Chapter 9

Autophagic Flux Failure in Neurodegeneration: Identifying the Defect and Compensating Flux Offset

Claudia Ntsapi, Chrisna Swart,
Dumisile Lumkwana and Ben Loos

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64197

Abstract

Protein degradation through autophagy is one of the key pathways that maintain proteostasis and neuronal viability. Dysregulation in autophagy has been associated with a number of major protein aggregation storage disorders that are characterized by increased cellular vulnerability and susceptibility to undergo cell death. Although the molecular machinery, the proteome, and the regulation of the autophagy system are becoming increasingly clear, the specific nature of its dysfunction in the context of neuronal disease pathogenesis remains largely unclear. Moreover, although the intricate network of autophagy regulatory proteins with key metabolic checkpoints is increasingly being revealed, the relationship between autophagy dysfunction, the changing rate of protein degradation in the specific pathology, and the aggregate prone behavior of specific candidate proteins remains less understood. Many questions remain and deserve urgent attention. When does a neuron respond with heightened autophagic activity and When does the system fail to degrade autophagy cargo? This book chapter will focus on some of the main challenges in the field of autophagy research, the identity, and nature of autophagic flux failure in neurodegeneration, current means to discern and measure autophagic flux dysfunction in neuronal tissue, and recent advances in compensating the flux offset. Specifically, the role of both macroautophagy and chaperone-mediated autophagy in neuronal function and dysfunction and the spatiotemporal changes in their rates of protein degradation will be discussed and their molecular interplay highlighted. Finally, current advances in the use of autophagy modulators to better control autophagy activity will be stressed and contextualized within the framework of re-establishing neuronal proteostasis to favorably control cellular fate.

Keywords: autophagic flux, proteotoxicity, Alzheimer's disease, cell death onset, neurodegeneration, autophagosome, lysosome

1. Introduction

Maintaining the delicate balance between protein synthesis and the degradation of cytotoxic aggregate-prone proteins is crucial for sustained cell growth and development [1]. In neurons, the continuous removal of deleterious intracellular components, including aberrant proteinaceous species and irreversibly damaged organelles, is governed by the machinery of two proteolytic systems: the ubiquitin proteasome system (UPS) and the autophagy-lysosome pathways [2]. While the activity of the UPS is limited to the degradation of short-lived cytosolic and nuclear proteins, the autophagy pathways are responsible for the bulk sequestration, degradation, and recycling of long-lived or misfolded cytosolic proteins and damaged organelles [3]. Defects in the autophagic pathways are particularly detrimental to neuronal cells, with heightened vulnerability to the accumulation of toxic cytoplasmic components [4, 5]. Autophagy is a highly conserved and tightly regulated pathway that is constitutively active in all cell types and is markedly induced under stress conditions [6]. Depending on the cargo targeted, and the mode of cargo delivery to the lysosome, autophagy is generally classified into macroautophagy, chaperone-mediated autophagy (CMA), and microautophagy [7]. All three autophagic pathways usually coexist in the same cell, but only macroautophagy (henceforth referred to as autophagy) and CMA have been implicated in the central nervous system and associated with specific neurodegenerative diseases [8]. In contrast to the distinctive vesicular formation, and the indiscriminate bulk degradation of cytoplasmic materials by autophagy, CMA's characteristic feature is selectivity, whereby cytosolic proteins containing a pentapeptide motif (KFERQ) are targeted and bound by the cytosolic chaperone heat-shock cognate protein of 70 kDa (hsc70) and its cochaperones [9]. An estimated 30% of all cytosolic proteins are thought to contain the KFERQ-like targeting motif, but this number is likely underrated given that post-translational modifications can also render proteins amiable to CMA-mediated degradation [9]. Similar to autophagy, CMA is constitutively active in all cell types studied thus far and upregulated in response to various stressors [10]. Importantly, the inability to upregulate CMA has been shown to render cells more vulnerable to cell death onset [11]. Neurons are highly efficient in autophagic cargo degradation [12], which contributes to their heightened vulnerability when autophagic flux, that is the rate of protein degradation though autophagy, is impaired. Changes in autophagic flux alter the cell's susceptibility to undergo cell death and it is becoming increasingly clear that the autophagic machinery is anchored within an energetic feedback loop that includes metabolic checkpoints that govern cell survival [13] (**Figure 1**). It is therefore critical in our understanding of autophagic flux deviation or dysfunction to reliably and robustly quantify this process, in vitro as well as in vivo. Autophagic flux is defined as the rate of cargo degradation within autophagosomes through autophagy [4]. Transmission electron microscopy (TEM), Western blotting, and fluorescence microscopy, all of which have been extensively described elsewhere [14], are widely used in this context. In brief, TEM remains a most powerful technique for assessing autophagy, as it allows the identification of the autophagic machinery structures at nm range [15]. Western blotting monitors endogenous microtubule-associated protein 1 light chain 3 (MAP1-LC3/Atg8/LC3) [16] as well as p62 (sequestosome/SQSTM1) degradation [17] as an indicator of autophagic flux. Importantly, the

http://dx.doi.org/10.5772/64197

amount of LC3 II correlates with the number of autophagosomes. p62/SQSTM1 is responsible for selecting cargo and to deliver proteins for degradation. It binds directly to LC3 and is codegraded by autophagy [18]. Therefore, the total amount of p62 expressed in a given cell inversely correlates with autophagic flux and provides an indication for the autophagic flux status. Fluorescence microscopy-based analysis techniques enable the counting of LC3 and p62 punctate as well as the quantification of the fluorescence signal at a single-cell level [19]. However, although above techniques are valuable in assessing whether autophagic flux has changed, they are most powerful when complemented with single-cell measurements that allow the assessment of the organelle pool size of autophagosomes (nA), autophagolysosomes (nAL), and lysosomes (nL), thus enabling to report on autophagic flux (J) and transition time (τ). Currently, there has been a major progression using such single cell-based assays to quantify flux using combinations of live cell imaging and photoswitchable fluorochromes [19–21]. These techniques are highly aligned with measuring the rate of cargo degradation, that is degradation per hour, and hence autophagic flux.

