

# Targeting autophagy in ALS

## A complex mission

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Several neurodegenerative diseases share a common neuropathology, primarily featuring the presence of abnormal protein inclusions in the brain containing specific misfolded proteins. Strategies to decrease the load of protein aggregates and oligomers are considered relevant targets for therapeutic intervention. Many studies indicate that macroautophagy is a selective and efficient mechanism for the degradation of misfolded mutant proteins related to neurodegeneration, without affecting the levels of the corresponding wild-type form. In fact, targeting autophagy with rapamycin treatment decreases the accumulation of protein aggregates and alleviates disease features in animal models of Huntington disease and other disorders affecting the nervous system. Recent evidence, however, indicates that the expression of several disease-related genes may actually impair autophagy activity at different levels, including omegasome formation, substrate recognition, lysosomal acidity and autophagosome membrane nucleation. A recent report from Zhang and co-workers indicates that treatment of an amyotrophic lateral sclerosis (ALS) mouse model with rapamycin actually exacerbates neuronal loss and disease progression, associated with enhanced apoptosis. This study reflects the need for a better understanding of the contribution of autophagy to ALS and other neurodegenerative diseases since this pathway may not only operate as a cleaning-up mechanism, but its impairment may be part of the pathological mechanisms underlying the

disease, whereas augmenting autophagy levels above a certain threshold could lead to detrimental effects in neuronal function and survival. Combinatorial strategies to repair the autophagy deficit and also enhance the activation of the pathway may result in a beneficial impact to decrease the content of protein aggregates and damaged organelles, improving neuronal function and survival.

The presence of abnormal protein aggregates or inclusions containing specific misfolded proteins is a common feature of most neurodegenerative disorders. These diseases include ALS, Alzheimer disease (AD), Parkinson disease (PD), Huntington disease (HD) and many others. Recent therapeutic strategies are focused in diminishing the load of aggregate-prone proteins through gene silencing (decrease synthesis) or by enhancing their clearance through degradation. The macroautophagy pathway (hereafter referred to as autophagy) is the main degradation route for long-lived proteins, damaged organelles and large protein aggregates. Although autophagy was initially thought to operate only as a central mechanism for survival under conditions of nutrient starvation, studies using brain-specific knockout mice for essential autophagy regulators (i.e., *atg5* and *atg7*) demonstrate a key role of autophagy in maintaining and balancing protein homeostasis (here referred to as *proteostasis*) in neurons at basal levels.<sup>1,2</sup>

Remarkably, a large body of in vitro evidence indicates that most aggregate-prone mutant proteins linked to neurodegeneration, and not the corresponding wild-type

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proteins, are efficient targets for autophagy-mediated degradation, including amyloid precursor protein (APP), huntingtin,  $\alpha$ -synuclein, the prion protein, ataxin-3, mutant superoxide dismutase-1 (SOD1) and many others.<sup>3</sup> Most of these mutant proteins, in addition to being recognized as specific substrates for autophagy, augment the amplitude of the autophagy flux. This suggests that neurons actively respond to the accumulation of protein aggregates by enhancing autophagy possibly as a detoxification mechanism. All these observations indicate that experimental strategies to enhance autophagy-mediated clearance may have an actual therapeutic impact to alleviate the central cause of neurodegeneration: accumulation of toxic protein aggregates. Indeed, different studies in HD animal models suggest that pharmacological strategies to enhance autophagy may decrease the accumulation of abnormal huntingtin aggregation, resulting in reduced neuronal loss, and improved motor performance.<sup>4,5</sup> Similarly, manipulating autophagy levels has a direct impact in disease features triggered by amyloid beta,<sup>6</sup>  $\alpha$ -synuclein<sup>7</sup> or mutant SOD1 expression.<sup>8</sup>

Although signs of autophagy activation have been reported in most cellular models of neurodegeneration, assessing autophagy activity in vivo (i.e., LC3 flux) is limiting due to technical difficulties. Recent reports, however, have changed our simplistic view for the participation of autophagy in neurodegenerative diseases. Accumulating examples actually suggest that defects in autophagy flux or in specific autophagy-regulatory processes rather than induction, may contribute to neurodegeneration in diverse diseases. Alterations in autophagy activity could then result in the accumulation of autophagosomes and inefficient cargo clearance, leading to the

