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a b s t r a c t

Autophagy is a lysosome-dependant intracellular degradation process that eliminates long-lived proteins as well as damaged organelles from the cytoplasm. An increasing body of evidence suggests that dysregulation of this system plays a pivotal role in the etiology and/or progression of neurodegenerative diseases including motor neuron disorders. Herein, we review the latest findings that highlight the involvement of autophagy in the pathogenesis of amyotrophic lateral sclerosis (ALS) and the potential role of this pathway as a target of therapeutic purposes. Autophagy promotes the removal of toxic, cytoplasmic aggregate-prone pathogenetic proteins, enhances cell survival, and modulates inflammation. The existence of several drugs targeting this pathway can facilitate the translation of basic research to clinical trials for ALS and other motor neuron diseases.

Keywords:
Authophagy
Amyotrophic lateral sclerosis
Protein aggregation

Contents

1. Introduction

There is still no effective treatment for amyotrophic lateral sclerosis (ALS), a fatal, progressive neurodegenerative disease characterized by the selective loss of upper and lower motor neurons (MNs) (Bucchia et al., 2015). The degeneration of MNs clinically leads to progressive paralysis and early death due to respiratory failure. About 90% of the ALS cases are sporadic, while 10% are familial, inherited with an autosomal dominant/recessive, or X-linked pattern (Marangi and Traynor, 2015). Over 30 causative genes have been identified in familial ALS, however only 10% of the sporadic forms present an involvement of those genes (Chen et al., 2013; Renton et al., 2014; Marangi and Traynor, 2015).

* Corresponding author.
E-mail address: stefania.corti@unimi.it (S. Corti).
[1] These authors equally contributed to this work.

ALS pathology is still not completely understood. Many pathways have been suggested to play a role in the disease pathogenesis including alteration of protein stability, conformation and degradation, disturbances of RNA metabolism, and axonal transport defects (Bucchia et al., 2015). In particular, mutated genes that cause ALS, such as superoxide dismutase 1 (*SOD1*), TAR DNA-binding protein 43 (*TARDBP* encoding for TDP-43), and RNA-binding protein *FUS*, encode proteins that can form aggregates (Al-Chalabi et al., 2012). Indeed, the most frequent protein cell inclusions detected in the central nervous system (CNS) of apparently sporadic patients are TDP-43 positive (Neumann, 2013). Remarkably, these aggregates are detected both in the presence of mutations in *TARDBP* gene and in other ALS causative genes such as *C9ORF72*, *SQSTM1*, *GRN*, *VCP*, *UBQLN2*, *OPTN*, and *NIPA1* (Marangi and Traynor, 2015).

Mutant aberrant or misfolded proteins, such as TDP-43 and others, aggregates in the cytoplasm, nucleus or extracellular matrix damaging the cellular organelles and leading to neuronal dysfunction (Walker and LeVine, 2000). The increased amount of misfolded proteins not

only induces endoplasmic reticulum (ER) stress and reduces protein translation, but it also enhances autophagy. On the other side, impairment of autophagy in neurons and MNs results in the accumulation of aggregate-prone proteins and cell death.

The first evidence of a link between the dysregulation of autophagy and ALS came from the observation that autophagic markers are upregulated in post mortem CNS samples of both ALS animal models and patients, suggesting that autophagy impairment could play an important role in motor neuron disease pathogenesis (Morimoto et al., 2007; Song et al., 2012).

2. The autophagic processes in healthy and ALS context

The quality of cellular components and the maintenance of homeostasis are key points for the healthy survival of neurons in their functional context. Both the ubiquitin-proteasome system (UPS) and the autophagy pathway make essential contributions to the maintenance of this homeostasis, the former acting in the ubiquitin-mediated processing and degradation of short-lived intracellular proteins, while the latter targets long-lived proteins and damaged organelles from cytoplasm to lysosomes for their digestion (Nijholt et al., 2011). The UPS recognizes only ubiquitinated proteins for proteasomal degradation, while autophagy, whose steps encompass the sequestration, transport to lysosomes, degradation, and utilization of degradation products, seems to be a non-selective elimination system, mediated by a specific organelle called "autophagosome". Macroautophagy, microautophagy and chaperone-mediated autophagy are the three kinds of autophagic mechanisms currently described (Nikoletopoulou et al., 2015). In chaperone-mediated autophagy, the Hsp70 protein holds a central role in selection and the presentation of ubiquitinated proteins to specific receptors on the lysosome, such as p62/*SQSTM1*, while in microautophagy there is a direct internalization of cellular proteins by lysosomes. Macroautophagy (Fig. 1) begins with the fusion of a double membrane (phagophore) around cellular compounds that have to be discarded, forming a so-called autophagosome when the membrane fusion is completed. Thereafter, these structures are transported along microtubules towards the perinuclear region of the cell to fuse with lysosomes, where their content is degraded.

