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Autophagy in Neurodegeneration

Latest developments on eating ourselves out of disease

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Resumo

As doenças neurodegenerativas constituem situações especialmente desgastantes para doentes e cuidadores e as suas formas esporádicas crescem em prevalência a par do envelhecimento da população. A autofagia é um processo celular de reconhecida importância tanto no processo etiológico como no tratamento desta patologia. Nesta revisão são compilados os resultados mais recentes sobre a modulação da autofagia nos processos neurodegenerativos. Observou-se que a autofagia está desregulada em células nervosas de doentes e em modelos celulares e animais de degeneração neuronal, sendo que foi evidente que a estimulação farmacológica da mesma melhorou o desempenho físico e cognitivo de modelos animais para estas doenças. Contudo, alguns estudos evidenciaram um processo mais complexo em que o fluxo autofágico aumentado ou desregulado pode funcionar como agente patológico. A descoberta da maquinaria autofágica tem-se revelado um dos avanços mais importantes da medicina moderna e portanto a modulação da autofagia no alívio da neurodegeneração é uma área central na investigação presente e futura e no desenvolvimento de futuras estratégias terapêuticas.

Abstract

Neurodegenerative diseases are devastating conditions for both patients and caregivers and their sporadic forms are increasingly common with population aging. Autophagy has conquered a relevant role in the study of pathological mechanisms associated with neurodegenerative disorders and their management. In this review, the latest results on the study of autophagic modulation in neurodegenerative processes are compiled and put in a broader context. Autophagy was shown to be impaired in both human neuronal tissues of affected patients and cellular and animal models of disease as it was evident that pharmacological enhancement of autophagy alleviates neurodegeneration in animal models of such diseases. A few studies however evidenced a more complex network where overactive or deregulated autophagic flux might be harmful. The disclosure of the autophagic machinery has proven to be one of the most important breakthroughs in modern medicine and thus modulating autophagy to relief neurodegeneration is a central field for present and future research and future therapeutic options for neurodegenerative diseases.

Keywords

- Autophagy;
- Neurodegenerative Diseases;
- Therapeutics;
- TOR Serine-Threonine Kinases;
- Alzheimer disease;
- Parkinson disease;
- Huntington disease;
- Amyotrophic lateral sclerosis.

1. Introduction

Neurodegenerative diseases are a group of incurable and debilitating conditions that result in progressive degeneration and/or death of nerve cells. This group of diseases has common cellular and molecular mechanisms including protein aggregation and inclusion body formation. The main constituents of aggregates in the most common neurodegenerative disorders are summarized in Table 1. Clinical features vary between diseases depending on the neuronal types that became dysfunctional or die, so can present different symptomatology as progressive dementia, motor abnormalities and disruptions in emotional, cognitive, and social behavior.

In general, Alzheimer's disease (AD) is characterized by progressive dementia with deterioration of social and behavioural skills and presents two major kinds of protein aggregates: extracellular amyloid plaques, containing amyloid- β ($A\beta$) aggregates and intracellular accumulation of neurofibrillary tangles composed mainly of hyperphosphorylated tau protein. Parkinson's disease (PD) is usually defined as a movement disorder characterized by motor symptoms, such as tremor, postural imbalance, bradykinesia and rigidity, having as histopathological hallmark an inclusion body termed Lewy body (LB) mainly constituted by α -synuclein (ASYN) insoluble fibrous aggregates. Huntington's disease (HD) is an incurable, hereditary brain disorder, which affects movement, mood and thinking skills, in which the histopathological hallmark consists in huntingtin (HTT) inclusions. Amyotrophic lateral sclerosis (ALS) causes progressive degeneration of upper and lower motor neurons accompanied by muscle atrophy and has some clinical overlap with frontotemporal dementia (FTD), which can also show compromise of functions such as speech and memory. Both pathologies show ubiquitinated protein aggregates in brain cells. Prion diseases, from which the most common is Creutzfeldt-Jakob disease, have a sudden and fast cognitive decline. Pathology can include amyloid plaques; nevertheless, disease is caused

by abnormally folded prion proteins. Peripheral nerve diseases like the familial amyloid polyneuropathy (FAP) are characterized by sensorimotor impairment and extracellular deposition of abnormal fibrils derived from misfolded, normally soluble transthyretin (TTR) molecules. Although HD and FAP are of autosomal dominant inheritance, most of AD, PD, ALS, FTD and Creutzfeldt-Jakob disease cases are of sporadic etiology. Nevertheless, genetic mutations identified in the familial counterparts of sporadic neurodegenerative diseases give rise to altered proteins that misfold and aggregate or are involved in cellular quality control systems such as the autophagy-lysosomal pathway (ALP).