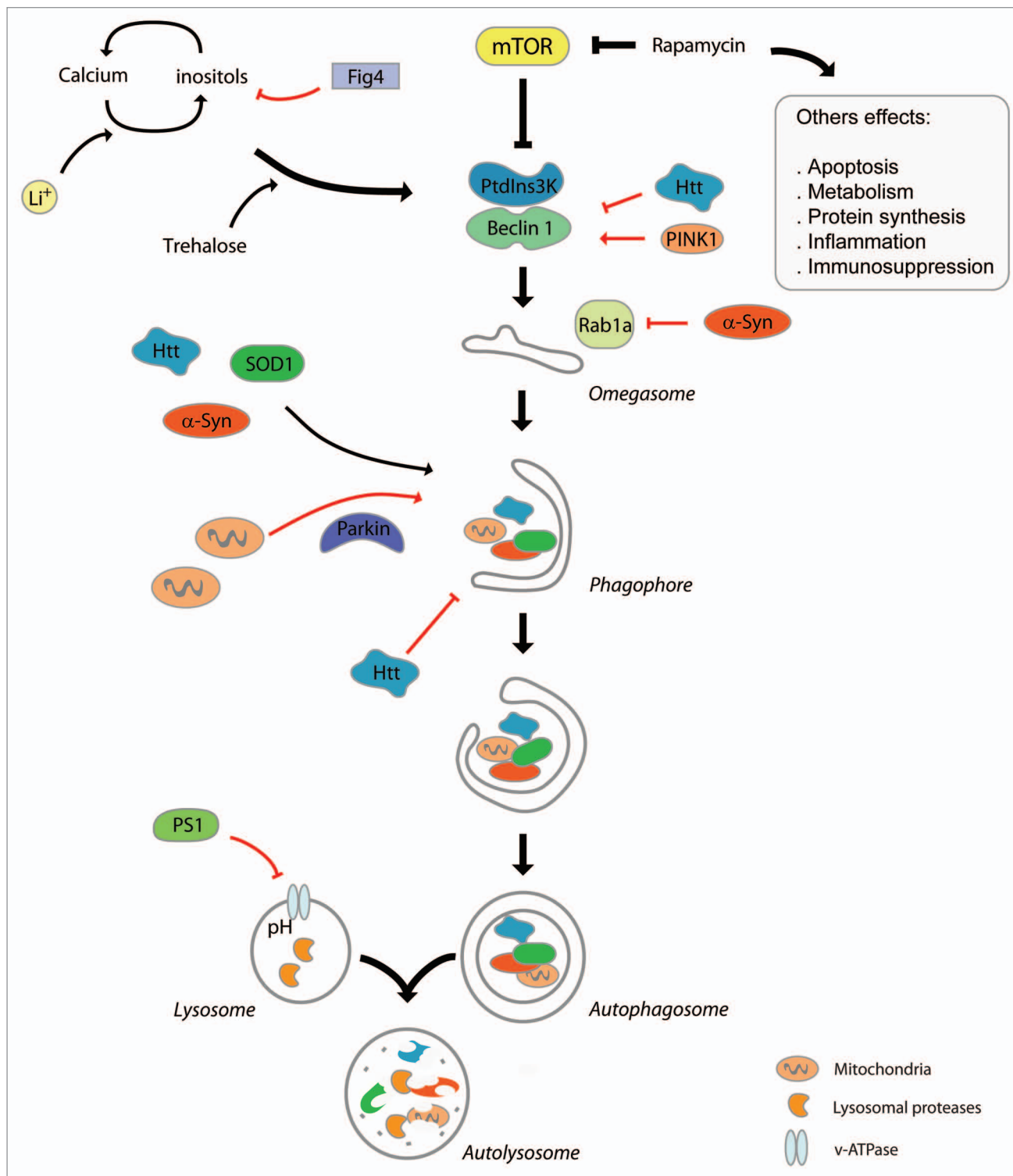
accumulation of damaged organelles and proteins ("cellular trash"), having a negative impact on general *proteostasis* (Fig. 1). For example, studies in HD models and human HD-derived tissue reveal an impairment of cargo recognition by autophagic vacuoles.<sup>9</sup> Autophagic vacuoles form at enhanced rates in HD-derived cells, but they fail to efficiently engulf cytosolic cargo in their lumen.<sup>9</sup> In addition, studies in AD models suggest that presenilin-1 (PS1) expression may directly control the acidification of lysosomes through targeting the v-ATPase to these vesicle compartments.<sup>10</sup> Then, AD-linked PS1 mutations lead to defective lysosomal proteolysis, possibly enhancing the accumulation of abnormal proteins resulting in neuronal loss.<sup>10</sup> Moreover, several studies report a physical interaction between disease-mutant proteins and autophagy regulators. Mutant huntingtin aggregates sequester the key autophagy regulator Beclin 1, which may decrease autophagy activity at the level of autophagosome membrane nucleation.<sup>11</sup> Recent data indicate that mitochondrial Parkin promotes the autophagic degradation of dysfunctional mitochondria, a phenomenon that may contribute to PD upon Parkin mutation.<sup>12</sup> In addition, Pink1, a gene product in which mutations are causative of autosomal recessive PD, interacts with Beclin 1 and modulates its pro-autophagy function.<sup>13</sup>  $\alpha$ -Synuclein expression also compromises autophagy by inhibition of Rab1a, which may alter the formation of the omegasome.<sup>14</sup> Finally, Beclin 1 protein levels are decreased in post-mortem brain samples derived from patients affected with AD, mild cognitive impairment and HD.<sup>6,11</sup> All together, these reports open a paradox where defining the impact of artificially-enhancing autophagy in the context of neurodegenerative diseases is