The essential steps of autophagy involve several autophagy-related (ATG) proteins, whose coding genes are highly conserved across eukaryotes.

The pathway modulating autophagy relies on the mammalian target of rapamycin complex 1 (mTOR), which inhibits autophagy by phosphorylating proteins such as ULK1, ATG13 and FIP200 that act upstream in phagophore formation.

The elongation of autophagosome is obtained by two conjugation steps involving the ATG12–ATG5–ATG16L complex and the phosphatidylethanolamine (PE)–light chain 3 (LC3) system. Microtubule-associated protein 1 LC3 is processed by ATG4 to produce cytosolic LC3-I, which is conjugated by ATG3 and ATG7 to PE to generate membrane-associated LC3-II on autophagosome precursors.

Ultimately, mature autophagosomes fuse with lysosomes, where hydrolyzing enzymes catalyze the digestion of cellular components. After the degradation is completed, membranes assembled in autophagic vesicles are returned to cellular membrane and recycled (Rubinsztein et al., 2015; Nikoletopoulou et al., 2015).

Neurons and other long-lived cells rely on the correct activity of both ubiquitin and autophagy degradation systems. Knockout mice for several ATG genes showed the accumulation of aggregates in the cytoplasm and led to neuropathological phenotypes (Hara et al., 2006; Komatsu et al., 2006).

The survival of MNs turns out to be particularly affected by autophagy disruption, as recently demonstrated by the experimental data (Ferrucci et al., 2011). Cytoplasmic inclusions containing LC3-II and p62/SQSTM1, two typical markers of autophagy, were detected in spinal MNs of ALS patients suggesting both activation and alteration of autophagy in the pathogenesis of sporadic ALS (Sasaki, 2011). In physiologic macroautophagy, the delivery of autophagosome to lysosomes,

