REVIEW ARTICLE

The role of autophagy-lysosome pathway in neurodegeneration associated with Parkinson's disease

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The ubiquitin-proteasome system (UPS) and autophagy-lysosome pathway (ALP) are the two most important mechanisms that normally repair or remove abnormal proteins. Alterations in the function of these systems to degrade misfolded and aggregated proteins are being increasingly recognized as playing a pivotal role in the pathogenesis of many neurodegenerative disorders such as Parkinson's disease. Dysfunction of the UPS has been already strongly implicated in the pathogenesis of this disease and, more recently, growing interest has been shown in identifying the role of ALP in neurodegeneration. Mutations of α-synuclein and the increase of intracellular concentrations of non-mutant α-synuclein have been associated with Parkinson's disease phenotype. The demonstration that α-synuclein is degraded by both proteasome and autophagy indicates a possible linkage between the dysfunction of the UPS or ALP and the occurrence of this disorder. The fact that mutant α-synucleins inhibit ALP functioning by tightly binding to the receptor on the lysosomal membrane for autophagy pathway further supports the assumption that impairment of the ALP may be related to the development of Parkinson's disease. In this review, we summarize the recent findings related to this topic and discuss the unique role of the ALP in this neurogenerative disorder and the putative therapeutic potential through ALP enhancement.

Introduction

Normal balance between the formation and degradation of cellular proteins is required for cell survival. The pathways by which most cytosolic and misfolded proteins are degraded are carried out by ubiquitin-proteasome system (UPS) and autophagy-lysosome pathway (ALP) (Ciechanover, 2005; Rubinsztein, 2006). Impairment of either of these systems may lead to the accumulation and aggregation of proteins resulting in cellular toxicity and eventual neurodegeneration as seen in Parkinson's disease, Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis and other related protein conformation disorders.

Recently, there has been a growing interest in identifying the role of the ALP in neurodegeneration (Martinez-Vicente and Cuervo, 2007). Defects in this pathway have been linked to neurodegenerative diseases (Ravikumar *et al.*, 2002, 2004), cancer (Kondo and Kondo, 2006) and cardiomyopathy (Nakai *et al.*, 2007). Since the UPS has been already extensively reviewed (Olanow and McNaught, 2006;
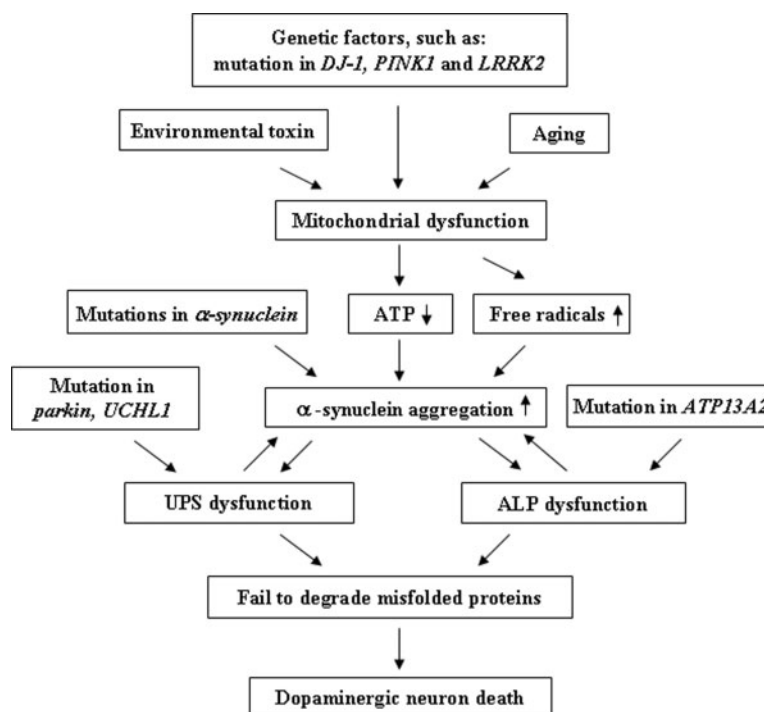
Fig. I Causes of protein aggregation and dopaminergic neuron death. Multiple factors, such as genetics, aging and environmental toxins, or combinations, have been implicated in the aetiology of Parkinson's disease. All these may directly or indirectly affect the function of protein degradation systems, including ubiquitin proteasome system (UPS) and autophagy-lysosome pathway (ALP), and thereby, cause the death of dopamine neurons.