Autophagy is traditionally sub-divided in micro-, macro- and chaperone-mediated-autophagy (CMA), although the majority of the authors using “autophagy” refer to macroautophagy. There is no consensus on whether autophagy is a disease inducing process¹ or rather a mechanism of defence²; as described below, the evidence tends to favour the representation of a rather complex process where the end result depends on the activating pathway, the selective autophagic process and molecular complexes implicated. A strong interplay occurs with organelles like the mitochondrion, the endoplasmic reticulum (ER) and the Golgi apparatus.³ An overview of the pathways implicated is provided in Figure 1.

In this paper we review the latest advances on the understanding of how the autophagic pathways can be implicated in the progression of neurodegenerative diseases, the main focus being on the development of putative therapeutic strategies targeting autophagy.

Table 1. Common neurodegenerative diseases and aggregated proteins		
Disease	Quality of aggregates*	Mutated autophagy genes ⁺
Alzheimer's disease (AD)	Cleavage of amyloid precursor protein (APP) yielding extracellular amyloid- β (A β) plaques; intracellular neurofibrillary tangles formed by hyperphosphorylated microtubule-associated protein tau	
Parkinson's disease (PD)	Intracellular accumulation of mutant α -synuclein protein forming the Lewy bodies	PINK1; PARK2
Huntington's disease (HD)	Nuclear inclusions of mutant Huntingtin (HTT) protein	ATG7
Amyotrophic lateral sclerosis (ALS)	TAR DNA binding protein (TDP-43)	SQSTM1/p62; optineurin
Frontotemporal dementia (FTD)	TDP-43 and/or tau	
*main pathologic findings in inclusion bodies, other constituents being also present		
+identified mutations in genes directly related to autophagy commonly seen in early-onset disease types		

2. Autophagy in neurodegeneration

A brief perusal of the literature is enough to readily identify the mechanistic target of rapamycin (mTOR) as a central molecule in the study of autophagic pathways and the possible therapeutic targets it can provide for the management of diverse neurological conditions. Research in this area is therefore sometimes divided in mTOR-dependent and mTOR-independent branches. However, because interfering with mTOR can block survival pathways and may do more harm than good, an interesting field of research comprises mechanisms that would enhance autophagy by circumventing the mTOR signalling pathway, for example by modulating the glycogen synthase kinase 3 β (GSK-3 β) or directly activating