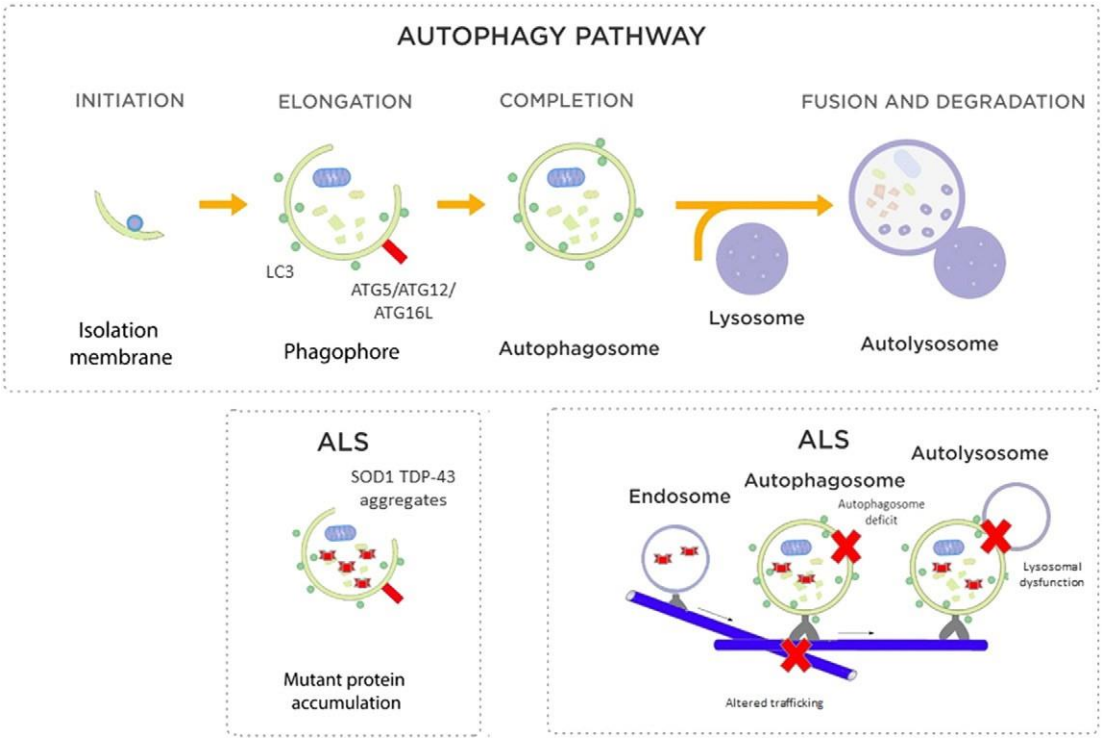

Fig. 1. Schematic view of functional autophagy in neurons (upper panel) and impairment of the process at different stages in ALS pathogenesis (lower panels). Accumulation of aggregates of proteins involved in familial ALS (SOD1 and TDP-43) within autophagic vesicles is marked in red. Defects of dynein/dynactin complex altering the trafficking of autophagosomes, as well as impaired activation of autophagic flux and lysosome function are highlighted (red crosses) as the main processes responsible for autophagy dysfunction in ALS.

in order to form an autolysosome, depends on microtubules in a dynein/dynactin-dependent manner (Mizushima, 2007). In the vacuolar membranes of ALS tissues an abnormal accumulation of lysosome-associated membrane proteins (LAMP-1 and LAMP-2) and LC3-I, which are the major protein markers of autophagosome, was observed, suggesting that immature autophagosomes could be the result of an alteration in the final fusion steps between autophagosomes and lysosomes (Tresse et al., 2010). Impairment of the retrograde axonal transport in SOD1 G93A mice may be responsible for the accumulation of autophagosomes as a result of a defect in the autophagosome–lysosome fusion, which occurs mainly in the soma (Nixon, 2007).

Despite several lines of evidence highlighting the role of autophagy in ALS pathogenesis (Chen et al., 2011), knocking-out motor neuron autophagy proved to be insufficient to cause ALS in mice, whereas the inhibition of the ubiquitin–proteasome system reproduced some cytopathological phenotypes of ALS through the disruption of 26S proteasome subunit (Tashiro et al., 2012).

Thus, it remains to be better clarified whether autophagy plays a pathogenetic or bystander role in ALS.

3. Accumulation of ALS pathogenetic proteins and autophagy

In ALS, the accumulation of aggregated misfolded proteins, such as TDP-43 and SOD1, leads to ubiquitin-containing inclusions in cell bodies and axons of MNs (Neumann et al., 2006; Shi et al., 2010). Aggregates in turn might cause UPS dysfunction (Crippa et al., 2010a) and compensatory induction of autophagy (Korolchuk et al., 2010). In spinal MNs from animal models of ALS (Li et al., 2008) and in post-mortem samples of ALS cases (Sasaki, 2011) the alteration of UPS and activation of autophagy have been observed (Li et al., 2008; Sasaki, 2011). Without cell division, neurons cannot reduce toxic products by dilution, thus resulting more sensitive to the accumulation of cytotoxic proteins. ER stress, which can progressively lead to neurodegeneration, is also caused by the aggregation of unfolded proteins. A possible protective factor with respect to this process is the upregulation of KDEL (Lys-Asp-Glu-Leu motif) receptor (KDELR), as it is involved in ER stress and protein quality control (Song et al., 2012).

The relationship between TDP-43 aggregates and autophagy was investigated by several groups. Aggregates containing TDP-43 can be induced by the inhibition of autophagy with 3-methyladenine (3-MA) (Wang et al., 2010) and by impairment of endosomal sorting complex required for transport (ESCRT) (Filimonenko et al., 2007). Conversely, the inhibition of mTOR using rapamycin results in the induction of autophagy, relocalization of TDP-43 to its proper nuclear compartment and reduction of its accumulation, and rescue of mRNA processing (Wang et al., 2013). In addition, enhancing autophagic flux through an mTOR-independent pathway with trehalose inhibits TDP-43 aggregate formation (Gomes et al., 2010). Moreover, autophagy-activating molecules increase TDP-43 clearance and improve neuronal survival in ALS models (Barmada et al., 2014).