Rubinsztein, 2006), in this article we will focus on the ALP and its unique role in Parkinson's disease. We will also discuss the putative therapeutic potential of autophagy upregulation.

Parkinson's disease is one of the most common neurodegenerative diseases in the elderly. Lewy bodies, cytoplasmic inclusions that contain aggregated proteins and degeneration of substantia nigra dopamine neurons represent the pathological hallmarks of the disorder (Taylor *et al.*, 2000; Braak *et al.*, 2003; Lansbury and Lashuel, 2006; McNaught and Olanow, 2006). Increasing numbers of proteins, such as *α*-synuclein, have been identified in Lewy bodies, but their role in the pathogenesis of both sporadic and familial Parkinson's disease is not well understood (Zarranz *et al.*, 2004; Chua and Tang, 2006; Mizuta *et al.*, 2006). Mutations of *α*-synuclein, such as *A53T*, *A30P* and *E46K* (Polymeropoulos *et al.*, 1997; Krüger *et al.*, 1998; Zarranz *et al.*, 2004), and the increase of intracellular concentrations of non-mutant *α*-synuclein, such as triplication of the *α*-synuclein gene, have been implicated in the cause of the disorder (Singleton *et al.*, 2003).

Beside excessive accumulation of misfolded proteins, several lines of evidence have converged to suggest that environmental neurotoxins, including herbicides, such as paraquat, and other mitochondrial poisons, such as rotenone, (Betarbet *et al.*, 2000; Sherer *et al.*, 2003), mutant proteins such as DJ-1 (Bonifati *et al.*, 2003), PINK1 (Valente *et al.*, 2004) and LRRK2 (West *et al.*, 2005), may contribute to mitochondrial dysfunction and increase cellular oxidative stress, all of which have been implicated in the aetiopathogenesis of Parkinson's disease. Increased oxidative stress, associated with depletion of ATP, is considered to contribute to the reduction of proteasome activity and aggregation of abnormal proteins (Norris and Giasson, 2005; Abou-Sleiman *et al.*, 2006; Lin and Beal, 2006; Schapira, 2006). Moreover, mutations of *parkin* (Foroud *et al.*, 2003; Kay *et al.*, 2007) and *UCH-L1*(Maraganore *et al.*, 2004; Das *et al.*, 2006), components of the UPS and mutations of *ATP13A2* that codes for a lysosomal ATPase (Ramirez *et al.*, 2006; Di Fonzo *et al.*, 2007) have also been shown to result in impaired protein degradation. Thus, a failure of protein degradation caused by various factors may lead to neuronal cell death related to Parkinson's disease and other neurodegenerative disorders (Webb *et al.*, 2003) (Fig. 1).