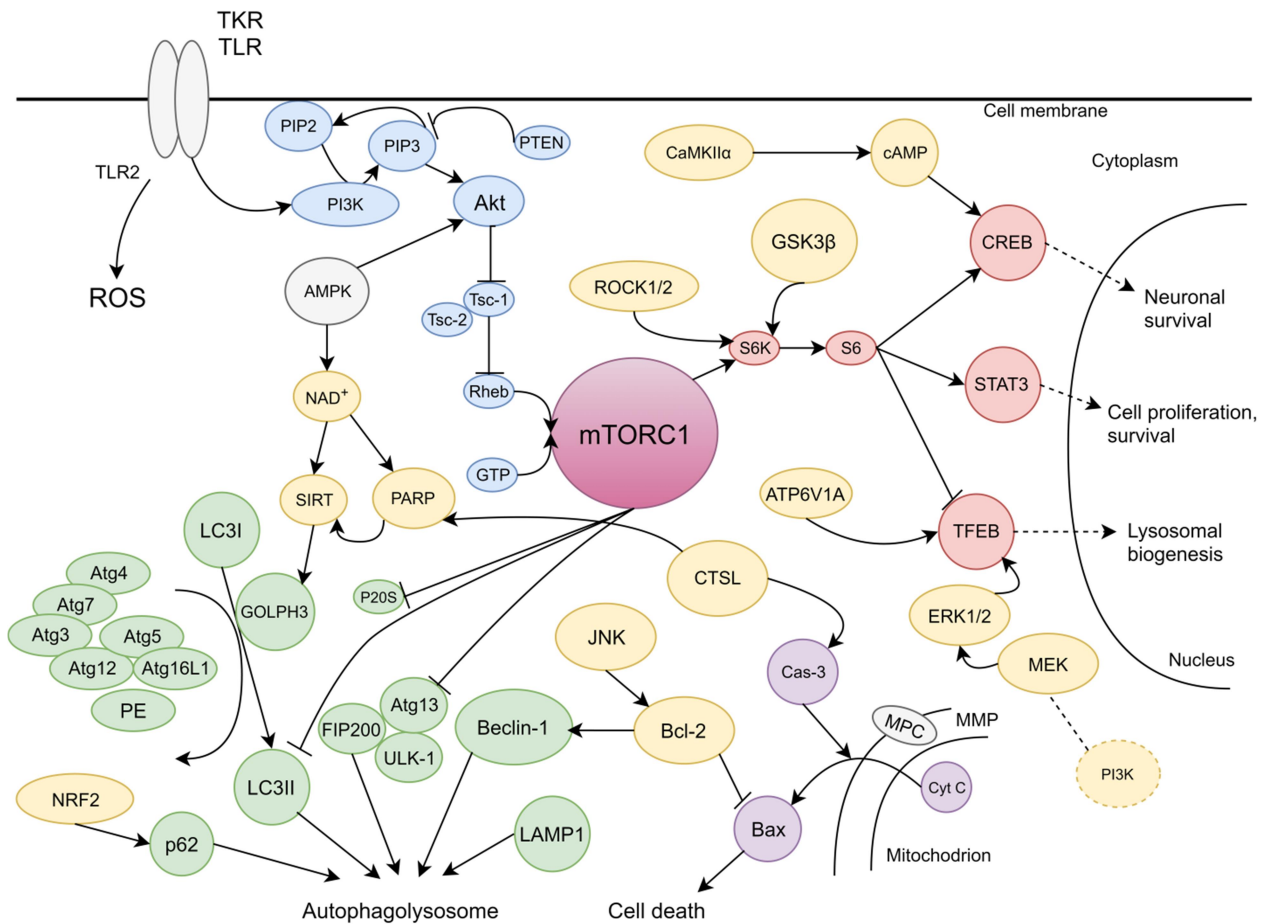
selected transcription factors. A synthetic curcumin analogue named C1 was able to do so in rat brain⁴, directly activating transcription factor EB (TFEB) and thus autophagy and lysosomal biogenesis in a Beclin-1-dependent, mTOR-independent manner. Curcumin was also reported to enhance autophagy and reduce amyloid precursor protein (APP) levels in SH-SY5Y cells (a human neuroblastoma-derived cell line) where oxidative stress was induced with paraquat⁵; (inducing reactive oxygen species - ROS - overproduction generally present in neurodegenerative conditions).

In order to evaluate the role of autophagy in neurodegeneration processes induced by an environmental neurotoxin, rats were intoxicated with trimethyltin, which blocked the late stages of autophagy, as shown by accumulation of microtubule-associated protein 1A/1B-light chain 3 II (LC3II, recruited to autophagosomes after conjugation of cytosolic LC3I⁶) and protein p62/sequestosome 1 (p62/SQSTM1, which binds to LC3 molecules and is used as a marker for autophagic flux⁷). Lithium treatment protected astrocytes through phosphorylation of GSK-3 β , rendering it inactive; this was achieved only for a high concentration (2 mM) of lithium when compared to a lower/initial concentration (0.5 mM).⁸ Under those conditions, reduced mobility of the autophagosomes could not be overcome by rapamycin (also named sirolimus, clinically used as an immunosuppressant), further highlighting the importance of autophagy modulation through the alternative GSK-3 β pathway. However, in another study, the same authors stated that lithium could have this effect in an autophagy-independent way in microglia,⁹ since improved cell survival was observed without a change in p62 levels.

Human post-mortem brain tissue showed augmented number of autophagosomes after stroke¹⁰ (which causes acute neurodegeneration) and accumulation of ASYN was seen in rodents after inducing infarction.¹¹ In the same study, both the knockdown (using si-RNA) and knockout of ASYN were proven to improve recovery through decreased phosphorylated