The positive effect of upregulating autophagy in ALS has been pointed out in SOD1 models (Hetz et al., 2009; Crippa et al., 2010a). Small heat shock protein HspB8 decreases the aggregation and promotes the clearance of mutant SOD1 in SOD1 G93A mice, with no impact on the turnover of the wild-type protein (Crippa et al., 2010a). The enhancement of macroautophagy, observed in X-box-binding protein-1 (XBP-1) knockout mice, where the pathway known as unfolded protein response is impaired, led to an increased clearance of mutant SOD1 aggregates in spinal cord (Hetz et al., 2009). Similarly, autophagy-linked FYVE protein may enhance the autophagic removal of misfolded SOD1 aggregates in SOD1 G93A mice (Han et al., 2015).

Though SOD1 is primarily a cytosolic protein, its partial deposition in the mitochondrial intermembrane space was shown to lead to mitochondrial dysfunction in mutant SOD1 transgenic mice (Magrane et al., 2009). The presence of damaged mitochondria is a pathological common finding in ALS MNs that may be associated to impaired autophagy-lysosomal system. Supporting this hypothesis, autophagic vacuoles engulfing damaged mitochondria have been observed along motor neuron axons in SOD1 G93A mice (Xie et al., 2015). Interestingly, the accumulation of vacuoles was linked to an altered dynein-driven retrograde transport of late endosomes, and was rescued by the overexpression of snapin which competes with SOD1 in binding dynein. Remarkably, this leads to MN survival and amelioration of the ALS disease phenotype in SOD1 G93A mice (Xie et al., 2015).

Beclin1, a key protein in autophagy regulation, has been observed to interact with SOD1 and to promote neurodegeneration in SOD1-mutated cells and animal models, as confirmed by an increased life span of Beclin1 haploinsufficient/SOD1 transgenic animals, that was accompanied by the up-regulation of p62/SQSTM1 and down-regulation of LC3-II (Nassif et al., 2014).

The dysregulation of autophagy in ALS patients is not limited to TDP-43 and SOD1 pathology.

Impairment of UBQLIN2 is associated to autophagic defects with even more evidence. *UBQLIN2* encodes a protein that promotes a correct identification of ubiquitinated misfolded protein by p62/SQSTM1. Mutations in *UBQLIN2* lead to neurodegeneration altering the autophagic process (Deng et al., 2011; Gellera et al., 2013).

Valosin-containing protein (VCP), an ATP-driven chaperone protein involved in autosomal dominant form of ALS (Johnson et al., 2011) promotes the maturation of ubiquitinated autophagosomes and is likely implicated in autophagy and in mitochondrial quality control (Yamanaka et al., 2012). VCP binds to ubiquitinated protein aggregates and has an important role in the removal of stress granules, dense cytoplasmic protein/RNA aggregates that are usually found in ALS pathologic tissues (Buchan et al., 2013). An observed consequence of mutated VCP is the accumulation of autophagosomes caused by the impairment of autophagosome/lysosome fusion (Ju et al., 2009), resulting in vacuole formation in the muscle tissues of patients with VCP-associated disease (Ju and Weihl, 2010; Weihl, 2006). Alteration of autophagy in VCP-related disease has been recently confirmed in neurons differentiated from patient-specific induced pluripotent stem cells (iPSCs) (Dec et al., 2014). Increased levels of TDP-43, ubiquitin, LC3 and p62/SQSTM1 have been observed in cells from patients suggesting a link between mitochondrial dysfunction and autophagosome formation (Nalbandian et al., 2015).

At present, the most common mutation in ALS involves an intronic hexanucleotide repeat expansion in *C9ORF72* gene (Ratti et al., 2012; Renton et al., 2014). Although its pathogenetic mechanism is still unclear, it is remarkable that the protein encoded by *C9ORF72* localizes with autophagosomes and its function is related to endocytic trafficking (Farg et al., 2014). However, the up-regulation of LC3-II, mediated by siRNA knockdown of *C9ORF72*, did not support the role of autophagy defect as the key pathogenic event in *C9ORF72*-associated pathology (Farg et al., 2014).