Protein clearance systems in Parkinson's disease
UPS

The UPS is responsible for a highly selective degradation of short-lived intracellular and plasma membrane proteins under basal metabolic conditions, as well as misfolded or damaged proteins in the cytosol, nucleus or endoplasmic reticulum. The system involves the targeting of susceptible proteins by ubiquitin and only the unfolded ubiquitinated proteins can pass through the narrow pore of the proteasome barrel. Dysfunction of the UPS and the
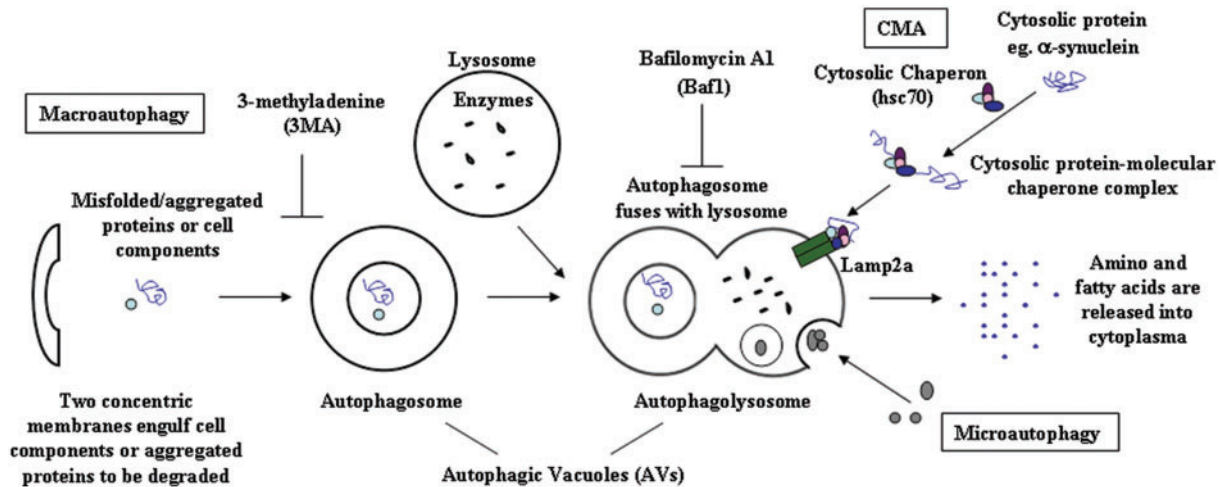
Fig. 2 Autophagy-lysosome pathway (ALP) in mammalian cells. Generally, the cytosolic proteins and cell components are degraded through macroautophagy pathway. Inhibition of the autophagosome formation by 3-methyladenine (3-MA) without markedly affecting protein synthesis or ATP levels (Seglen and Gordon, 1982), or inhibition of the fusion of autophagosome with lysosome by bafilomycin AI (BafAI) through inhibiting vacuolar type H^+-ATPase (V-ATPase), may lead to the dysfunction of macroautophagy. In microautophagy, the lysosomal membrane itself deforms to engulf the cytosolic substrates. Specific cytosolic proteins that can be recognized by a cytosolic chaperone, the heat-shock cognate protein of 70 kDa (hsc70), which targets them to the surface of lysosomes, may be degraded through CMA pathway.

resultant accumulation of misfolded proteins have been strongly implicated in the pathogenesis of Parkinson's disease (Larsen and Sulzer, 2002; McNaught *et al.*, 2003; Olanow and McNaught, 2006; Rubinsztein, 2006), which is supported by molecular genetic studies of Parkinson's disease-causing genes, including *α-synuclein*, *parkin* and *UCH-L1* (Foroud *et al.*, 2003; Maraganore *et al.*, 2004; Das *et al.*, 2006; Kay *et al.*, 2007). *Parkin* mutations in autosomal recessive juvenile parkinsonism showed a decrease in ubiquitin-ligase enzymatic activity in the substantia nigra (Shimura *et al.*, 2000, 2001), which also support the hypothesis that failure of the UPS leads to the neurodegeneration underlying Parkinson's disease.

ALP

General function
The ALP can be divided into three distinct pathways based on the ways substrates reach the lysosomal lumen: macroautophagy (generally referred to as autophagy), microautophagy and chaperone-mediated autophagy (CMA) (Cuervo *et al.*, 2004; Levine and Klionsky, 2004) (Fig. 2). Autophagy can be induced within short periods of nutrient deprivation, and CMA can be induced after prolonged nutrient deprivation, while microautophagy is not activated by nutritional deprivation or stress. In contrast to the UPS, the major inducible pathway autophagy is likely to be the primary mechanism involved in the degradation of long-lived, stable proteins and is the only mechanism by which entire organelles such as mitochondria are recycled. Large membrane proteins and protein complexes (including oligomers and aggregates) that fail to pass through the narrow proteasome barrel can

be degraded by autophagy (Klionsky and Emr, 2000; Cuervo *et al.*, 2004; Levine and Klionsky, 2004; Hideshima *et al.*, 2005).

Autophagy is a multi-step process, involving the formation of double membrane structures known as autophagosomes. Later, the autophagosome fuses with lysosomes to form autophagolysosomes, where their contents are then degraded by hydrolytic enzymes. The autophagosome and autophagolysosome are collectively referred to as autophagic vacuoles, which are considered to be the characteristic components of autophagy (Takeuchi *et al.*, 2005). Finally, the inner membrane structure within the autophagolysosome disintegrates while its contents are digested, and the vacuolar contents are recycled to provide amino acids and energy as needed by the cells (Fig. 2). Microautophagy is responsible for the gradual, continuous turnover of cytosolic proteins, pinched off from the lysosome membrane even under resting conditions. CMA is a secondary response that temporally follows autophagy. In CMA, a specific cytosolic protein–molecular chaperone (heat-shock cognate protein of 70 kDa, hsc70) complex binds to the lysosomal membrane receptor, lamp2a, and is then transported into lysosomes for degradation by hydrolases (Crotzer and Blum, 2005).