very complex, and the therapeutic outcomes are difficult to predict. Disease-related aggregate-prone proteins could be recognized as autophagy substrates and induce the pathway, and at same time they could preclude the autophagy activity by interacting with components of the pathway. This paradox highlights the need to establish the possible existence of a threshold level for autophagy manipulation for therapeutic gain in a disease context.

One of the main targets explored for therapeutic intervention is the mTOR (mammalian target of rapamycin) pathway. mTOR kinase normally represses autophagy by regulation of Atg1 and Atg13, and the inhibition of a downstream class III phosphatidylinositol 3-kinase (PtdIns3K) complex containing Beclin 1. Rapamycin treatment has protective effects in several mouse models of neurodegeneration, including HD,<sup>4,15</sup> PD<sup>16</sup> and AD.<sup>17</sup> In addition, rapamycin delays aging, augmenting the life span of mice.<sup>18</sup> The positive effects of rapamycin administration observed in all those disease models have been indirectly attributed at least in part to its pro-autophagic effects. However, the mTOR pathway operates as a crucial rheostat for controlling mRNA translation, cell growth, metabolism and inflammation, among other processes.<sup>19</sup>

ALS is the most common adult-onset motoneuron neurodegenerative disease, characterized by highly selective degeneration of upper and lower motor neurons resulting in paralysis and premature death.<sup>20</sup> Most ALS cases are sporadic, lacking a clear hereditary component, but mutations in SOD1 are associated with a fraction of hereditary ALS cases. In addition, post-translational modifications of wild-type SOD1 lead to its misfolding and aggregation, possibly contributing to sporadic ALS.<sup>21</sup> Many studies in cellular