dynamamin-related protein 1 (Drp1), 3-nitrotyrosine, cleaved caspase-3 and LC-3II/I ratio, meaning modulation of respectively mitochondrial fragmentation, oxidative stress, apoptosis and autophagy. This suggested a role for autophagy in post-stroke changes but whether it had a neuroprotective or neurodegenerative effect was controversial, which could be important for the understanding of neurodegenerative diseases where neuronal cell death also occurs. A study on rats after spinal cord ischemia-reperfusion injury suggested that the opposite effects from autophagy could depend on its extension and persistence; in this sense, independent administration of 3-methyladenine (3-MA, an autophagy inhibitor) and rapamycin (autophagy enhancer) immediately and 48 hours after injury showed that autophagic clearance could improve survival on an initial phase (8 hours after injury, inhibiting apoptosis and inflammation) but was rather deleterious when sustained (at 72 hours, showing lower motor scores and decreased survival).¹² Silibinin (SLB, derived from a flavonoid with antioxidant properties) improved survival in a mouse model of middle cerebral artery occlusion and protected from H₂O₂-induced oxidative stress in cortical neurons by disfavoring Bax-mediated apoptosis; this effect was abrogated by wortmannin (PI3K inhibitor), suggesting a role for SLB on regulating autophagy.¹³

Although not a direct determinant of disease, such alterations (e.g. ROS production), that are transversal to neurodegenerative processes, could render the neuron more susceptible to harm, and trigger autophagy as an effort to try and rescue the cell after insult, as further suggested in a study linking the accumulation of lysosomal-associated membrane protein 1 (LAMP1, implicated in fusion of late endosomes with lysosomes¹⁴) in hippocampal neurons to post-epileptic states.¹⁵

Figure 1. Cellular pathways leading to autophagolysosome formation

Molecular pathways referred in the text and the relations between them are depicted in a simplified way. Arrows indicate a positive influence while truncated lines represent inhibition. Akt- protein kinase B; AMPK - 5' adenosine monophosphate-activated protein kinase; Atg - autophagy-related protein; Bax - Bcl-2-associated X protein; Bcl-2 - B-cell lymphoma 2; CaMKII α - Calcium/calmodulin-dependent protein kinase type II alpha chain; cAMP - cyclic adenosine monophosphate; Cas-3 - caspase 3; CREB - cAMP response element-binding protein; CTSL - cyclic adenosine monophosphate; CytC - cytochrome c; ERK 1/2 - extracellular signal-regulated kinases 1 and 2; FIP200 - focal adhesion kinase family interacting protein of 200 kD; GOLPH3 - Golgi phosphoprotein 3; GSK3 β - glycogen synthase kinase-3 β ; GTP - guanosinotriphosphat; JNK- c-jun N-terminal kinases; LAMP1 - lysosome-associated membrane glycoprotein 1; LC3 - microtubule-associated proteins 1A/1B light chain 3; MEK- mitogen-activated protein kinase kinase; MMP - mitochondrial membrane permeabilization; MPC- mitochondrial pyruvate carrier; mTORC1 - mammalian target of rapamycin complex 1 (mTOR is the central molecule of the mTORC1 complex); NAD - nicotinamide adenine dinucleotide; NRF2 - nuclear factor (erythroid-derived 2)-like 2; P20S- 20S proteasome; p62- nucleoporin 62 (also SQSTM1, sequestosome-1); PARP - poly ADP-ribose polymerase; PE - phosphatidylethanolamine; PI3K - phosphoinositide 3-kinase; PIP2- phosphatidylinositol-4,5-bisphosphat; PIP3 - phosphatidylinositol-3,4,5-trisphosphate; PTEN - phosphatase and tensin homolog; Rheb - Ras (Rat sarcoma) homolog enriched in brain; ROCK1/2 - Rho-associated protein kinases 1 and 2; ROS - reactiveoxygen species; S6K - protein S6 kinase; SIRT- sirtuin; STAT - signal transducer and activator of transcription 3; TFEB - transcription factor EB; TKR - tyrosine kinase receptor; TLR - toll-like receptor; Tsc - tuberous sclerosis protein; ULK - serine/threonine-protein kinase Unc-51-like kinase 1.

2.1. Autophagy in Alzheimer's disease - AD

Gene therapy increasing p62 levels in the brain of APP/PS1 mice rescued cognitive deficits by increasing autophagic clearance of both soluble amyloid- β (A β) and plaques; removing the LC3-interacting region (LIR) from p62 or directly inhibiting autophagy impeded this clearance.¹⁶ Haploinsufficiency for AT-1/SLC33A1 (that translocates acetyl-CoA from the cytosol to the ER) in mice rescued AD but not HD or ALS phenotypes, while

inhibition of acetyltransferases ATase1 and ATase2, inducing autophagy, caused a decrease in soluble A β and phosphorylated tau and an increase in synaptic plasticity and rodent lifespan.¹⁷ This study illustrated how intracellular toxic protein aggregates form in the secretory pathway in AD, whereas in HD and ALS this occurred in different cellular compartments, highlighting the targeting of ER acetyltransferases as a putative therapeutic strategy.