There are many steps at which the dysfunction of the ALP may occur, including the failure of autophagosome formation or autophagosome fusion with lysosomes, deficiency of enzymes in lysosomes and the dysfunction of the molecular charperone or lysosomal membrane receptor, any of which may consequently cause the aggregation of unwanted proteins leading to the death of cells.

Autophagy is an important process in a variety of human diseases caused by toxic, aggregate-prone, intracytosolic

Table I The pathogenesis and autophagy in some of the PCDs and LSDs

Neurodegenerative disease	Pathogenesis	Protein degradation	Number of AV	Effect of autophagy enhancement
Parkinson's disease	Aggregation of α-synuclein mutants (A53T, A30P, E46K)	UPS and ALP	Increased in dopamine neurons	Degradation of aggregated proteins
Huntington's disease	Aggregation of mutant huntingtin	UPS and ALP	Increased in HD neurons	Degradation of aggregated proteins
Alzheimer's disease	Aggregation of neurofibrillary tangles, β-amyloid-containing neuritic plaques	UPS and ALP	Increased	Enhance degradation of aggregated proteins
Amyotrophic lateral sclerosis (ALS)	Aggregation of mutant SODI	UPS and ALP	?	Reduces the toxicity of mutant SODI proteins
Lysosomal storage diseases (LSDs)	Accumulation of substrates targeted by lysosomal enzymes that are genetic defect	ALP	unchanged	Compensate for the defects in lysosome function caused by deficiency of lysosomal enzymes

PCD = protein conformation disorder; ? = not clear/not known.

proteins, which become inaccessible to the proteasome when they form oligomers (Ravikumar et al., 2002, 2004; Webb et al., 2003; Rubinsztein et al., 2007). It has been reported that suppression of the basal autophagy gene *Atg5* (*autophagy-related gene 5*) or *Atg7* (*autophagy-related gene 7*) in the CNS leads to accumulation of polyubiquitinated proteins with neurodegeneration in mice (Kuma et al., 2004; Hara et al., 2006; Komatsu et al., 2006; Massey et al., 2006) and increases the susceptibility to certain types of apoptosis (Larsen and Sulzer, 2002; Boya et al., 2005; Ravikumar et al., 2006), further providing the evidence that there is a relationship between autophagy deficiency and the development of neurodegenerative diseases. One of the examples for neurodegeneration caused by deficiency of enzymes in lysosomes is lysosome storage disorder, which is an inherited metabolic disease, characterized by an abnormal build-up of various toxic materials in the cells as a result of the lysosomal enzyme deficiencies (Fukuda et al., 2006; Kiselyov et al., 2007) (Table 1).

Involvement of ALP in Parkinson's disease

Mutations of α-synuclein and the increase of intracellular concentrations of non-mutant α-synuclein have been implicated in the pathogenesis of Parkinson's disease (Polymeropoulos et al., 1997; Krüger et al., 1998; Singleton et al., 2003; Zarranz et al., 2004). In addition to the UPS, α-synuclein is also cleared by autophagy (Webb et al., 2003; Cuervo et al., 2004; Lee et al., 2004; Bandhyopadhyay and Cuervo, 2007), which supports the hypothesis that impaired autophagic degradation of α-synucleins is an important mechanism of neurodegeneration in Parkinson's disease (Cuervo et al. 2004).

Furthermore, it has been shown that wild-type α-synuclein, but not the mutant α-synuclein, is selectively translocated into lysosomes for degradation by the CMA pathway (Cuervo et al., 2004). The failure of CMA to clear mutant α-synucleins may be explained by their exceptionally high affinities for the lysosomal membrane receptors that mediate the autophagy pathway as compared with the wild-type α-synucleins (Cuervo et al., 2004). The binding of mutant α-synucleins

to the receptors probably blocks the lysosomal uptake and inhibits the degradation of not only mutant α-synucleins, but also other CMA substrates. Although the blockage of CMA could potentially result in a compensatory activation of other degradation pathways, these auto-activated pathways may not be able to sustain efficient rates of protein degradation. Thus, the accumulated mutant α-synucleins and other substrates further perturb cellular homoeostasis and contribute to neuronal toxicity.

The findings that lysosomal malfunction accompanies α-synuclein aggregation in a progressive mouse model (Meredith et al., 2002), and that mutations in *ATP13A2*, a lysosomal ATPase, lead to a failure of autophagy execution and aggregation of α-synuclein in Parkinson's disease (Ramirez et al., 2006; Di Fonzo et al., 2007) further support the hypothesis that ALP dysfunction is an important mechanism of neurodegeneration.