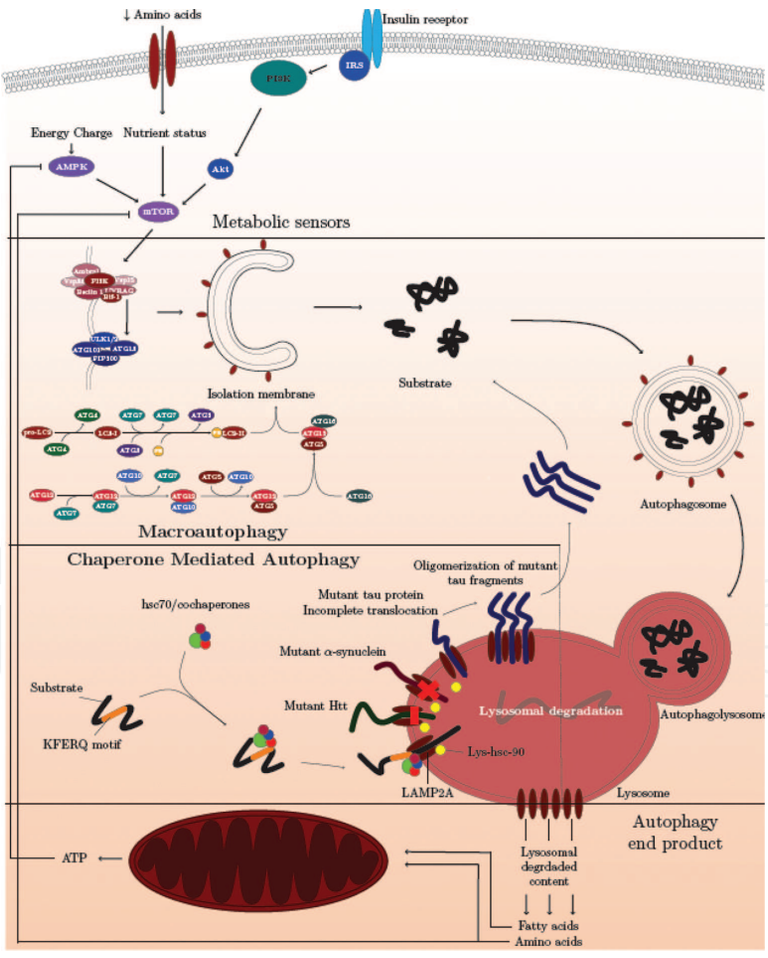

Figure 1. Changes in macroautophagy (MA) and chaperone-mediated autophagy (CMA) impact on cell vulnerability. The autophagic machinery is anchored within an energetic feedback loop that includes key metabolic checkpoints governing proteostasis and cell survival.

2. Macroautophagy and chaperone-mediated autophagy in neurodegeneration: from spatiotemporal changes to complete pathway failure

A unifying theme in neurodegenerative diseases is the failure of the proteolytic systems to adequately dispose unwanted, deleterious proteins [22]. The first pathological evidence of dysfunctional autophagy related to neurodegeneration came from electron microscopy studies of the Alzheimer's disease (AD) brain showing amyloid plaque-associated dystrophic neurites displaying massive autophagic vacuole (AV) accumulation [23]. Similar observations have been made in multiple animal models of AD [24] as well as in brains of patients with Parkinson's disease (PD) [25] and Huntington's disease (HD) [26]. Although the exact mechanism underlying autophagic dysfunction in neurodegeneration remains unclear, AV buildup may result from increased autophagic induction, impairment of downstream degradative processes in the autophagic pathway, or a decreased rate of autophagosome formation combined with insufficient lysosomal fusion [27]. The role of chaperone-mediated autophagy (CMA) in neurodegeneration is twofold: On the one hand, CMA contributes to the removal of pathogenic proteins, but, on the other hand, CMA itself becomes functionally affected by the toxicity of abnormal proteins [28] (**Figure 1**). In the following section, we will focus specifically on the three candidate pathologies with emphasis on the variability of autophagy and CMA dysfunction.

2.1. Alzheimer's disease

Evidence indicates that abnormal autophagy at the level of induction or autophagosome formation may contribute to AD pathogenesis as the expression of Beclin 1, an essential initiator of autophagy, was found to be decreased in AD patients [29, 30], possibly due to an increase in caspase 3-mediated cleavage of Beclin 1 [31]. However, a genome-wide study reported an upregulation of autophagy in AD due to transcriptional upregulation of positive regulators of autophagy as well as reactive oxygen species (ROS)-dependent activation of type III PI3 kinase, a critical kinase for the initiation of autophagy [32]. Furthermore, accumulation of electron-dense autolysosomes in the AD brain indicates lysosomal proteolytic failure [23, 33]. The morphology of such accumulated AVs resembles those resulting from selectively blocking lysosomal proteolysis through deletion of specific cathepsins or addition of lysosomal inhibitors [34, 35]. The most common cause of early-onset, familial AD is autosomal-dominant mutation in presenilin 1 (PS1) and PS2 [36], which enhance the disproportionate release of aggregation prone Aβ. However, not all AD-linked PS mutations manifest with this effect. Apart from its role in the cleavage of γ-secretase, PS1 was suggested to function in calcium homeostasis [37]. Calcium flux regulates both autophagic induction and lysosomal fusion, and PS mutations appear to aggravate this dysfunction [24] and may represent a mechanistic link unifying these pathologies [38]. Therefore, presenilins may affect autophagic flux by facilitating two crucial aspects, firstly, vesicle fusion and secondly, lysosomal function [39]. In fact, PS1 is involved in lysosomal acidification and autophagosome-lysosome fusion, and recent findings demonstrated its association with defective proteolysis of autophagic substrates in

AD patients [39, 40]. It was suggested that familial AD-linked PS1 mutations may have a loss-of-function effect on lysosomal proteolysis that leads to AV accumulation and impaired autophagic substrate turnover in AD [41]. Moreover, defective axonal transport of AVs is being implicated in AD pathogenesis. Under normal conditions, immature AVs are transported retrogradely toward the soma for degradation, but in the AD brain, a significant buildup of AVs is found within dystrophic neurites, an event that could be mimicked by inhibiting autophagosome delivery to lysosomes in healthy cells [34]. The exact molecular defects underlying axonal transport failure remain, however, largely unclear. Neuronal damage may be further inferred via inflammatory reactions generated by brain amyloid deposits. Such reactions may affect both neuronal and glial functions [42], with glial autophagy specifically affecting amyloid processing during the advanced stages of the disease [43].

Moreover, pathogenic variants of proteins, such as mutant tau associated with AD and other proteinopathies, block CMA leading to increased levels of neurofibrillary tangles [44]. When mutant tau binds to the lysosomal surface protein LAMP-2A, it is only partially internalized and the portion that gains entry is trimmed resulting in smaller amyloidogenic tau fragments at the lysosomal membrane [44]. Tau fragment oligomerization disrupts lysosomal membrane integrity and blocks CMA function. In addition, tau oligomers released from the lysosomes upon membrane rupture may act as a nucleating agent to further seed tau aggregation. It was suggested that alterations in mTOR signaling and autophagy occur at early stages of the disease [45]. A significant increase in Aβ (1–42) levels associated with a reduction in autophagy (Beclin 1 and LC3) was observed in postmortem tissue from the inferior parietal lobule of AD, amnestic mild cognitive impairment (MCI), and preclinical AD (PCAD) subjects. Hyperactivation of the PI3K/Akt/mTOR was evident in MCI and AD subjects, but not in PCAD subjects, indicating that autophagy is dynamically altered early on in the disease pathogenesis of AD.

2.2. Parkinson's disease

Faulty CMA has been widely reported in both familial [28] and sporadic PD [46]. An important role for CMA in familial PD was indicated by sequence analysis showing the presence of CMA-targeting motifs in the majority of PD-related proteins. The two most predominantly mutated proteins affected in PD, α-synuclein, and leucine-rich repeat kinase 2 (LRRK2) have been shown to undergo lysosomal degradation through CMA [28]. Mutant variants of these proteins fail to reach the lysosomal lumen despite recognition by cytosolic hsc70 and successful delivery to the lysosomal membrane [28, 47]. Aberrant interactions of these toxic proteins with lysosomal surface protein LAMP-2A obstruct internalization [28]. Importantly, such toxic interactions not only impede the degradation of these proteins but also obstruct the degradation of other CMA substrates [28, 47]. In sporadic PD, post-translational modifications caused by environmental or cellular stressors may reduce dopamine-modified α-synuclein susceptibility to CMA degradation in a manner similar to mutant α-synuclein [46]. Moreover, the persistent binding of modified forms of α-synuclein to the lysosomal membrane promotes the formation of highly toxic α-synuclein oligomers or protofibrils. Studies show that an increase in the cellular levels of either α-synuclein [28] or LRRK2 [47] beyond a tolerable threshold has similar inhibitory effects on CMA activity even in the absence of modifications. Aberrant α-

synuclein not only inhibits CMA but also inhibits autophagy [48], while the overexpression of α-synuclein blocks autophagosome formation [49]. The block in autophagy through α-synuclein overexpression presents early, prior to autophagosome formation, suggesting an effect on Atg9, the only transmembrane autophagic protein.