Sequestosome 1 (*SQSTM1*) may represent another potential link between autophagy and ALS, as deletions and point mutations of this gene, encoding the ubiquitin-binding protein p62/SQSTM1, have been detected in ALS (Hirano et al., 2013; Teyssou et al., 2013). p62/SQSTM1 is a scaffold that acts as a mediator between ubiquitinated proteins and LC3-II in order to facilitate their removal via the autophagy (Matsumoto et al., 2011). Several neurodegenerative diseases present aggregates containing p62/SQSTM1 (Laurin et al., 2002). The different effects on autophagy exerted by p62/SQSTM1 mutations, which are located across multiple domains of the protein, need further investigation (Teyssou et al., 2013).

Optineurin (OPTN1) is an autophagy adaptor, whose mutations are linked to ALS (Maruyama et al., 2010; Del Bo et al., 2011). In addition to its roles of degradating foreign pathogens (xenophagy) (Wild et al., 2011) and binding to protein aggregates (Korac et al., 2013), OPTN1 is found in neuronal inclusions of patients affected by ALS and other neurodegenerative diseases (Schwab et al., 2012; Mori et al., 2012). The role of OPTN1 in ALS has been further elucidated by the recent discovery of

loss of function mutations in *TBK1* gene in familial ALS cases (Cirulli et al., 2015). The interaction between TBK1 and OPTN1 is a key factor in autophagy and inflammation (Maruyama et al., 2010; Maruyama and Kawakami, 2013; Thomas et al., 2013; Kachaner et al., 2012). TBK1 enhances the autophagic turnover of bacteria-bound ubiquitylated proteins (Wild et al., 2011; Gleason et al., 2011), through the phosphorylation of OPTN and SQSTM1 (Morton et al., 2008; Pilli et al., 2012) and promoting the interaction of OPTN with LC3. TBK1 co-localization with OPTN and SQSTM1 within autophagosomes, even in cells carrying *SOD1*, *TARDBP* and *FUS* mutation, suggests a still undisclosed role of these proteins in aggregate formation in ALS (Keller et al., 2012). A cargo-specific subtype of autophagy seems to be activated by aggregates, thus degradating ubiquitinated proteins through the lysosome (Scotter et al., 2014). The SQSTM1 and OPTN deliver ubiquitinated proteins to the autophagosome thanks to their function as cargo receptors and LC3-interaction region motifs. Additionally, the autophagic turnover of damaged mitochondria, involving PARKIN/ubiquitin ligase pathway, is prone to OPTN activity (Wong and Holzbaur, 2014).

Overall, OPTN, SQSTM1, VCP and TBK1 play a critical role in pathological inclusions and degradation mechanisms. An interesting aspect is the selective motor neuron death, caused by alteration of this pathway, which spares other cell types. Mutations in *OPTN*, *SQSTM1*, or *TBK1* could be responsible for a deficit in cellular riboproteostasis, due to the impairment of autophagic mechanisms (Ramaswami et al., 2013).

Dynactin 1 (*DCTN1*), another ALS-causing gene (Puls et al., 2003), mediates the transfer of autophagosome within the cell to facilitate fusion with lysosomes (Jahreiss et al., 2008; Kimura et al., 2008). In Perry syndrome, a complex neurodegenerative disease manifesting mainly with parkinsonism and caused by specific *DCTN1* mutations, autophagic impairment is suggested by neuronal aggregates containing p62 in autoptic specimens (Farrer et al., 2009).

Additionally, ALS2/alsin, a guanine nucleotide exchange factor for the small GTPase Rab5 mutated in juvenile forms of ALS, is involved in endosome fusion. ALS2 loss has recently been demonstrated to aggravate SOD1 H46R-mediated toxicity by affecting endosome-autophagosome trafficking (Hadano et al., 2010).

Overall, a key point to understand the pathogenesis of motor neuron disease relies on interactions between ALS mutated genes/proteins and perturbation of autophagy. The pathogenetic context within such interactions that occur is emerging as a pivotal process in ALS and deserves further investigation.