Neuronal PAS domain protein 4 (NPAS4) has recently been identified as a brain-specific transcription factor which regulates GABAergic (inhibitory) synapses by modulating brain-derived neurotrophic factor (BDNF) gene expression. Overexpression of NPAS4 in cortical neurons reduced tau levels with no interference on mRNA or in the main tau kinases GSK3 β and CDK5, along with increased LC3II puncta formation and p62 degradation¹⁸, suggesting tau degradation occurred with enhanced autophagic flux; however, neither autophagic blockage (3-MA, chloroquine), nor proteasome inhibition (MG132) could fully reverse the effects of NPAS4.

Hypercystinemia, a known risk factor for AD, was shown to up-regulate mTORC complex-1 (mTORC1), whereas mTORC1 inhibition with rapamycin or direct autophagy induction with tat-Beclin-1 peptides (tat being a HIV1-derived vehicle) could lower amyloid levels in knockout mice for cystathionine- β -synthase (which converts homocysteine to cystathionine).¹⁹ Altered calcium homeostasis and mitochondrial membrane potential (MMP) were also shown to disturb the autophagic process in cells derived from patients with homocystinuria, suggesting that stabilising calcium homeostasis could be an ancillary therapy by lowering ER stress.²⁰

The harmful effects of autophagy were studied by injecting miR-299-5p in the ventricles of APP^{swe}/PS1^{dE9} mice, improving cognitive performance while down-regulating both Atg5 and caspase-dependent apoptosis²¹; along with the observation of low cerebrospinal fluid levels of this micro RNA in AD patients, the authors proposed this micro

RNA as a neuroprotector against AD. The plant-derived β -asarone mediated an increase in mTOR phosphorylation and p62 expression, in relation to a decrease in Akt phosphorylation (PI3K/Akt/mTOR/CREB pathway) and diminished levels of $A\beta_{1-42}$, Beclin-1, LC3II and acetylcholinesterase²² (AChE; note that acetylcholine, ACh, is low in AD and AChE inhibitors are clinically employed), down-regulating autophagy and improving learning and memory ability, as observed in APP/PS1 transgenic mice. Acting on the same pathway through mTORC1 phosphorylation at Ser2481, but stimulating autophagy, the well-studied mTOR inhibitor rapamycin was able to increase cell viability in $A\beta_{1-42}$ -treated PC12 cells²³ (through Beclin-1 up-regulation mediating the stabilization of MMP and calcium homeostasis) and improved learning and memory in rats on which $A\beta_{1-42}$ was stereotaxically injected into the hippocampus²⁴ (related to augmented defences against reactive oxygen species - ROS - and lowering calcium concentration), raising the question of whether the putative harmful effect of down-regulating autophagy was masked by the modulation of AChE in the β -asarone study.

Sirtuin-1 (SIRT1), a deacetylase, was largely stimulated by resveratrol (RSV, a natural polyphenol) in PC12 cells previously treated with the neurotoxic peptide $A\beta_{25-35}$ ²⁵ through TyrRS-PARP1-SIRT1 pathway signalling. RSV-induced expression of tyrosyl transfer-RNA synthetase (TyrRS) stimulated the poly ADP-ribose polymerase 1 (PARP1) that modulates nuclear expression, increasing autophagosomes formation and degradation of p62, both effects abolished by 3-MA. Sirtuin-2 (SIRT2) loss-of-function induced by administration of adenylyate kinase 1 (AK1, a specific SIRT2 inhibitor) or using SIRT2 knockout transgenic mice improved microtubule stabilization,²⁶ allowing for better autophagic flux and improving the clearance of $A\beta$ oligomers and cell survival. This evinces a modifying therapeutic modality through acetylation of tubulin, which facilitates vesicle trafficking and senescent

mitochondria degradation; this strategy was also studied in PD⁵³ and Prion disease⁸⁶ (see below).