Several genes related to PD participate in the removal of damaged mitochondria via the specialized form of autophagy, termed mitophagy [49]. In nearly 50% of autosomal recessive PD, and about 15% of sporadic early-onset PD cases, the PARK2 gene is mutated. The gene product of PARK2, PARKIN, is a ubiquitin E3 ligase containing a ubiquitin-like domain, two RING finger domains, and a conserved region between the RING domains [50]. PARKIN, a cytosolic protein, plays an important role in eliminating dysfunctional mitochondria [51]. It is recruited to the membrane of damaged mitochondria and promotes their autophagic degradation [52]. Degradation of mitochondria is both dependent on the expression of PARKIN and the presence of Atgs. Another PD-related protein, PTEN-induced kinase 1 (PINK1), interacts with PARKIN. p62 connects ubiquitinated proteins to LC3 for degradation via the autophagic pathway [18] and the loss of mitochondrial membrane potential promotes p62 accumulation on clustered mitochondria in a PARKIN-dependent fashion. It remains, however, less clear whether p62 is required for mitophagy [53, 54].

2.3. Huntington's disease

Wild-type Huntingtin protein (Htt) is a short-lived, regulatory protein usually degraded through the ubiquitin proteasome system (UPS) [55]. In HD, the long polyQ may affect the UPS by obstructing the system with mutant Htt (mHtt) [55, 56]; however, the exact affliction remains less clear. In HD, a unique situation arises compared to other neurodegenerative proteinopathies: Apart from autophagy being dysfunctional, wild-type Huntingtin protein (Htt) plays multiple roles in regulating the dynamics of the autophagic process [57]. mHtt contributes toward the induction of autophagy through mTOR sequestering and inactivation [58]. Importantly, the autophagosomes detected, while increased in abundance, appear devoid of contents indicating cargo recognition failure [59]. Hence, a situation arises where aggregated proteins and damaged organelles are not readily degraded despite the increase in autophagic induction. The presence of mHtt results in defective autophagy, leading to increased accumulation of protein aggregates, which in turn leads to compensatory upregulation of autophagy, resulting in accumulation of mHtt and subsequent toxicity [57]. mHtt affects autophagosome motility and prevents their fusion with lysosomes, further contributing to the heightened autophagosome pool size [60]. However, the exact point in disease pathogenesis during which the specific molecular defects manifests remains elusive. Fusion dynamics may be affected early on in the disease leading to compensation through alternative pathways followed by autophagic failure to recognize mHtt and subsequent toxicity, or *vice versa* [57]. In order to implement a successful autophagic therapeutic strategy in neurodegeneration, such defects need to be precisely mapped and quantified, in order to correct and offset a specific flux deviation.

http://dx.doi.org/10.5772/64197

3. Spatiotemporal changes of MA and CMA flux in the pathogenesis of neurodegeneration

Functional autophagic flux involves both the execution of autophagosome formation and lysosomal clearance, and dual evaluation is required when studying disease pathology [38]. The presence of autophagosomes alone is not a measure of functional autophagy and autophagic flux; the net rate of autophagosome content degradation [19, 61] reflects the efficiency of the process. Many neurodegenerative diseases have been characterized by a low autophagic flux leading to accumulation of diseased proteins and neurotoxicity [62]. Reports on autophagic flux are often contradictory as dysfunction in multiple steps of the pathway may be implicated. In the case of HD, for example, human and rodent samples have been reported to display increased numbers of autophagosomes while, at the same time, maintaining basal, or even increased, levels of autophagic flux compared to wild-type controls [59, 63]. In AD, decreased expression of autophagic induction proteins and increased activity of autophagy-suppressing molecules indicate impaired autophagic induction [30, 64]. However, accumulating intermediate AVs containing partially digested cargo indicates intact autophagic induction and failure instead of substrate clearance [23, 30]. Given the number of pathological events occurring in the lysosomal network of AD neurons, such changes in autophagic status are likely to reflect different stages of AD progression. During normal ageing, autophagy is downregulated; however, transcriptional regulation thereof seems to be upregulated in AD brains [32, 65]. This upregulation may represent a compensatory attempt to increase flux affected by the defective autophagosome maturation that occurs in AD neurons [38]. Impaired autophagy was suggested to occur early in the onset of AD and the dysregulated overcompensation in the advanced stages instead. It becomes clear that a fine dissection and quantification of autophagic flux [19] are required to better elucidate the extent of pathway failure and to better align autophagy modulating drugs to compensate for the existing offset.

4. Autophagy biomarkers?

There is currently an urgent need for validated biomarkers to guide clinical diagnosis in the early stages of neurodegenerative disease progression, to estimate disease risk, to evaluate disease stages, and to monitor progression and/or response to therapy before the brain is irreversibly damaged [66]. Some of the earliest pathogenic events in AD have also been linked to the Aβ clearance systems, which consists of an interconnected vesicular network of endosomes, lysosomes, and autophagosomes [67]. These alterations are followed by an increase in lysosomal biogenesis, autophagy impairment, and loss of function in genes and proteins related to the lysosomal system in AD [23]. A recent study investigated whether alterations in the lysosomal system are mirrored in the CSF of AD patients and found that the lysosomal proteins LAMP-1 and LAMP-2 were significantly upregulated in the CSF of AD patients [68]. Moreover, strongly reduced BECN1 levels have been observed in the affected brain regions of presymptomatic AD patients compared with controls [69]. APP-transgenic mice with a homozygous BECN1 deletion ($BECN1^{-/-}$) died during embryogenesis [70], whereas mice

containing a heterozygous deletion, that is BECN1$^{+/-}$, revealed increased Aβ plaque deposition, neuronal loss, and prominent accumulation of dysfunctional lysosomes containing electron-dense material [30].

These data indicate that the autophagy profile changes substantially in the disease pathogenesis, increasing the complexity of treating neuronal autophagy dysfunction. Brain imaging studies of AD disease progression have previously been monitored by the presence of tangle-bearing neurons in selective brain areas classified into Braak stages 0–VI [71, 72]. In Braak stages V and VI, the clinical diagnosis of dementia is made as NFC-associated neuropathology is spread throughout most parts of the neocortex [71, 72]. However, few studies have investigated the alterations in gene expression patterns throughout the entire course of AD progression. These and above data strongly highlight that an assessment of the autophagy proteome, autophagic as well as CMA flux parameters, and a correlation with clinical data or Braak stages would be highly beneficial in advancing successful implementation of autophagy modulation in the clinical scenario.

