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Perspective

Therapeutic induction of autophagy to modulate neurodegenerative disease progression

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There is accumulating evidence that aggregating, misfolded proteins may have an impact on autophagic function, suggesting that this could be a secondary pathological mechanism in many diseases. In this review, we focus on the role of autophagy in four major neurodegenerative diseases: Alzheimer disease (AD), Huntington's disease (HD), Parkinson's disease (PD) and amyotropic lateral sclerosis.

Keywords: autophagy; neurodegenerative disease; Alzheimer disease; Huntington's disease; Parkinson's disease; amyotropic lateral sclerosis; mTOR; rapamycin; IP$_3$; lithium

Introduction to autophagy

Macroautophagy (which we will call autophagy) is initiated by the formation of a cup-shaped double-membrane structure (the phagophore) in the cytoplasm. The origin of this structure is still under investigation, but currently the endoplasmic reticulum, Golgi, mitochondria and the plasma membrane are all proposed to be potential sources (which may not be mutually exclusive)[1–3]. The phagophore edges expand and then seal to engulf intracytoplasmic cargo, such as protein oligomers, organelles and ribosomes, thereby sequestering the cargo in a double-membrane called an autophagosome. Autophagosomes are then trafficked along microtubules towards the microtubule-organizing centre, where they mature through fusion with multivesicular bodies and early and/or late endosomes, before fusing with lysosomes. The autophagosomal contents are then degraded by lysosomal hydrolases and the degradation products are then transported back into the cytoplasm to be recycled.

It has been well established that autophagy regulates important biological functions, such as cell survival, cell death, cell metabolism, development, aging, infection and immunity. At a cellular level, the involvement of autophagy in the cell death and cell survival processes appears to be complex. The visualization of autophagosomes in dying cells has led certain groups to conclude that autophagy can serve as a nonapop-

totic form of programmed cell death[4]. Although cells can manifest a clear increase in the numbers of autophagosomes shortly before or during their death, this phenomenon is sometimes due to defects in autophagosomal maturation and, hence, decreased, rather than increased, autophagy[5, 6]. Most evidence indicates that autophagy is primarily a pro-survival rather than a pro-death mechanism, and in the context of neurodegenerative disorders, an emerging consensus is that induction of autophagy is a neuroprotective response and that defective autophagy promotes pathology.

Autophagy malfunction and neurodegenerative diseases

There is accumulating evidence that aggregating, misfolded proteins may have an impact on autophagic function, suggesting that this could be a secondary pathological mechanism in many diseases. In this review, we focus on the role of autophagy in four major neurodegenerative diseases: Alzheimer Disease (AD), Huntington's Disease (HD), Parkinson's Disease (PD) and Amyotropic Lateral Sclerosis (ALS).

Huntington's disease

Huntington's Disease (HD) is an autosomal dominant disorder caused by the expansion of the polyglutamine repeat in the N-terminus of the Huntington gene. The mutant protein is aggregate-prone and forms many extra-nuclear inclusions in the typical adult-onset case. (In the rarer juvenile-onset cases, inclusions can form in the nucleus). The first clues about the ability of autophagy to influence the accumulation and toxicity of aggregate-prone intracytoplasmic proteins associated

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with neurodegeneration were made in cell-based models of Huntington's disease, which showed that chemical inhibition of autophagy slowed the clearance of these proteins and enhanced their toxicity, while autophagy induction enhanced mutant protein clearance and was protective[7]. Interestingly, the mutant protein has a high dependence on autophagy for its clearance, while the wild-type protein turnover is hardly affected by autophagy. This phenomenon may be due to the fact that oligomeric forms of the mutant protein are not accessible to the proteasome, and so need to be cleared via autophagy. On the other hand, the wild-type protein can be very effectively and rapidly cleared by the ubiquitin-proteasome system.

Alzheimer's disease

Post-mortem analysis of AD brains reveal abnormal structures consisting of amyloid plaques and intracellular neurofibrillary tangles comprising hyperphosphorylated protein tau[8]. Several genetic defects have been identified to cause rare familial forms of AD, such as mutations in amyloid precursor protein (*APP*) and presenilin (*PSEN*) 1[9, 10]. Within neurons, autophagosomes and endosomes appear to be formed in processes and travel towards lysosomes concentrated in the perinuclear region of the cell body. A prominent feature of AD is the accumulation of autophagosomes, many containing amyloid-peptide. It has been proposed that defects in retrograde transport and therefore impaired vesicle movement within dystrophic neurons, especially those with neurofibrillary tangles may contribute to defective delivery of autophagosomes to lysosomes in Alzheimer's disease[11]. It is still unclear when this occurs during the course of the condition.