In the search for new therapeutic possibilities for AD, the novel AVN-211, a selective serotonin 6 receptor (5-HT6) antagonist (5HT6 receptor activity was linked to mTOR activation), could improve learning, behavioural and cognitive performances,²⁷ having a greater *in vivo* effect than memantine (an N-methyl-D-aspartate - NMDA - receptor antagonist used in AD that lowers the excitatory activity of glutamate), intepirdine and idalopirdine (two selective 5-HT6 receptor antagonists being tested in clinical trials for AD) and showing a similar or better anxiolytic effect in comparison with fenobam, rufinamide, lorazepam and buspirone, while lacking side effects or significant toxicity in rodent and primate (*Rhesus* macaques) models. The substance KT5823-mediated inhibition of nitric oxide/protein kinase G (NO/PKG) improved performance in the Morris Water Maze (which evaluates spatial memory and learning) in rodents in which spatial memory deficits were induced by A β ₁₋₄₂, along with increased LC3II/I ratio and levels of Atg7 and Beclin-1.²⁸

In an interesting study, five compounds previously shown to hinder the formation of A β ₁₋₄₂ aggregates were tested in APPOSK mice²⁹ (with the Osaka E693 Δ mutation promoting A β oligomerization). The results showed a clear advantage for the common antibiotic rifampicin, which prevented A β aggregation and promoted a decrease in levels of p62 while maintaining conversion of LC3I to LC3II, re-establishing the autophagic flux. This compound was also shown to be effective in inhibiting tau and ASYN aggregate formation in cell-free conditions by favouring the formation of monomers, evidencing its broad spectrum in neurodegenerative disorders; although it was unable to clear already established plaques, the fact that it is a safely used molecule, with no significant side-effects, highlights its possible preventive role in neurodegenerative processes involving protein oligomerization.

A new modality of GSK-3 inhibition with L807mts, a molecule that the kinase converts into its own inhibitor, was both potent and safe in stimulating clearance of A β in 5XFAD mice, an AD model, also lowering inflammation, stimulating autophagy and improving cognitive and social behaviour tests.³⁰ Differentiated rat neuronal PC12 cells were treated with icariside II (ICS II), a novel phosphodiesterase 5 (PD-5) inhibitor, in order to evaluate its protective role against oxidative damage induced by hydrogen peroxide (H₂O₂); ROS generation was attenuated and autophagic cell death was inhibited through regulation of GSK3 β , proposing a protective role for autophagy inhibitors in oxidative states of the nervous system.³¹

The disaccharide trehalose that comprises two D-glucose molecules joined by an α,α -1,1 link induced an autophagy-upregulation effect (elevated LC3-II and p62) that was shown to be mTOR-, Beclin-1- and Atg5-independent, thus explaining the recent growing interest sparked by this compound. In an *in vitro* study, neither maltose nor glucose administration had impact on APP and LC3 levels,³² showing that its action could not be mimicked by other disaccharides or its metabolites and was therefore not linked to the supply of glucose, but were rather trehalose-specific.

Mice fed with a diet rich in palmitic acid showed increased inflammation markers, low levels of neuroprogenitor cells and formation of A β deposits in the hippocampus, in comparison with controls after 16 months of high fat diet, with a concomitant reduction in autophagic markers,³³ evincing that changing dietary intake could influence the development of sporadic AD. In another study, high caloric intake was shown to augment mTOR, S6K, and decrease LC3 levels measured in the hippocampus, which translated in learning and memory difficulties in rodents³⁴; caloric restriction on the other hand showed opposite variations of the same markers and correlated with improved memory and learning abilities. In a trial of tart-cherry dietary supplementation, lower hippocampal inflammatory levels and improved

working memory, but no significant changes in motor performance, were associated with enhanced autophagic flux in aged mice.³⁵ In APPsw/Tg2576 mice, a supplement of pomegranate extract resulted in reduced beta-cleavage of APP in the brain and increased levels of synaptic structural proteins (PSD-95, Munc18-1, SNAP25) and synaptophysin³⁶; under the same experimental conditions, increased phosphorylation of calcium/calmodulin-dependent protein kinase II α (increased ratio p-CaMKII α /CaMKII α) and cyclic AMP-response element binding protein (ratio p-CREB/CREB) were accompanied by decreased neuroinflammatory activity and enhanced autophagy in a PI3K/Akt/mTOR-dependent, rapamycin-like manner. Ghrelin, an orexigenic circulating hormone up-regulated in starvation states, was shown to have positive effects on SH-SY5Y cells transfected with a mutant APP gene (AD model), namely through up-regulation of both the ubiquitin-proteasome system (lower levels of ubiquitinated proteins and p27, consistent with augmented 20S and 26S proteasome enzyme activity) and autophagy (increased LC3II and Beclin-1), along with a trophic influence on neural cells by increasing growth hormone secretagogue receptor type 1 expression³⁷ (GHS-R1, a G protein-coupled receptor for ghrelin).