5. MA and CMA in disease-specific target protein clearance

Although knowledge of how autophagy and CMA are linked is limited, these two pathways have been shown to provide an integrated cytoprotective response against various proteotoxic challenges [11]. Indeed, experimental inhibition of either pathway has been shown to result in compensatory upregulation of the other, revealing a close "cross talk" between these systems [11, 73] (**Figure 2**). For example, blockage of CMA through Lamp-2A silencing in cultured cells not only leads to the constitutive upregulation of autophagy [11, 73] but also sensitizes cells to various stressors, such as oxidative stress [11, 74]. The autophagy–CMA compensatory response appears to be sequential rather than simultaneous, further stressing the need for a time-dependent flux profile assessment in the disease pathogenesis. For example, autophagy is rapidly upregulated as a transitory response to starvation [75], while CMA is sequentially upregulated in response to long-term starvation following the downregulation of autophagy [76]. In some instances, autophagy and CMA have been shown to degrade the same substrate proteins, but to varying degree. For example, wild-type α-synuclein [28], mutant HTT [77], and mutant tau protein [44] are all degraded by autophagy and CMA (**Figure 2**). Therefore, it is possible that the compensatory upregulation of these pathways may attenuate a specific disease pathogenesis by preferentially targeting and eliminating a specific candidate mutant protein aggregate. Indeed, autophagy has been shown to serve as the primary route for mutant HTT degradation [58, 78] and to eliminate both soluble and mutant tau protein aggregates in vitro and in vivo models [79, 80]. It would therefore be expected that, in the presence of CMA dysfunction, autophagy would be upregulated, thereby enabling CMA-defective cells to maintain their normal protein degradative capacity to sustain cell viability. However, in cortical neurons and differentiated SHSY5Y cells, CMA blockage due to the overexpression of mutant α-synuclein was not found to result in the compensatory upregulation of autophagic activity [81]. Instead, it led to the accumulation of autophagosomes, cytoplasmic release of vacuolar hydrolases, and eventually induced autophagic cell death of primary cortical

http://dx.doi.org/10.5772/64197

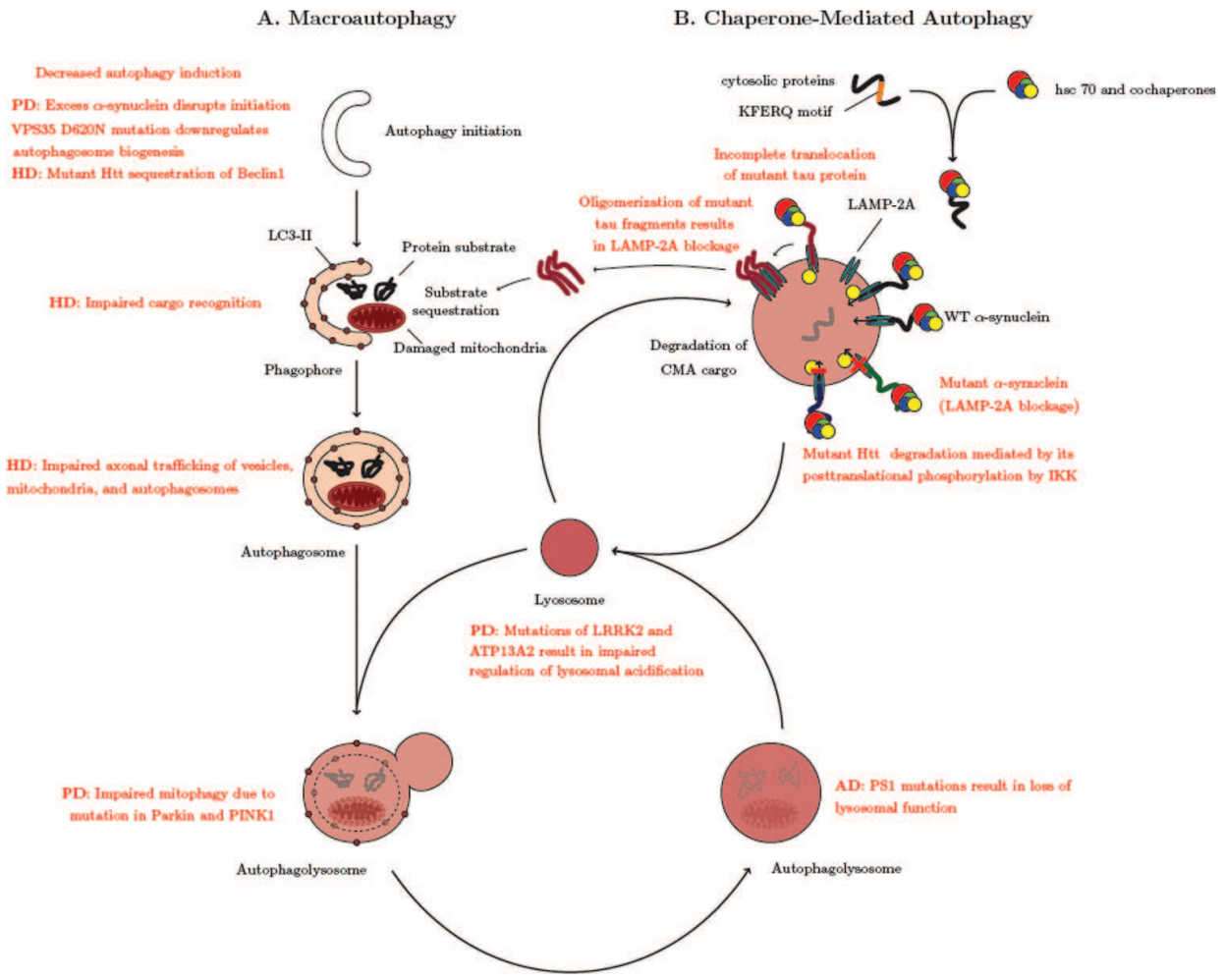

Figure 2. Substantial cross talk exists between macroautophagy (MA) and chaperone mediated autophagy (CMA), with precision defect localization. This calls for the need to precisely map and quantify both MA and CMA fluxes, in order to correct and offset the pathological flux deviation, re-establishing proteostasis.

neurons [82, 83]. In this regard, the interaction between autophagy and CMA may be a detrimental, calling for the need to accurately determine how these pathways are sequentially activated and why the molecular interplay does not always operate functionally.

6. Flux modulation and future outlook

Autophagy can be modulated through mammalian target of rapamycin (mTOR)-dependent and mammalian target of rapamycin (mTOR)-independent pathways using either pharmacological agents or lifestyle interventions. Autophagy upregulation has been shown to clear various types of aggregate prone proteins in vitro [84–86] as well as in vivo [58, 87, 88].Agents such as rapamycin, rilmenidine, lithium, and trehalose have been used in various disease models of AD, PD, and HD and have been shown to reduce the disease pathology (**Table 1**). However, the application of these drugs in different cell types, at different concentrations, or

varying time durations makes conclusive flux modulation challenging. In addition, autophagic flux was not always assessed, as the techniques and the approach to accurately measure autophagy activity have often evolved in parallel. For example, rapamycin has been used to induce autophagy and has been shown to protect against AD and HD when administered in COS-7 cells for 15 and 48 h [84, 86] and in PD models when given at 2 μg/ml in PC12 cells for 48 h [85] (**Table 1**). The same can be reported in in vivo studies where rapamycin has been used at different concentration and durations, for example 2.24or 14 mg/kg for 13 weeks or 16 months, respectively [89, 90]. Rilmenidine has been shown to reduce aggregate prone proteins associated with HD when administered at 1 μM incortical neurons and PC12 for 8 and 24 h, respectively [87]. Lithium has been shown to increase the clearance of mutant Huntingtin and α-synuclein when given at 10 nM [91] and 15 mM [92] in Hela cells (**Table 1**).

Intervention	Pathology	Pathology specificity	Model system	Concentration applied	Duration applied	Mode of flux assessment	References
Rapamycin	AD	Autophagy induction and autophagosome clearance	COS7	0.2 μg/ml	48 h	—	Berger et al. [86]
	HD	Cargo recognition	CO7	0.2 μg/ml	15 h	—	Ravikumar et al. [84]
	PD	Cargo recognition and autophagy induction	PC12	0.2 μg/ml	48 h	—	Webb et al. [85]
	AD	Autophagy induction and autophagosome clearance	Mouse	2.24 mg/kg	1X per day	WB (LC3II, p62), FM (LC3 II)	Spilman et al. [89]
Rilmenidine	HD			1 μM	8 and 24 h	FM & WB (LC3 II)	Rose et al. [87]
Lithium	HD		Hela	15 mM	48 h/5 days	FM (LC3) WB (p62)0	Wu et al. [92]

The application of these drugs in different cell types, at different concentrations or varying durations applied, makes conclusive flux modulation challenging and calls for enhanced method standardization.