Parkinson's disease

Parkinson's disease is characterized by the selective degeneration of neurons in the substantia nigra and the presence of aggregated α-synuclein-containing intracellular inclusions known as Lewy bodies. Overexpression of wild type α-synuclein is sufficient to cause human PD, as this occurs in families who have duplications of this locus[12, 13]. Excess α-synuclein impairs autophagy in mammalian cells and transgenic mice. Conversely, a reduction in α-synuclein levels enhances autophagy in cells and in mice[14, 15]. Experiments using inhibitors and activators of autophagy confirm that wild–type α-synuclein is degraded by this pathway[16, 17] and in an α-synuclein transgenic mouse model, delivery of a Beclin-1 encoding lentivirus that induces autophagy ameliorates the synaptic and dendritic pathology and decreases α-synuclein accumulation in the limbic system[18].

Amyotropic lateral sclerosis (ALS)

ALS is an adult onset neurodegenerative disease involving selective death of motor neurons in the brain and spinal cord[19]. Mitochondrial damage and abnormal protein inclusions such as Lewy bodies[20], Skein inclusions[21] and Bunina inclusions[22] are the characteristic pathological features in ALS. ALS can be caused by mutations in a number of different genes. Mutations in DCTN1, which encodes for p150, a subunit of dynactin have been implicated in familial ALS[23]. Since the dynein apparatus is required for transport of autophagosomes to lysosomes, this may explain the accumulation of aggregate-prone proteins in this form of the disease[24, 25].

A mutation in an ESCRT (Endosomal Sorting Complexes Required for Transport) protein also causes ALS. Cells depleted of ESCRT have inhibited autophagic degradation, due to impaired autophagosome-lysosome fusion, causing accumulation of ubiquitinated aggregates[26]. This may explain some of the pathology in patients with ALS who have a mutation in this complex. Another protein mutated in ALS is VCP (Valosin-Containing Protein), which also appears to regulate autophagosome removal[27]. VCP mutant models accumulate autophagosomes, which fail to mature into autolysosomes[28].

It is likely that decreases in autophagic flux will contribute to the progression of this disease by enhancing the accumulation of aggregate-prone proteins, dysfunctional mitochondria, and increasing susceptibility to cell death.

Therapeutic implications for up-regulation of autophagy

As many diseases that are caused by intracytoplasmic aggregate-prone proteins are associated with gain-of-function mutations, a viable therapeutic strategy might be to reduce the accumulation of the toxic protein in the cytoplasm. Indeed promoting the clearance of aggregate-prone proteins via pharmacological induction of autophagy has proved to be a useful mechanism for protecting against the toxic effects of these proteins in a range of cell and animal models[7, 29, 30].

