimental paradigms that lead to the conclusion of ACD generally use autophagy-null states or chemical inhibitors like 3-MA or wortmannin which have multiple autophagy-independent

Table 1. Selected strategies of autophagy flux restoration in models of neurodegeneration (mammalian where available)

Strategy	Neurodegenerative Disease	Changes to Pathology	Reference
Pharmacological			
Rapamycin	Alzheimer's disease	Autophagy induction; reductions in A β and cognitive recovery in AD mice	[71],[112]
	Huntington's disease	Reductions in Htt aggregate formation, improvements in behavioral tests in mice	[111]
	Parkinson's disease	Reductions in α -synuclein accumulation, alleviation of neurodegenerative behavior in mice	[113],[114]
Rilmenidine	Huntington's disease	Autophagy induction; enhanced clearance of mutant Htt, improved motor performance in mice	[110]
Spermidine	Parkinson's disease	Autophagy induction; Improved motor performance in fruit fly, reduced dopaminergic neuron loss in nematodes	[115]
Arctigenin	Alzheimer's disease	Autophagy induction; Reduction in A β plaques through inhibition of formation and enhanced clearance, improved memory in mice	[117]
GTM-1	Alzheimer's disease	Autophagy induction and increased flux; removal of A β oligomers, cognitive improvements in mice	[116]
Glucosylceramide inhibitors	Niemann-Pick Type-C 1	Correction of autophagic flux; improved clearance of cholesterol and autophagic vesicles in mouse and cat models, prolonged neuron survival	[119],[120]
Genetic			
TFEB	Alzheimer's disease	Upregulation of lysosomal and autophagy genes;	[124]
	Huntington's disease	enhanced clearance of tau, α -synuclein, and mutant	[25]
	Parkinson's disease	Htt aggregates	[67]

effects. Thus, autophagy may be permissive for certain forms of cell death, but may not be sufficient – we are not aware of any data showing that “specific” autophagic hyperactivation by overexpressing a complete Atg gene induces cell death. Lysosomal dysfunction can also lead to aberrant autophagosome accumulation through blockade of their degradation, and is associated with neurodegenerative pathologies. In such cases there is reduced autophagic flux and the failure to successfully clear intracellular protein aggregates and ROS means the toxins can propagate unchecked and cause further damage, ultimately leading to cellular demise. These differing mechanisms influence treatment strategies. While autophagy inhibitors may improve survival in ACD by over-activation of autophagosome biogenesis, their application is likely

to be of little use in lysosomal dysfunction. In the latter instance, therapies should instead focus on the restoration of lysosomal function and autophagic flux. Therefore, it is clear that targeting autophagy to prevent cell death is not a case of ‘one size fits all’, but rather, careful consideration is needed before selecting a treatment strategy.

ACKNOWLEDGEMENTS

DCR is funded by a Wellcome Trust Principal Research Fellowship and the NIHR Biomedical Research Unit in Dementia at Addenbrooke's Hospital. This review is supported by Plymouth University and Northcott Medical Foundation.

Received date: 2015-04-14; Accepted date: 2015-05-11

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