EM, electron microscopy; FM, fluorescence microscopy; and WB, Western blotting.

Table 1. Autophagic flux modulators in key model systems of neurodegenerative disease.

Although it becomes clear that major promise exists to achieve favorable therapeutic effects through autophagy upregulation, it remains largely unclear what the concentration or dose

and the duration of exposure should be. In addition, AD, PD, and HD affect the autophagic pathway in different compartments and subtypes of autophagy, changing autophagic flux distinctively. Increased autophagic induction prior to developing AD-like pathology in 3xTg-AD mice reduces levels of soluble Aβ and tau, but induction after formation of mature plaques and tangles has no effect on either pathology or cognition [90]. In a scenario where the lysosomal clearance of autophagosomes is halted, activation of autophagy will result in an increase in the harmful accumulation of intermediate AVs [93]. In the case of Aβ, it was found that autophagosomes in AD brains may be a major reservoir of Aβ [94]; therefore, enhancement of new autophagosome formation without the parallel increase in their degradation may lead to an increase in Aβ production and subsequent toxicity [95]. Ideally, modification of autophagic failure should improve autophagosome clearance via the lysosome. Thus, restoring normal lysosomal proteolysis may hold a key to optimal therapeutic interventions against AD [33]. Currently, such therapeutic compounds are not yet available. With regard to the role of Htt in regulating autophagy, it is necessary to identify therapeutic targets that are able to both restore Htt function and normalize defects associated with key autophagic processes [57]. CMA regulation also represents a potential therapeutic target given the cross talk that exists between autophagic pathways [96]. Currently, it is, however, unclear to what extent autophagic flux is being affected. This demands a better quantitative assessment of autophagic flux as well as subsequent improved alignment of autophagy modulators, to allow for precision in compensating flux offset. Taken together, upregulation of autophagy may be beneficial, especially in the early stages of disease pathogenesis; however, the precise molecular target within the autophagy machinery as well as the approach and timing of the intervention has to be strongly aligned with the particular disease specific autophagic flux deviation. Future studies will undoubtedly better address these challenges, thereby impacting on the therapeutic success brought about by autophagic flux control.

Acknowledgements

The authors acknowledge financial support from the South African National Research Foundation (NRF), the Medical Research Council as well as the Cancer Association of South Africa (CANSA). We also wish to thank Andre du Toit, Stellenbosch University, South Africa, for assistance in figure development.

Author details

Claudia Ntsapi, Chrisna Swart, Dumisile Lumkwana and Ben Loos*

*Address all correspondence to: bloos@sun.ac.za

Department of Physiological Sciences, Faculty of Natural Sciences, University of Stellenbosch, Stellenbosch, South Africa

References

[1] Ryazanov AG, Nefsky BS. Protein turnover plays a key role in aging. Mech. Ageing Dev. 2002;123:207–213. doi:10.1016/S0047-6374(01)00337-2

[2] Goldberg AL. Protein degradation and protection against misfolded or damaged proteins. Nature. 2003;426:895–899. doi:10.1038/nature02263

[3] Rubinsztein DC. The roles of intracellular protein-degradation pathways in neurodegeneration. Nature. 2006;443: 780–786. doi:10.1038/nature05291

[4] Nixon R A. Autophagy induction and autophagosome clearance in neurons: relationship to autophagic pathology in Alzheimer's disease. J. Neurosci. Off. J. Soc. Neurosci. 2008;28:6926–6937. doi:10.1523/JNEUROSCI.0800-08.2008

[5] Winslow AR, Rubinsztein DC. Autophagy in neurodegeneration and development. Biochim. Biophys. Acta. 2008; 1782:723–729. doi:10.1016/j.bbadis.2008.06.010

[6] Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. Nature. 2008; 451:1069–1075. doi:10.1038/nature06639

[7] Mizushima N. Autophagy: process and function. Genes Dev. 2007; 21:2861–2873. doi: 10.1101/gad.1599207

[8] Cuervo AM. Chaperone-mediated autophagy: selectivity pays off. Trends Endocrinol. Metab. 2010; TEM 21: 142–150. doi:10.1016/j.tem.2009.10.003

[9] Dice JF. Peptide sequences that target cytosolic proteins for lysosomal proteolysis. Trends Biochem. Sci. 1990; 15: 305–309. doi:10.1016/0968-0004(90)90019-8

[10] Kiffin R, Christian C, Knecht E, Cuervo, AM. Activation of chaperone-mediated autophagy during oxidative stress. Mol. Biol. Cell. 2004; 15: 4829–4840. doi:10.1091/mbc.E04-06-0477

[11] Massey AC, Kaushik S, Sovak G, Kiffin R, Cuervo AM. Consequences of the selective blockage of chaperone-mediated autophagy. Proc. Natl. Acad. Sci. USA. 2006b; 103: 5805–5810. doi:10.1073/pnas.0507436103

[12] Mizushima N, Yamamoto A, Matsui M, Yoshimori T, and Ohsumi Y. In vivo analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker. Mol. Biol. Cell 2004;15: 1101–1111. doi:10.1091/mbc.E03-09-0704

[13] Loos B, Lockshin R, Klionsky DJ, Engelbrecht AM and Zakheri Z. On the variability of autophagy and cell death susceptibility. Autophagy. 2013; 9(9): 1270–1285. doi:10.4161/auto.25560

[14] Klionsky DJ, Cuervo AM, Seglen PO. Methods for monitoring autophagy from yeast to human. Autophagy. 2007; 3:181–206. doi:10.4161/auto.3678

[15] Klionsky DJ, Abdelmohsen K, Abe A, Abedin MJ, Abeliovich H, Acevedo-Arozena A, Adach H, Adams CM, Adam PD, Adeli K and Adhihetty PJ. Guidelines for the use and interpretation of assays for monitoring autophagy. Autophagy 2016; 12(1): 1–222. doi: 10.1080/15548627.2015.1100356

[16] Rubinsztein DC, Cuervo AM, Ravikumar B, Sarkar S, Korolchuk VI, Kaushik S, Klionsky DJ. In search of an "autophagomometer". Autophagy 2009; 5:585–589. doi: 10.4161/auto.5.5.8823

[17] Bjørkøy G, Lamark T, Brech A, Outzen H, Perander M, Øvervatn A, Stenmark H, Johansen T. p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. J Cell Biol 2005; 171:603-14. doi: 10.1083/jcb.200507002

[18] Pankiv, S., Clausen, T.H., Lamark, T., Brech, A., Bruun, J.-A., Outzen, H., Øvervatn, A., Bjørkøy, G., and Johansen, T. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. J. Biol. Chem. 2007;282: 24131–24145. doi:10.1074/jbc.M702824200

[19] Loos B, du Toit A and Hofmeyr JHS. Defining and measuring autophagosome flux—concept and reality. Autophagy. 2014;10: 2087–2096. doi:10.4161/15548627.2014.973338

[20] Koga H, Martinez-Vicente M, Arias E, Kaushik S, Sulzer D and Cuervo AM. Constitutive upregulation of chaperone-mediated autophagy in Huntington's disease. J. Neurosci. Off. J. Soc. Neurosci. 2011;31: 18492–18505. doi:10.1523/JNEUROSCI.3219-11.2011