In addition to the primary causes of ALP dysfunction already discussed, aging may be another important factor related to the dysfunction of protein control systems since it has been found that the activities of both the UPS and ALP are decreased in almost all old organisms (Keller et al., 2004; Cuervo et al., 2005; Martinez-Vicente et al., 2005; Kiffin et al., 2007). Since age is one of the chief risk factors for Parkinson's disease (Dauer and Przedborski, 2003; Jankovic, 2005; Nagatsy and Sawada, 2006), it is reasonable to postulate that the aging brain is particularly vulnerable to dysfunction of the ALP.

Auto-regulation of autophagy in Parkinson's disease

Alterations of the UPS have been implicated in the pathogenesis of Parkinson's disease. Along with ubiquitinated protein aggregates, affected neurons often contain structures related to autophagy. For example, an increased number of autophagic vacuoles and related structures of autophagy have been found in Parkinson's disease patients (Anglade et al., 1997), animal models of Parkinson's disease (Öztap and Topal, 2003) and in other disorders, including Huntington's disease (Sapp et al., 1997; Kegel et al., 2000; Petersen et al., 2001) and Alzheimer's disease (Nixon et al.,
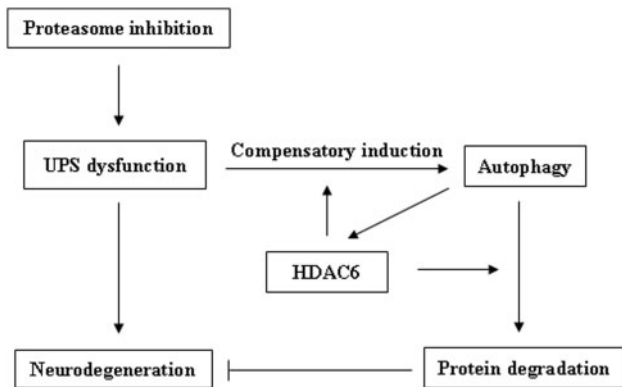
Fig. 3 Autophagy compensates for impaired UPS function. Proteasome inhibition-induced dysfunction of UPS leads to neurodegeneration. When UPS is impaired, autophagy can be compensatively induced to help remove the excessive unwanted proteins caused by UPS dysfunction and rescues neurodegeneration. The activity of HDAC6, a microtubule-associated deacetylase that interacts with poly-ubiquitinated proteins, is essential for autophagy to compensate for impaired UPS function. HDAC6 rescues neurodegeneration associated with UPS dysfunction in an autophagy-dependent manner (Pandey *et al.*, 2007).

2005; Butler and Bahr, 2006; Zheng *et al.*, 2006) (Table 1). This increase in autophagic markers raises the argument whether autophagy is a cause or a protective factor of neuron death. It has been suggested that the increased number of autophagic vacuoles is responsible for the neuronal cell death, but an alternative view is emerging that autophagy is induced to protect neurons by enhancing degradation of abnormal proteins that might trigger injury or apoptosis in the early stages of cell death (Butler *et al.*, 2006; Bandhyopadhyay and Cuervo, 2007).

Several lines of evidence suggest that once the UPS is inhibited, autophagy is upregulated and the remaining aggregated proteins are degraded (Iwata *et al.*, 2005; Massey *et al.*, 2006). Thus, this is considered to be a default pathway when an aggregate-prone substrate cannot efficiently be cleared by the proteasome (Rideout *et al.*, 2004; Olanow and McNaught, 2006). The auto-regulative mechanism that accelerates the degradation of misfolded proteins as a defence or protection may be one of the explanations of the increased number of autophagic vacuoles in the brains of Parkinson's disease patients (Anglade *et al.*, 1997), possibly in response to dysfunction of the UPS (Fig. 3). However, with pathogenic deterioration, this compensatory auto-regulative mechanism is ultimately unable to maintain the cellular balance and eventually results in neuronal death (Trojanowski and Lee, 2000). This auto-regulative concept to explain the increased the number of autophagic vacuoles is also supported by the finding that autophagic structures occur in the early stage of the MPTP-injected mouse model of Parkinson's disease (Öztap and Topal, 2003) and in a progressive mouse model of Parkinson's disease with lysosomal malfunction accompanied by α-synuclein aggregation (Meredith *et al.*, 2002).