4. Autophagy as therapeutic target

Based on the above findings, it has been hypothesized that activation of autophagy may serve as a therapeutic target for ALS. Despite the growing evidence that supports this putative mechanism, the enhancement of autophagy proved to be insufficient to rescue the phenotype in several studies (Fornai et al., 2008a,b; Zhang et al., 2014), or, in some cases, to lead to the worsening of the pathology (Zhang et al., 2011). Autophagy should be more deeply studied, since its tight regulation may play an important role in the protection of neurons against neurodegeneration in ALS.

Elimination of damaged mitochondria and aggregated proteins via the autophagy was able to slow ALS progression (Crippa et al., 2010b; Fornai et al., 2008a; Hetz et al., 2009; Xie et al., 2015). On the other hand, a reduction of neuronal loss, even though without a complete rescue of the pathology, can be obtained with the autophagy inhibitor 3-MA or knocking-down ATG5 or ATG12 (Wong et al., 2011). This would suggest that at least in some cases an excessive activation of the autophagy flux might be mainly responsible for cell death (Cherra and Chu, 2008; Cheung and Ip, 2009; Scarlatti et al., 2009).

A number of potential therapeutic compounds promoting autophagy via the mTOR-dependent and independent fashion have been identified to promote the clearance of ALS protein aggregates in MNs. Table 1 briefly summarizes autophagy modulation-based therapeutic strategies that have been investigated in ALS models.

Rapamycin, an mTOR dependent inducer of autophagy, has been shown to up-regulate autophagy, reducing aberrant protein aggregation in ALS (Sarkar et al., 2008; Harrison et al., 2009; Malagelada et al., 2010; Dehay et al., 2010; Caccamo et al., 2011; Hetz et al., 2009). However, rapamycin was demonstrated to worsen motor neuron loss and to reduce the survival of SOD1 G93A mice (Zhang et al., 2011) when administrated in advanced stages of the disease, while at early stages it may exert positive effects (Zhang et al., 2013). Food restriction, another autophagy promoter, demonstrated a potential protective role on SOD1 G93A mice spinal MNs (Staats et al., 2013).

Considering the mTOR-independent pathway as a possible target to modulate autophagy, lithium has been shown to influence ALS progression in SOD1 G93A mice through inhibition of inositol monophosphatase and the phosphoinositol cycle. However, these putative beneficial effects are still controversial, as demonstrated by several published clinical trials (Fornai et al., 2008a,b; UKMND-LiCALS Study Group et al., 2013). The compound L-690,330, carbamazepine and valproic acid may decrease inositol and inositol triphosphate levels (Sarkar, 2013), thus showing neuroprotective effects in neurodegenerative models (Sarkar, 2005; Fornai et al., 2008a; Feng et al., 2008; Forlenza et al., 2012). Trehalose and resveratrol have shown an important role in reducing protein aggregation and fostering neuronal survival (Gomes et al., 2010; Kim et al., 2007).

Trehalose, a disaccharide acting as a chemical chaperone, can promote the clearance of several aggregation-prone proteins linked to neurodegeneration such as α-synuclein, huntingtin and Tau, though its mechanism has yet to be fully understood. In a recent study, trehalose

Table 1
Autophagy-promoting molecules tested for ALS.

Autophagy target pathway	Molecule	Trial phase	Findings	References
TORC1 inhibition	Rapamycin	Preclinical stage	In immunodeficient ALS mice rapamycin, an MTOR-dependent autophagic activator, prolongs survival because it increases autophagy without exerting immunosuppression. The goal should be the development of molecules that selectively target autophagy without effects on immune response.	Staats et al. (2013)
AMPK activation	Lithium	Preclinical/clinical stage	Lithium demonstrates neuroprotective effects in mouse models, influencing disease onset and duration and prolonging survival. Lithium delays motor neurons death and gliosis, promotes the replacement of damaged mitochondria and the removal of alpha-synuclein, ubiquitin, and SOD1 aggregates. In humans the disease progression in lithium-treated patients was slower than in riluzole-treated ones.	Fornai et al. (2008a,b),
			This double-blind, randomized controlled trial of lithium versus placebo in ALS showed no significant results concerning effects of lithium on survival at 18 months.	UKMND-LiCALS Study Group et al. (2013)
AMPK activation	Trehalose	Preclinical stage	In mouse models trehalose, MTOR-independent autophagic inducer, demonstrates neuroprotective properties modifying disease onset and duration. Trehalose protects motor neuron survival, decreases aberrant protein aggregation and modulates autophagic pathways. It shows protective effects on the mitochondria, skeletal muscle and cell apoptosis.	Zhang et al. (2014)

was demonstrated to reduce SOD1 and p62/SQSTM1 aggregation, as well as ubiquitinated protein accumulation, thereby inhibiting the pro-apoptotic pathway in SOD1 G93A mice (Zhang et al., 2014).