[21] Tsvetkov AS, Arrasate M, Barmada S, Ando DM, Sharma P, Shaby BA and Finkbeiner S. Proteostasis of polyglutamine varies among neurons and predicts neurodegeneration. Nat Chem Biol 2013; 9: 586–592. doi:10.1038/nchembio.1308

[22] Morimoto RI, Driessen AJM, Hegde RS and Langer T. The life of proteins: the good, the mostly good and the ugly. Nat. Struct. Mol. Biol. 2011; 18: 1–4. doi: 10.1038/nsmb0111-1

[23] Nixon RA, Wegiel J, Kumar A, Yu WH, Peterhoff C, Cataldo A and Cuervo AM. Extensive involvement of autophagy in Alzheimer disease: an immuno-electron microscopy study. J. Neuropathol. Exp. Neurol. 2005; 64: 113–122. doi:10.1093/jnen/64.2.113 113-122

[24] Cataldo AM, Peterhoff CM, Schmidt SD, Terio NB, Duff K, Beard M, Mathews PM and Nixon RA. Presenilin mutations in familial Alzheimer disease and transgenic mouse models accelerate neuronal lysosomal pathology. J. Neuropathol. Exp. Neurol. 2004; 63: 821–830. doi:10.1074/jbc.M801279200

[25] Anglade P, Vyas S, Javoy-Agid F, Herrero MT, Michel PP, Marquez J, Mouatt-Prigent A, Ruberg M, Hirsch EC and Agid Y. Apoptosis and autophagy in nigral neurons of

patients with Parkinson's disease. Histol. Histopathol. 1997; 12: 25–31. doi:10.1111/jnc.13266

[26] Petersén A, Larsen KE, Behr GG, Romero N, Przedborski S, Brundin P and Sulzer D. Expanded CAG repeats in exon 1 of the Huntington's disease gene stimulate dopamine-mediated striatal neuron autophagy and degeneration. Hum. Mol. Genet. 2001; 10: 1243–1254. doi:10.1093/hmg/10.12.1243

[27] Barnett A and Brewer GJ. Autophagy in aging and Alzheimer's disease: pathologic or protective? J. Alzheimers Dis. JAD 2011; 25: 385–394. doi:10.3233/JAD-2011-101989

[28] Cuervo AM, Stefanis L, Fredenburg R, Lansbury PT and Sulzer D. Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy. Science. 2004; 305: 1292–1295. doi:10.1126/science.1101738

[29] Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H and Levine B. Induction of autophagy and inhibition of tumorigenesis by beclin 1. Nature. 1999; 402: 672–676. doi:10.1038/45257

[30] Pickford F, Masliah E, Britschgi M, Lucin K, Narasimhan R, Jaeger PA, Small S, Spencer B, Rockenstein E, Levine B, et al. The autophagy-related protein beclin 1 shows reduced expression in early Alzheimer disease and regulates amyloid β accumulation in mice. J. Clin. Invest. 2008; 118: 2190–2199. doi:10.1172/JCI33585

[31] Rohn TT, Wirawan E, Brown RJ, Harris JR, Masliah E and Vandenabeele P. Depletion of Beclin-1 due to proteolytic cleavage by caspases in the Alzheimer's disease brain. Neurobiol. Dis. 2011; 43: 68–78. doi:10.1016/j.nbd.2010.11.003

[32] Lipinski MM, Zheng B, Lu T, Yan Z, Py BF, Ng A, Xavier RJ, Li C, Yankner BA, Scherzer CR., et al. Genome-wide analysis reveals mechanisms modulating autophagy in normal brain aging and in Alzheimer's disease. Proc. Natl. Acad. Sci. USA. 2010; 107: 14164–14169. doi:10.1073/pnas.1009485107

[33] Yang DS, Stavrides P, Mohan PS, Kaushik S, Kumar A, Ohno M, Schmidt SD, Wesson DW, Bandyopadhyay U, Jiang Y., et al. Therapeutic effects of remediating autophagy failure in a mouse model of Alzheimer disease by enhancing lysosomal proteolysis. Autophagy 2011b; 7: 788–789. doi:10.4161/auto.7.7.15596

[34] Boland B, Kumar A, Lee S, Platt FM, Wegiel J, Yu WH and Nixon RA. Autophagy induction and autophagosome clearance in neurons: relationship to autophagic pathology in Alzheimer's disease. J. Neurosci. Off. J. Soc. Neurosci. 2008; 28: 6926–6937. doi:10.1523/JNEUROSCI.0800-08.2008

[35] Koike M, Nakanishi H, Saftig P, Ezaki J, Isahara K, Ohsawa Y, Schulz-Schaeffer W, Watanabe T, Waguri S, Kametaka S., et al. Cathepsin D deficiency induces lysosomal storage with ceroid lipofuscin in mouse CNS neurons. J. Neurosci. Off. J. Soc. Neurosci. 2000;20: 6898–6906. doi:10.1016/S0168-0102(00)81020-2

[36] Querfurth HW and LaFerla FM. Alzheimer's disease. N. Engl. J. Med. 2010;362: 329–344. doi:10.1056/NEJMra0909142

[37] Supnet C and Bezprozvanny I. Presenilins function in ER calcium leak and Alzheimer's disease pathogenesis. Cell Calcium. 2011;50: 303–309. doi:10.1016/j.ceca.2011.05.013

[38] Orr ME and Oddo S. Autophagic/lysosomal dysfunction in Alzheimer's disease. Alzheimers Res. Ther. 2013;5: 53. doi:10.1186/alzrt217

[39] Neely KM, Green KN and LaFerla FM. Presenilin is necessary for efficient proteolysis through the autophagy-lysosome system in a γ-secretase-independent manner. J. Neurosci. Off. J. Soc. Neurosci. 2011;31: 2781–2791. doi:10.1523/JNEUROSCI.5156-10.2010

[40] Esselens C, Oorschot V, Baert V, Raemaekers T, Spittaels K, Serneels L, Zheng H, Saftig P, De Strooper B, Klumperman J., et al. Presenilin 1 mediates the turnover of telencephalin in hippocampal neurons via an autophagic degradative pathway. J. Cell Biol. 2004;166: 1041–1054. doi:10.1083/jcb.200406060

[41] Lee JH, Yu WH, Kumar A, Lee S, Mohan PS, Peterhoff CM, Wolfe DM, Martinez-Vicente M, Massey AC, Sovak G., et al. Lysosomal proteolysis and autophagy require presenilin 1 and are disrupted by Alzheimer-related PS1 mutations. Cell. 2010;141: 1146–1158. doi: 10.1016/j.cell.2010.05.008

[42] Mosher KI and Wyss-Coray T. Microglial dysfunction in brain aging and Alzheimer's disease. Biochem. Pharmacol. 2014;88: 594–604. doi:10.1016/j.bcp.2014.01.008

[43] Pomilio C, Pavia P, Gorojod RM, Vinuesa A, Alaimo A, Galvan V, Kotler ML, Beauquis J and Saravia F. Glial alterations from early to late stages in a model of Alzheimer's disease: evidence of autophagy involvement in Aβ internalization. Hippocampus. 2016;26: 194–210. doi:10.1002/hipo.22503

[44] Wang Y, Martinez-Vicente M, Krüger U, Kaushik S, Wong E, Mandelkow EM, Cuervo AM and Mandelkow E. Tau fragmentation, aggregation and clearance: the dual role of lysosomal processing. Hum. Mol. Genet. 2009;18: 4153–4170. doi: 10.1093/hmg/ddp367