A recent report has shown that histone deacetylase 6 (HDAC6) is an essential mechanistic link in this compensatory induction of autophagy when the UPS is impaired in *Drosophila melanogaster* (Pandey *et al.*, 2007).

The mitochondrial toxin paraquat (1, 1′-dimethyl-4, 4′-bipyridinium dichloride), a widely used herbicide, is thought to be a putative aetiological factor in the development of Parkinson's disease. The facts that paraquat induces the accumulation of autophagic vacuoles and increases the degradation of long-lived proteins in the cytoplasm of human neuroblastoma SH-SY5Y cells (Gonzalez-Polo *et al.*, 2007*a*) indicate that enhanced oxidative stress possibly actives autophagy during the early stage of mitochondrial dysfunction and helps to resist the enhanced oxidative stress (Gonzalez-Polo *et al.*, 2007*b*). It seems that autophagy is induced in the early stage and impaired in the later period of the neurodegerative process (Boland and Nixon, 2006; Bandhyopadhyay and Cuervo, 2007).

Although cells can activate different compensatory mechanisms in response to various insults in order to prevent or minimize damage, this is often not enough to completely avoid injury to cells. Strategies focusing not only on ameliorating the symptoms of Parkinson's disease, but also on neuroprotection, or neurorescue that can favourably modify the natural course of the disease will be the most important aspect of therapeutic development (Jankovic, 2006). Thus far, there are no effective therapies to slow or prevent neuronal degeneration in this disorder. Since the formation of inclusion bodies that contain the misfolded and aggregated proteins is one of the its pathological features, development towards the effective and safe targeting of aggregate-prone proteins will be an important aim for therapies in Parkinson's disease (Eriksen *et al.*, 2005).

Although dysfunction of the UPS has been implicated in Parkinson's disease, it is undesirable to upregulate proteasome activity as a therapeutic strategy since proteasomes degrade not only toxic proteins but also key short-lived intracellular regulators (for example, the tumour suppressor p53), whose steady-state levels are dependent on their degradation rates. Enhancing the degradation of such proteins may have deleterious consequences, such as cancer in the case of p53. On the other hand, upregulating autophagy may be beneficial, since in contrast to many UPS substrates, autophagy substrates are typically long-lived proteins and are not believed to be selectively degraded.