A contribution from Chinese traditional medicine, the *Ampelopsis grossedentata*-derived dihydromyricetin (DHM) alleviated learning and memory impairment, this time in miR-34a-overexpressing aged brains (downregulating miR-34a and upregulating SIRT1) in D-galactose-treated mice, a model for AD, creating target options for any neurodegenerative process where the autophagic flux is disturbed.³⁸

2.2. Autophagy in Parkinson's disease - PD

Aggregates of α -synuclein (ASYN) are known to be pathologic in PD and other synucleinopathies. Glucocerebrosidase (GBA) trapped in the ER in models for the GBA-N370S mutation (the strongest genetic risk factor for PD) was shown to interfere with ASYN

clearance,³⁹ representing a possible therapeutic target upstream in the pathologic cascade. In rAAV6-infected cells overexpressing ASYN, dimethyl fumarate (DMF, already used in multiple sclerosis, where a positive effect on autophagy was also observed⁴⁰) was shown to regulate the nuclear factor erythroid 2-related factor 2 (NRF2) - p62 axis to enhance ASYN delivery to the autophagosome, while knockout mice for Nrf2 did not replicate the effects of DMF⁴¹; ASYN-induced microgliosis also decreased, indicating a less pro-inflammatory phenotype (down-regulation of interleukin 1 β and inducible nitric oxide synthase were observed). In cells treated with ASYN pre-formed fibrils (which had a stronger effect in reducing cell viability in comparison to treatment with monomeric ASYN), trehalose had no effect on ASYN levels but improved cell survival.⁴² In an AAV ASYN rat model for PD, trehalose lowered the number of aggregates of A53T ASYN (mutant ASYN resulting from an amino acid change) and improved behavioural skills⁴³. Treatment with trehalose also showed a neuroprotective effect in a mild, sustained MPP+ exposure model for PD (although it was not effective following an acute intoxication due to irreversible lysosomal damage).⁴⁴

Pre-treatment with the mitochondrial pyruvate carrier (MPC) modulator MSDC-0160 increased the mobility time both in a spontaneous locomotion test and the rotarod test in MPTP-treated mice, improved survival of cultured midbrain dopaminergic cells and ASYN-treated *Caenorhabditis elegans*, thus showing effectiveness in multiple models by reducing the levels of mTORC1 phosphorylation (but not of mTORC2, mimicking rapamycin).⁴⁵ In a *post mortem* analysis of neurons and microglia from PD specimens, TLR2 (toll-like receptor 2) co-localized with Lewy bodies, along with increased levels of cytokines, microglial-activating chemokines, ROS and p62,⁴⁶ showing a link between TLR2 and inflammation. Rapamycin and TLR2 inhibitors could lower ASYN in SH-SY5Y cells previously treated with TLR2 agonists. In the same experimental setup, NG25, a transforming growth factor-beta-activated kinase 1 (TAK1) inhibitor that ultimately blocks the c-Jun N-terminal kinase

(JNK) activation and production of inflammatory cytokines, clearly ameliorated ASYN deposits. NogoA, a myelin-associated protein that inhibits axonal regeneration was shown to be upregulated in MPP⁺ treated mice and miRNA knockout of this protein inhibited the activation of signal transducer and activator of transcription 3 (STAT3) by mTOR in a rapamycin-like fashion⁴⁷, protecting against PD-like toxicity.