[45] Tramutola A, Triplett JC, Di Domenico F, Niedowicz DM, Murphy MP, Coccia R, Perluigi M and Butterfield DA. Alteration of mTOR signaling occurs early in the progression of Alzheimer disease (AD): analysis of brain from subjects with pre-clinical AD, amnestic mild cognitive impairment and late-stage AD. J. Neurochem. 2015;133: 739–749. doi:10.1111/jnc.13037

[46] Martinez-Vicente M, Talloczy Z, Kaushik S, Massey AC, Mazzulli J, Mosharov EV, Hodara R, Fredenburg R, Wu DC, Follenzi A., et al. Dopamine-modified alpha-synuclein blocks chaperone-mediated autophagy. J. Clin. Invest. 2008;118: 777–788. doi: 10.1172/JCI32806

[47] Orenstein SJ, Kuo SH, Tasset I, Arias E, Koga H, Fernandez-Carasa I, Cortes E, Honig LS, Dauer W, Consiglio A., et al. Interplay of LRRK2 with chaperone-mediated autophagy. Nat. Neurosci. 2013;16: 394–406. doi:10.1038/nn.3350

[48] Winslow AR, Chen CW, Corrochano S, Acevedo-Arozena A, Gordon DE, Peden AA, Lichtenberg M, Menzies FM, Ravikumar B, Imarisio S., et al. α-Synuclein impairs macroautophagy: implications for Parkinson's disease. J. Cell Biol. 2010;190: 1023–1037. doi:10.1083/jcb.201003122

[49] Lynch-Day MA, Mao K, Wang K, Zhao M and Klionsky DJ. The role of autophagy in Parkinson's disease. Cold Spring Harb. Perspect. Med. 2012;2: a009357. doi:10.1101/cshperspect.a009357

[50] Schapira AHV. Mitochondria in the aetiology and pathogenesis of Parkinson's disease. Lancet Neurol. 2008;7: 97–109. doi:10.1002/ana.410440714

[51] Narendra D, Tanaka A, Suen DF and Youle RJ. Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. J. Cell Biol. 2008; 183: 795–803. doi:10.1083/jcb.200809125

[52] Gasser T. Mendelian forms of Parkinson's disease. Biochim. Biophys. Acta 2009; 1792: 587–596. doi:10.1016/j.bbadis.2008.12.007

[53] Geisler S, Holmström KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ and Springer W. PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. Nat. Cell Biol. 2010; 12: 119–131. doi:10.1038/ncb2012

[54] Narendra D, Kane LA, Hauser DN, Fearnley IM and Youle RJ. p62/SQSTM1 is required for Parkin-induced mitochondrial clustering but not mitophagy; VDAC1 is dispensable for both. Autophagy 2010; 6: 1090–1106. doi:10.4161/auto.6.8.13426

[55] Schipper-Krom S, Juenemann K, Reits EAJ, Schipper-Krom S, Juenemann K and Reits EAJ. The ubiquitin-proteasome system in Huntington's disease: are proteasomes impaired, initiators of disease, or coming to the rescue? Biochem. Res. Int. Biochem. Res. Int. 2012; e837015. doi:10.1155/2012/837015

[56] Kisselev AF, Akopian TN, Woo KM and Goldberg AL. The sizes of peptides generated from protein by mammalian 26 and 20 S proteasomes. Implications for understanding the degradative mechanism and antigen presentation. J. Biol. Chem. 1999; 274 (6): 3363–3371. doi:10.1074/jbc.274.6.3363

[57] Martin DDO, Ladha S, Ehrnhoefer DE and Hayden MR. Autophagy in Huntington disease and Huntingtin in autophagy. Trends Neurosci. 2015; 38: 26–35. doi:10.1016/j.tins.2014.09.003

[58] Ravikumar B, Vacher C, Berger Z, Davies JE, Luo S, Oroz LG, Scaravilli F, Easton DF, Duden R, O'Kane CJ., et al. Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. Nat. Genet. 2004; 36: 585–595. doi:10.1038/ng1362

[59] Martinez-Vicente M, Talloczy Z, Wong E, Tang G, Koga H, Kaushik S, de Vries R, Arias E, Harris S, Sulzer D., et al. Cargo recognition failure is responsible for inefficient autophagy in Huntington's disease. Nat. Neurosci. 2010;13: 567–576. doi:10.1038/nn.2528

[60] Wong YC and Holzbaur ELF. The regulation of autophagosome dynamics by huntingtin and HAP1 is disrupted by expression of mutant huntingtin, leading to defective cargo degradation. J. Neurosci. Off. J. Soc. Neurosci. 2014; 34: 1293–1305. doi:10.1523/JNEUROSCI.1870-13.2014

[61] Klionsky DJ, Abdalla FC, Abeliovich H, Abraham RT, Acevedo-Arozena A, Adeli K, Agholme L, Agnello M, Agostinis P, Aguirre-Ghiso JA, et al. Guidelines for the use and interpretation of assays for monitoring autophagy. Autophagy. 2012; 8: 445–544. doi:10.4161/auto.19496

[62] Menzies FM, Moreau K and Rubinsztein DC. Protein misfolding disorders and macroautophagy. Curr. Opin. Cell Biol. 2011; 23: 190–197. doi:10.1016/j.ceb.2010.10.010

[63] Kegel KB, Kim M, Sapp E, McIntyre C, Castaño JG, Aronin N and DiFiglia M. Huntingtin expression stimulates endosomal-lysosomal activity, endosome tubulation, and autophagy. J. Neurosci. Off. J. Soc. Neurosci. 2000; 20: 7268–7278. doi:10.1016/j.toxlet.2014.01.039

[64] Funderburk SF, Wang QJ and Yue Z. The Beclin 1–VPS34 complex—at the crossroads of autophagy and beyond. Trends Cell Biol. 2010; 20: 355–362. doi:10.1016/j.tcb.2010.03.002

[65] Ginsberg SD, Mufson EJ, Counts SE, Wuu J, Alldred MJ, Nixon RA and Che S. Regional selectivity of rab5 and rab7 protein upregulation in mild cognitive impairment and Alzheimer's disease. J. Alzheimers Dis. JAD 2010; 22: 631–639. doi:10.3233/JAD-2010-101080

[66] Blennow K, Hampel H, Weiner M, Zetterberg H. Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. Nat. Rev. Neurol. 2010; 6: 131–144. doi:10.1038/nrneurol.2010.4

[67] Ihara Y, Morishima-Kawashima M, Nixon R. The ubiquitin-proteasome system and the autophagic-lysosomal system in Alzheimer disease. Cold Spring Harb. Perspect. Med. 2012; 2: 1–27. doi:10.1101/cshperspect.a006361

[68] Armstrong A, Mattsson N, Appelqvist H, Janefjord C, Sandin L, Agholme L, Olsson B, Svensson S, Blennow K, Zetterberg H, Kågedal K. Lysosomal network proteins as potential novel CSF biomarkers for Alzheimer's disease. Neuromolecular Med. 2014; 16: 150–160. doi:10.1007/s12017-013-8269-3

[69] Small SA, Kent K, Pierce A, Leung C, Kang MS, Okada H, Honig L, Vonsattel JP, Kim TW. Model-guided microarray implicates the retromer complex in Alzheimer's disease. Ann. Neurol. 2005; 58: 909–919. doi:10.1002/ana.20667

[70] Qu X, Yu J, Bhagat G, Furuya N, Hibshoosh H, Troxel A, Rosen J, Eskelinen EL, Mizushima N, Ohsumi Y, Cattoretti G, Levine B. Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. J. Clin. Invest. 2003; 112: 1809–1820. doi:10.1172/JCI20039