Targeting the mTOR-dependent pathway

Chemical induction of autophagy protects cells against the toxic insults of aggregate-prone proteins associated with neurodegeneration by promoting their clearance[7] and also by reducing susceptibility to caspase activation and apoptosis[31]. The first known drug used in people identified as an autophagy inducer was rapamycin, which was already in clinical use for other indications. In mammalian cells, rapamycin inhibits the kinase activity of mTOR by forming a complex with the immunophilin FK506-binding protein of 12 kDa (FKBP12) that binds to and inactivates mTOR, leading to the upregulation of autophagy[29, 32, 33]. Rapamycin treatment enhances the clearance of mutant huntingtin fragments, reduces aggregate formation and protects against toxicity in cell, Drosophila and mouse models of Huntington's disease (HD)[29, 31, 34]. In cell models, rapamycin promotes the clearance of other intracytoplasmic, disease-associated, aggregate-prone proteins, including certain mutant proteins associated with spinocerebellar ataxias, mutant forms of α-synuclein implicated in PD, and mutant tau responsible for FTD[29, 35, 36]. It is likely that autophagy regulates clearance of SDS-soluble species of these proteins, and that the formation of large aggregates visible by light microscopy is influenced by autophagy clearing the smaller aggregate precursors. In Drosophila models of these diseases, the benefits of rapamycin appear to be autophagy-dependent, as this drug had no effects on
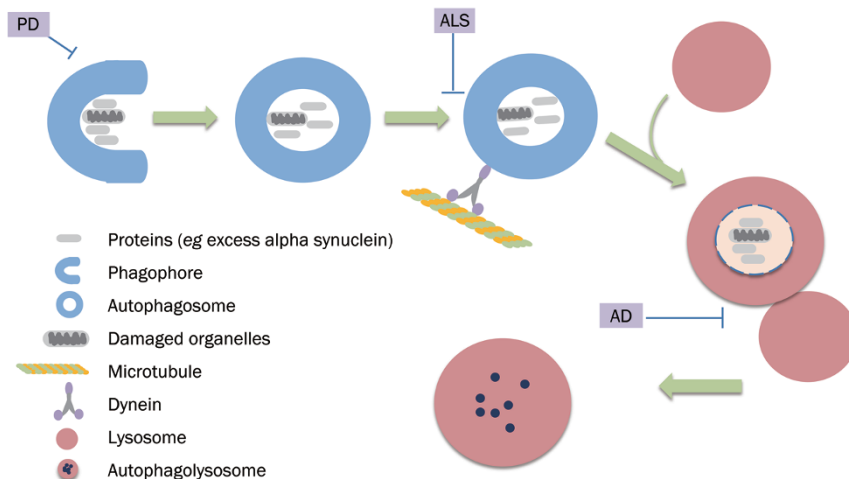
Figure 1. A schematic of autophagy showing stages disrupted in selected neurodegenerative diseases. In starvation conditions, or growth factor deprivation, autophagy is stimulated due to mammalian target of rapamycin (mTOR) inhibition. Autophagy begins with the formation of a cup-shaped double-membrane structure, termed a phagophore. This is followed by membrane elongation and fusion to form a complete autophagosome, engulfing mutant and/or aggregate-prone proteins such as excess alpha-synuclein from the cytoplasm. Autophagosomes are then trafficked along microtubule tracks via dynein motors towards the lysosomes where autophagosome-lysosome fusion occurs, generating an autophagolysosome. Excess alpha-synuclein disrupts autophagic initiation in Parkinson's disease (PD). In amyotropic lateral sclerosis (ALS), mutations in the dynein/dynactin complex inhibit autophagosome trafficking to lysosomes. Inefficient autophagosome-lysosome fusion and defects in lysosomal acidification contributes to pathogenesis in forms of Alzheimer's disease (AD).

the proteinopathy toxicity in flies expressing these mutant proteins when the activity of different autophagy genes were reduced[29, 37, 38]. Consistent with the Drosophila data, the rapamycin analogue CCI-779 reduces both mutant huntingtin and ataxin-3 levels and ameliorates toxicity in mouse models of HD and spinocerebellar ataxia type 3, respectively[39, 40].

Targeting the mTOR-independent pathway

The disadvantage of TOR targeting is that its substrates control several cellular processes besides autophagy, including repression of ribosome biogenesis and protein translation[29, 41]. These processes likely contribute to the complications seen with its long-term use. Accordingly, there have been a series of studies to identify novel autophagy-upregulating compounds with the subsequent discovery of pathways that are independent of the target of rapamycin[42].

For example, lithium, a mood stabilising drug used for the long-term treatment of affective disorders, facilitates the clearance of mutant huntingtin in HD cell and Drosophila models by reducing IP_3 levels, and reduces mutant protein-associated aggregation and toxicity[43]. Consistent with the role of IP_3 in autophagy, pharmacological inhibition of the IP_3R by xestospongin B also induces autophagy[44]. Additionally, sodium valproate and carbamazepine, which inhibit inositol synthesis and therefore decrease IP_3 levels, also reduce the accumulation and aggregation of mutant huntingtin and its toxicity in HD cell models, and protect against neurodegeneration in Drosophila models of HD[43, 45, 46]. Rilmenidine, an imidazoline receptor 1 agonist, induces autophagy, enhances mutant huntingtin clearance and reduces toxicity in an HD mouse model[30].

Since this drug is a centrally-acting hypertensive agent with minimal side-effects, it is currently being tested in a safety trial in HD patients.

Future perspectives

For many diseases, the upregulation of autophagy is a promising therapeutic target. Combining knowledge of the potential mechanisms of autophagy compromise in neurodegenerative proteinopathies with knowledge of the range of signalling pathways and drugs available to control autophagy may enhance the development of optimal therapeutics.

Acknowledgements

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