Autophagy has been largely recognized as an inducible process because its activity can be regulated by nutrient or growth factor-deprivation, stress or pathogenic invasion (Levine, 2005; Kiffin *et al.*, 2006). The autophagic activity is maintained at low levels in the brain even with nutrient starvation, a condition that usually induces autophagy in most other organs (Mizushima *et al.*, 2004); however, neural cells have the ability to induce autophagy in response to factors other than nutrient limitation. Thus, autophagy enhancement might be a novel strategy for the treatment of neurodegenerative disease (Menzies
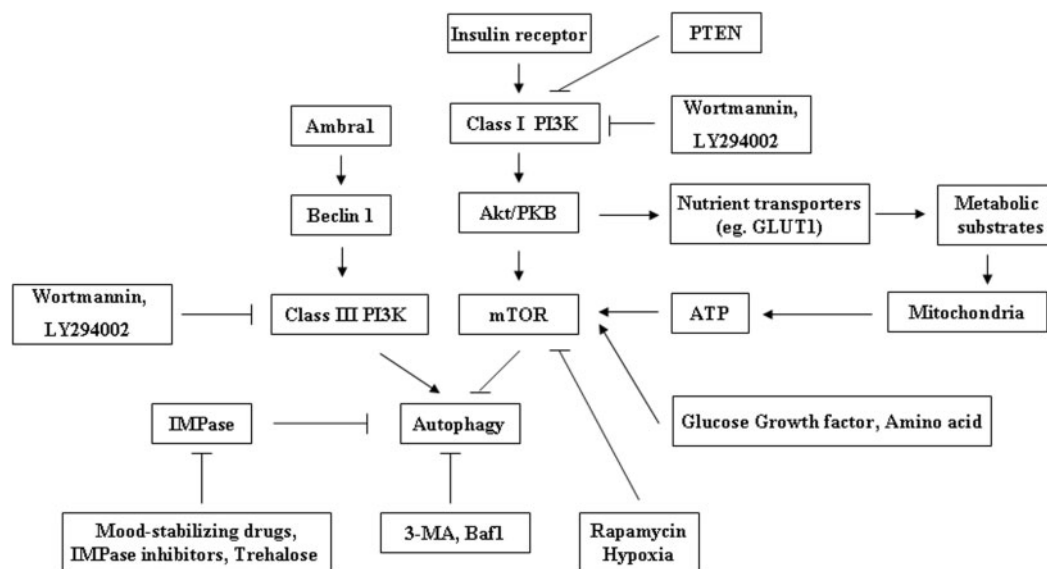
Fig. 4 Signalling pathways in the regulation of autophagy. Autophagy is activated in response to nutrient starvation, differentiation and developmental triggers. It is an adaptive process responding to metabolic stresses that results in degradation of intracellular proteins and organelles. There are three classes of phosphatidylinositol 3-kinase (PI3K) in mammalian cells. Class I PI3K is an inhibitor of autophagy. Stimulation of class I PI3K pathway through insulin receptor results in the activation of the mammalian target of rapamycin (mTOR), thereafter, inhibits autophagy. Phosphatase and tensin homologue (PTEN) enhance the autophagy through the inhibition of class I PI3K. Class III PI3K is an activator of autophagy and plays a crucial role at an early step of autophagosome formation. The beclin I/PI3K-III complex is involved in the formation of autophagosomes and initiation of autophagy. Ambra1 (activating molecule in beclin1-regulated autophagy), is a positive regulator of the beclin1-dependent programme of autophagy (Fimia *et al.*, 2007). Autophagy is regulated through energy metabolism. During the nutrient starvation or ATP deficiency due to mitochondrial complex I inhibition, autophagy is enhanced through the mTOR pathway. At the sequestration step, 3-MA interferes with the activity of class III PI3K to interrupt autophagy (Blommaart *et al.*, 1997; Petiot *et al.*, 2000). Wortmannin, LY294002 inhibit class III PI3K to reduce autophagy. Furthermore, neither wortmannin nor LY294002 displays selectivity for different members of the class I PI3K. At higher concentrations, wortmannin inhibits PI3K-related enzymes, such as mTOR. Rapamycin inhibits mTOR to induce autophagy. Mood-stabilizing drugs, such as lithium, carbamazepine and VPA, induce autophagy through inhibition of inositol monophosphatase (IMPase), which is mTOR-independent pathway.

et al., 2006). Utilizing different signalling pathways (Fig. 4), autophagy may participate in cell growth, proliferation, cell survival and death.

Enhancement of autophagy through the mTOR-dependent pathway

Although various manipulations can be used *in vitro* to upregulate autophagy, most of these strategies are not suitable for clinical application. One pharmacological strategy for upregulating autophagy in mammals, however, is to use rapamycin or its analogues through the inhibition of mammalian target of rapamycin (mTOR), a negative regulator of autophagy (Klionsky and Emr, 2000). Rapamycin has been reported to protect against huntingtin-induced neurodegeneration in fly and mouse models of Huntington's disease through autophagy induction, indicating its therapeutic potential (Ravikumar *et al.*, 2002, 2004, 2006; Berger *et al.*, 2006; Rubinsztein, 2006). Also, rapamycin-induced autophagy has been shown to degrade all forms of α-synuclein, as demonstrated in a stable inducible PC12 cell model (Webb *et al.*, 2003), suggesting that it may be considered as a potential therapeutic agent in Parkinson's disease. Furthermore, enhancement of autophagy by rapamycin may have additional cytoprotective effects against a range of subsequent pro-apoptotic insults (Pan *et al.*, 2006; Ravikumar *et al.* 2006; Williams *et al.*, 2006). Thus, the drug may work primarily by inducing autophagic degradation of mutant aggregate-prone proteins and, therefore, may be useful in neurodegenerative diseases that are associated with an increased rate of apoptosis (Jankovic, 2005). Secondary beneficial effects of rapamycin-induced autophagy may be an enhanced clearance of mitochondria with a reduction of cytosolic cytochrome *c* release and downstream caspase activation (Ravikumar *et al.*, 2006).

Although rapamycin has been used clinically as an antibiotic and immunosuppressant without serious adverse effects, long-term use of the drug as an inhibitor of mTOR may potentially be associated with complications since the mTOR proteins control several cellular processes, including repression of ribosome biogenesis and protein translation, and transcriptional induction of compensatory metabolic pathways (Sarbassov *et al.*, 2005).