[71] Braak H and Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol. (Berl.) 1991; 82: 239–259. doi:10.1007/BF00308809

[72] Braak H, Alafuzoff I, Arzberger T, Kretzschmar H, Del Tredici K.. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. Acta Neuropathol. (Berl.) 2006; 112: 389–404. doi:10.1007/s00401-006-0127-z

[73] Vogiatzi T, Xilouri M, Vekrellis K and Stefanis L. Wild type alpha-synuclein is degraded by chaperone-mediated autophagy and macroautophagy in neuronal cells. J. Biol. Chem. 2008;283: 23542–23556. doi:10.1074/jbc.M801992200

[74] Kaushik S, Massey AC, Mizushima N, Cuervo AM. Constitutive Activation of chaperone-mediated autophagy in cells with impaired macroautophagy. Mol. Biol. Cell. 2008; 19: 2179–2192. doi:10.1091/mbc.E07-11-1155

[75] Massey AC, Kaushik S, Cuervo AM. Lysosomal chat maintains the balance. Autophagy. 2006a; 2: 325–327. doi:10.4161/auto.3090

[76] Cuervo AM, Knecht E, Terlecky SR, Dice JF. Activation of a selective pathway of lysosomal proteolysis in rat liver by prolonged starvation. Am. J. Physiol. 1995; 269: C1200–1208.

[77] Bauer PO, Goswami A, Wong HK, Okuno M, Kurosawa M, Yamada M, Miyazaki H, Matsumoto G, Kino Y, Nagai Y, Nukina N. Harnessing chaperone-mediated autophagy for the selective degradation of mutant Huntingtin protein. Nat. Biotechnol. 2010;.28: 256–263. doi:10.1038/nbt.1608

[78] Sarkar S, Perlstein EO, Imarisio S, Pineau S, Cordenier A, Maglathlin RL, Webster JA, Lewis TA, O'Kane CJ, Schreiber SL, Rubinsztein DC. Small molecules enhance autophagy and reduce toxicity in Huntington's disease models. Nat. Chem. Biol. 2007;.3: 331–338. doi:10.1038/nchembio883

[79] Krüger U, Wang Y, Kumar S, Mandelkow EM. Autophagic degradation of tau in primary neurons and its enhancement by trehalose. Neurobiol. Aging. 2012; 33: 2291–2305. doi:10.1016/j.neurobiolaging.2011.11.009

[80] Rodríguez-Navarro JA, Rodríguez L, Casarejos MJ, Solano RM, Gómez A, Perucho J, Cuervo AM, García de Yébenes J, Mena MA. Trehalose ameliorates dopaminergic and tau pathology in parkin deleted/tau overexpressing mice through autophagy activation. Neurobiol. Dis. 2010; 39: 423–438. doi:10.1016/j.nbd.2010.05.014

[81] Xilouri M, Vogiatzi T, Vekrellis K, Park D and Stefanis L. Abberant α-synuclein confers toxicity to neurons in part through inhibition of chaperone-mediated autophagy. PLoS One. 2009;4: e5515. doi:10.1371/journal.pone.0005515

[82] Bains M, Florez-McClure ML, Heidenreich KA. Insulin-like growth factor-I prevents the accumulation of autophagic vesicles and cell death in Purkinje neurons by increasing the rate of autophagosome-to-lysosome fusion and degradation. J. Biol. Chem. 2009; 284: 20398–20407. doi:10.1074/jbc.M109.011791

[83] Kroemer G and Jäättelä M. Lysosomes and autophagy in cell death control. Nat. Rev. Cancer. 2005; 5: 886–897. doi:10.1038/nrc1738

[84] Ravikumar B, Duden R and Rubinsztein DC. Aggregate-prone proteins with polyglutamine and polyalanine expansions are degraded by autophagy. Hum. Mol. Genet. 2002;11: 1107–1117. doi:10.1093/hmg/11.9.1107

[85] Webb JL, Ravikumar B, Atkins J, Skepper JN, Rubinsztein DC. Alpha-synuclein is degraded by both autophagy and the proteasome. J. Biol. Chem. 2003; 278: 25009–25013. doi:10.1074/jbc.M300227200

[86] Berger Z. Ravikumar B, Menzies FM, Oroz LG, Underwood BR, Pangalos MN, Schmitt I, Wullner U, Evert BO, O'Kane CJ, Rubinsztein DC. Rapamycin alleviates toxicity of different aggregate-prone proteins. Hum Mol Genetics. 2006;15(3): 433–442. doi: 10.1093/hmg/ddi458

[87] Rose C, Menzies FM, Renna M, Acevedo-Arozena A, Corrochano S, Sadiq O, Brown SD and Rubinsztein DC. Rilmenidine attenuates toxicity of polyglutamine expansions in a mouse model of Huntington's disease. Hum Mol Genetics. 2010; 19(11): 2144–2153. doi:10.1093/hmg/ddq093

[88] Hebron ML, Lonskaya I and Moussa CEH. Nilotinib reverses loss of dopamine neurons and improves motor behavior via autophagic degradation of α-synuclein in Parkinson's disease models. Hum Mol Genetics. 2013. 22(16): 3315–28. doi: 10.1093/hmg/ddt192

[89] Spilman P, Podlutskaya N, Hart MJ, Debnath J, Gorostiza O, Bredesen D, Richardson A, Strong R and Galvan V. Inhibition of mTOR by rapamycin abolishes cognitive deficits and reduces amyloid-βlevels in a mouse model of Alzheimer's disease. PLoS One. 2010;5: e9979. doi:10.1371/journal.pone.0009979

[90] Majumder S, Richardson A, Strong R and Oddo S. Inducing Autophagy by rapamycin before, but not after, the formation of plaques and tangles ameliorates cognitive deficits. PLoS One 2011;6: e25416. doi:10.1371/journal.pone.0025416

[91] Sarkar S, Krishna G, Imarisio S, Saiki S, O'Kane CJ and Rubinsztein DC. A rational mechanism for combination treatment of Huntington's disease using lithium and rapamycin. Hum. Mol. Genet. 2008;17: 170–178. doi:10.1093/hmg/ddm294

[92] Wu S, Zheng SD, Huang HL, Yan LC, Yin XF, Xu HN, Zhang KJ, Gui JH, Chu L, Liu XY. Lithium down-regulates histone deacetylase 1 (HDAC1) and induces degradation of mutant huntingtin. J. Biol. Chem. 2013; 288 (49): 35500–35510. doi:10.1074/jbc.M113.479865

[93] Liang JH and Jia JP. Dysfunctional autophagy in Alzheimer's disease: pathogenic roles and therapeutic implications. Neurosci. Bull. 2014; 30: 308–316. doi:10.1007/s12264-013-1418-8

[94] Yu WH, Cuervo AM, Kumar A, Peterhoff CM, Schmidt SD, Lee JH, Moha PS, Mercken M, Farmery MR, Tjernberg LO, et al. Macroautophagy - a novel Beta-amyloid peptide-generating pathway activated in Alzheimer's disease. J. Cell Biol. 2005;171: 87–98. doi: 10.1083/jcb.200505082

[95] Nixon RA. Autophagy, amyloidogenesis and Alzheimer disease. J. Cell Sci. 2007;120: 4081–4091. doi:10.1242/jcs.019265

[96] Bejarano E and Cuervo AM. Chaperone-mediated autophagy. Proc. Am. Thorac. Soc. 2010;7: 29–39. doi:10.1513/pats.200909-102JS.