Furthermore, resveratrol and curcumin exhibit a neuroprotective activity in models of neurodegenerative disorders, working as indirect mTOR-dependent activators of autophagy (Jeong et al., 2012; Jiang et al., 2013; Wu et al., 2011).

Arimoclomol, an inducer of heat shock proteins, increased SOD1 G93A mice lifespan, delayed disease onset, improved the neuromuscular function and increased motor neuron survival, even if administered after the symptom onset (Kieran et al., 2004; Kalmar et al., 2008). The reduction of ubiquitin-positive aggregates in MNs suggests that arimoclomol presents an anti-aggregation activity in this ALS model (Kalmar et al., 2008).

Another aspect that has to be evaluated is that in progressive and complex neurodegenerative disorders such as ALS, cell death is not limited to neurons, but extends also to non-neuronal cells that interact with neurons. The modulation of autophagy and stress responses in astrocytes and other non-neuronal populations may be an effective, complementary approach in the development of a disease-modifying therapy for ALS that deserves further investigation (Finsterwald et al., 2015).

In order to maintain neuronal and motor neuronal integrity, an equilibrium between the formation and degradation of autophagic vacuoles should be maintained. This balance is typically lost in the pathologic context, manifesting in the overproduction or accumulation of autophagic vesicles. In this regard, possible therapeutic approaches may induce the up-regulation of proteins related to autophagosome maturation, such as those involved in endosomal fusion and multivesicular body formation, counteracting their physiological loss due to aging. Sasaki (2011) hypothesized that in the early phase of motor neuron disease, the autophagosome–lysosome fusion or the degradation ability of lysosomes may display a normal function, justifying an early therapeutic intervention through moderate activation of autophagy aimed to reduce protein aggregation in MNs. Conversely, at advanced disease stages, the disruption of the autophagic flux is responsible for the increase of autophagosome production, with the release of lysosomal enzymes that further induce cell death and accelerate the pathological process (Massey et al., 2006). The mechanisms involved in the switch in autophagy between early and late ALS phases are not fully understood and need further investigation. From this perspective, it is important to determine the role of aging in the clearance of autophagosomes, since ALS typically occurs in late adulthood.

Given these premises, at early ALS phases, therapeutic enhancement of autophagy may aid in re-establishing this autophagic balance. On the contrary, augmenting the autophagic process at later disease phases, when lysosomal function is less efficient, may be less beneficial, due to an increase in autophagosome synthesis that overwhelms the clearance capacity of lysosomes (Bové et al., 2011).

5. Conclusions

An increasing body of evidence points to a central role of autophagy in motor neuronal function and pathology. Intracellular autophagy is the major process for the degradation of abnormal protein aggregates, which are hallmarks of familial and sporadic ALS. Impairment in autophagy is present in degenerating MNs, although the direct link between autophagy disruption and cell loss remains to be fully undisclosed. Nevertheless, experimental data suggest the enhancement of autophagy as a possible therapeutic strategy for ALS. At this early stage of research, testing known compounds that act on the autophagy (Table 1) is increasing the knowledge on the possible therapeutic effect to target this pathway in ALS. Several autophagic inductors such as rapamycin, lithium, and trehalose are expected to exert a positive effect on ALS, however the findings of possible negative effects in experimental models suggest caution in clinical translation.

A known critical issue in this field of research is the transition from in vitro and in vivo models to humans. Currently available ALS models do not entirely recapitulate the human pathology, thus raising the question whether screening therapeutic compounds in such models may prove beneficial in humans. In this regard, the advent of iPSC-based models may represent a valuable approach for future research (Faravelli et al., 2014).

Further studies to unravel the impact of autophagy in ALS pathogenesis and to elucidate the role of the interaction between proteins involved in ALS and the autophagic pathway will accelerate the discovery and development of effective therapies for motor neuron diseases.

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