Enhancement of autophagy through an mTOR-independent pathway

Recent studies have identified novel small-molecule enhancers (SMER) of mammalian autophagy, which boost the clearance of autophagy substrates including mutant

huntingtin and *A53T α-synuclein*. These agents seem to act either independently or downstream to the target of rapamycin (Sarkar *et al.*, 2007*b*).

Lithium, one of the mood-stabilizing drugs, can increase the clearance of aggregate-prone proteins, including mutant *huntingtin* and the *A30P* and *A53T* mutants of α-synuclein through induction of autophagy by inhibiting inositol monophosphatase (IMPase) (Sarkar *et al.*, 2005; Sarkar and Rubinsztein, 2006). The stimulation of autophagy by lithium is thought to involve a novel mTOR-independent pathway by decreasing free inositol (Sarkar *et al.*, 2005; Sarkar and Rubinsztein, 2006). Since inositol depletion is a common mechanism for mood-stabilizing drugs, including lithium, carbamazepine and valproic acid, by enhancing autophagy, these may have an important therapeutic potential in the treatment of Parkinson's disease.

In addition, trehalose, a disaccharide present in many non-mammalian species, has been reported to enhance the clearance of mutant *huntingtin* and the *A30P* and *A53T* mutants of α-synuclein and protect cells against various environmental stresses through autophagy induction in an mTOR-independent pathway (Sarkar *et al.*, 2007*a*). Furthermore, the combination of trehalose and mTOR inhibition by rapamycin has been shown to exert an additive effect on the clearance of these aggregate-prone proteins because of increased autophagic activity (Sarkar *et al.*, 2007*a*).

Deleterious effects of autophagy

The discovery of the molecular basis of autophagy has uncovered its importance during development, life extension and in pathological conditions, such as cancer, certain myopathies and neurodegenerative diseases. Besides, autophagy may play an important cytoprotective role by facilitating removal of protein aggregates before they become toxic (Kuma *et al.*, 2004; Baehrecke, 2005). In addition, autophagy can function in cell death by execution of self-killing programme of irreversibly injured cells (Bursch, 2001; Dickson, 2007). In some extreme instances of programmed cell death, cells can be completely degraded through autophagic digestion. Accumulating evidence demonstrates that late-stage neuronal cell loss generally occurs via autophagy and it has been suggested that over activation of autophagy in neurons is the eventual cause of 'physiological' death (Takacs-Vellai *et al.*, 2006).

Autophagy has been linked to disease processes by various morphological studies (Klionsky and Emr, 2000). Thus, whether autophagy protects against disease or causes it may depend on the specific situation and stage in the pathological process (Rubinsztein *et al.*, 2005). Although many issues remain unclear, there is emerging evidence that abnormal regulation of autophagic pathways may lead to apoptosis and cell death (Degterev *et al.*, 2005; Chu, 2006; Klionsky, 2006).

From a therapeutic viewpoint, it is not certain that prolonged upregulation of autophagy would be without risks because autophagy enhancement may cause a lower steady-state level of mitochondrial load and a decrease in oxidative phosphorylation (Ravikumar *et al.*, 2006; Rubinsztein, 2006). However, the finding that the activities of some respiratory complexes can be reduced by 25–80% before respiration or ATP synthesis in brain mitochondria are affected (Murphy, 2001) indicates that upregulation of autophagy will not result in eventual total depletion of mitochondria. It is, therefore, possible to induce autophagy and reduce mitochondrial load to the levels that have substantial protective effects against proteinopathies but do not adversely affect cellular respiration. Hence, the ability to maintain proper autophagic activity, rather than massive upregulation of autophagy, should be the therapeutic goal in diseases associated with protein aggregation.

Conclusion

Knowledge of the ALP has advanced rapidly in the last few years and its dysfunction has emerged as a theme in neurodegenerative disorders. It is believed that activating autophagy, which may have beneficial effect on the clearance of misfolded and aggregated proteins and prevention of neurodegeneration, may become a new therapeutic target in neurodegenerative disorders, such as Parkinson's disease. However, due to the dual role of autophagy in cell survival and death, it is imperative to keep in mind that the regulation of autophagy is a very delicate process. Inappropriate or prolonged activation of autophagy may lead to the complete demise of the cells involved. Thus, to determine when and for how long this activation should be maintained will be another major challenge in the future development of the therapeutic strategy.

Acknowledgements

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