**Figure 1 (See opposite page).** The impact of autophagy in neurodegeneration. The canonical autophagy pathway is activated by nutrient starvation upon derepression of mammalian target of rapamycin (mTOR). In addition to inducing autophagy, rapamycin treatment also triggers other effects including regulation of apoptosis, metabolism, protein synthesis and immunological responses. An alternative mTOR-independent pathway is initiated by a decrease in global inositol phosphate (IP3) levels, which is modulated by inositol monophosphatases and calcium levels, and could be induced by lithium and trehalose treatments. Autophagosome formation begins with the nucleation of a Beclin 1 and class III PtdIns 3-kinase protein complex to form the growing phagophore membrane. Autophagosome membranes could originate from the endoplasmic reticulum through the omegasome structure. Many mutant misfolded proteins related to neurodegeneration are degraded by autophagy including huntingtin (Htt),  $\alpha$ -synuclein ( $\alpha$ -Syn) and mutant SOD1. The degradation of these proteins by autophagy is possibly related to their propensity to aggregate. In addition, the expression of disease-related genes directly alters the autophagy pathway. For example, Htt and Pink1 interact with Beclin 1, modifying its pro-autophagy function.  $\alpha$ -Syn inhibits Rab1a, which may alter the formation of the omegasome. Parkin is essential to mitophagy. Htt also impairs cargo recognition by autophagic vacuoles. Mutant presenilin-1 (PS1) alters the transport of v-ATPase from the secretory pathway to the lysosome, affecting lysosomal pH. In ALS, genetic mutations in *FIG4* influence the autophagy pathway by altering the metabolism of phosphoinositides.



**Figure 1.** For figure legend, see page 2.

and animal models of ALS indicate an enhanced autophagy activity in the disease, in addition to the occurrence of autophagy-mediated clearance of mutant SOD1 and also TDP43, another ALS-linked protein (reviewed in ref. 20). In

addition, we have described genetic strategies that augment autophagy levels in the central nervous system correlated with increased SOD1 degradation and a significant delay in ALS progression.<sup>8</sup> However, no studies are available addressing the

direct role of autophagy in ALS in vivo. In this issue of Autophagy, Zhang and co-workers<sup>22</sup> established the possible impact of treating mutant SOD1 transgenic mice with rapamycin. Surprisingly, the authors observed an enhancement in disease

progression and decreased life span upon treating pre-symptomatic ALS mice with rapamycin. In addition, the authors corroborated the inactivation of mTOR signaling, the upregulation of LC3-II, p62 and Beclin 1 levels, in addition to the accumulation of autophagic vacuoles in the spinal cord of mutant SOD1 transgenic mice. Unexpectedly, the authors could not detect any alteration in the levels of mutant SOD1 upon rapamycin treatment, even though it is a known target of autophagy-mediated degradation.

Zhang and co-workers described increased mitochondrial impairment after rapamycin treatment, associated with increased Bax levels and enhanced caspase-3 activation, suggesting possible proapoptosis effects. This study opens the possibility that autophagy activity/flux may be actually altered in SOD1-mediated ALS, and then enhancing autophagy may poison the cells with the accumulation of autophagosomes. Alternatively, since a relevant component of ALS pathogenesis is neuroinflammation and glial activation,<sup>23</sup> it could be possible that the immunosuppressive effects of rapamycin may contribute to accelerate disease progression. Another possibility is that rapamycin treatment could alter other cellular or metabolic events related to neuroprotection in ALS. It is important to highlight the fact that mutations in two other genes linked to ALS, charged multivesicular body protein-2B (*CHMP2B*) and the lipid phosphatase Fig4, lead to drastic alterations in the autophagy pathway,<sup>20</sup> reinforcing the concept that autophagy impairment may contribute to ALS (Fig. 1).

More studies are required to directly manipulate autophagy levels in the context of ALS and then define the possible impact in disease progression and pathogenesis. In this context, the use of rapamycin analogs or molecules that induce mTOR-independent autophagy,<sup>3,20</sup> such as the disaccharide trehalose or combinations with lithium, among other small molecules, will be useful to solve this fundamental question. Genetic manipulation of Beclin 1 or related molecules could be used as tools to test this hypothesis

in ALS, as already demonstrated in AD and PD models.<sup>6,7</sup> However, there is still a necessity for developing new small molecules to target autophagy with higher specificity and lower side effects. In summary, the current status of the field highlights the need for a deeper understanding of the exact contribution of autophagy to neurodegeneration. Defining whether or not autophagy (i) operates as an active survival pathway triggered by the accumulation of protein aggregates, (ii) operates as a pathological mechanism due to its impairment or (iii) if autophagy actually has a “dual dynamic” role in the disease process is fundamental to move forward possible therapeutic intervention. Combinatorial strategies to repair the autophagy deficit and also enhance the activation of the pathway may result in a beneficial impact to decrease the content of protein aggregates and damaged organelles, improving neuronal function and survival.

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