In a 6-hydroxydopamine (6-OHDA, which is injected in the nigrostriatal dopaminergic pathway leading to ROS-induced cytotoxicity) rodent model for PD, paeoniflorin, a Chinese herb-derived substance, was shown to be neuroprotective by inhibiting acid-sensing ion channels (ASIC),⁴⁸ improving behavioural symptoms and reducing the loss of dopaminergic cells (effects further observed with the known ASIC blockers psalmotoxin-1 and amiloride). Since knockdown of ASIC1 α decreased ASYN aggregation through enhanced autophagy in PC12 cells, effects of pharmacologic inhibition of these channels could be related to increased autophagic activity. β -Asarone also exerted neuroprotective effects in 6-OHDA rats via the JNK/Bcl-2/Beclin-1 pathway (the non-treated mice overexpressing phospho-c-jun marker of JNK-mediated autophagic cell death), showing better behavioural and motor results.⁴⁹ In another study, 6-OHDA-treated SH-SY5Y cells showed accumulation of LAMP1, LC3II and p62,⁵⁰ suggesting a blockade of the autophagic flux, while the cathepsin-L (CTSL) inhibitor Z-FY-CHO promoted autophagy, decreased p62 and blocked both caspase-3 and PARP, suggesting the nuclear translocation of CTSL as enhancing the apoptotic cascade while disturbing the autophagy-apoptosis balance. A dual agonist (DA-JC1) of the incretins glucagon-like peptide 1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP) attenuated motor and behavioural deficits caused by treatment with 6-OHDA in rats,⁵¹ suggesting a role for antidiabetics in the management of PD.

In MPTP/p-treated mice (p standing for probenecid), metformin prevented SNpc dopaminergic neural death, raised the quantity of striatal dopamine (DA), improved motor

function and decreased microglia overactivation-induced neuroinflammation through potentiation of the autophagy-inducing 5' adenosine monophosphate-activated protein kinase (AMPK).⁵² The same study showed that metformin was able to raise LC3II levels while decreasing ROS production and apoptosis in MPP⁺-treated SH-SY5Y cells. Sirtuin-2 inhibition with AK1 in sporadic PD patient-derived hybrid cells restored the acetylation of α -tubulin, which facilitated autophagy (increased number of mitochondria and autophagic vacuoles in motion) and intra-peritoneal injection of MPTP in sirtuin-2 knock-out mice failed to impair motor behaviour.⁵³ Sirtuin-2-dependent deacetylation of ASYN slowed aggregate clearance *in vitro* and increased death of dopaminergic neurons from rats injected with an ASYN acetylation-mimic mutant, while targeted acetylation of ASYN on the lysine residues 6 and 10 facilitated its autophagic degradation⁵⁴; thus inhibiting sirtuin-2 or increasing acetylation of ASYN could have therapeutic potential in PD.

The substance piperine (PIP), acting on phosphatase 2A (PP2A), lowered mTORC1 levels through dephosphorylation of Akt kinase,⁵⁵ thus stimulating the autophagic clearing of damaged mitochondria in rotenone-treated SK-N-SH cells (another model for PD) and striatal dopaminergic cell survival in rotenone-injected mice.

Variations in the lysosomal K⁺ channel transmembrane protein 175 (TMEM175) were linked to sporadic PD in genome-wide association studies; hippocampal cells derived from rats deficient in TMEM175 had increased levels of ASYN and showed a decrease in lysosomal catalytic activity (owing to increased lysosomal pH) and mitochondrial respiration.⁵⁶ The K⁺ ionophore nigericin decreased cytosolic pH in SH-SY5Y cells and enhanced autophagy dependent on the PTEN-induced putative kinase 1 (PINK1)/parkin axis.⁵⁷ In cell lines derived from PD patients with loss-of-function mutations in PINK1 and parkin (which causes accumulation of dysfunctional mitochondria leading to nigrostriatal degeneration and early-onset PD), the pro-apoptotic Nip3-like protein X (Nix) improved

mitophagy and mitochondrial function (increased ATP synthesis),⁵⁸ thus suggesting a mechanism to circumvent Parkin deficiency in early-onset PD.

An alternative therapeutic target was tested in progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD), diseases that course with PD-like symptoms, where fasudil, a clinically used Rho-associated protein kinases (ROCK1 and ROCK2) inhibitor, was able to reduced tau levels through autophagy enhancement,⁵⁹ suppressing phosphorylation of ribosomal protein S6 kinase beta-1 (p70S6K1) by mTOR; reduction of tau mRNA in neuroblastoma cells and PSP and CBD mouse brains, as well as less severe rough eye phenotype in a *Drosophila* tauopathy model were also observed. Fasudil was also tested in PD models where it could reverse injury caused by A53T ASYN by enhancing its autophagic degradation in a JNK-1/Bcl-2/Beclin-1-dependent manner,⁶⁰ the same pathway targeted by β -asarone.

2.3. Autophagy in Huntington's disease - HD

Expansion of cysteine-adenosine-guanine (CAG) repeats is a genetic defect common to a group of disorders caused by mutant proteins with polyglutamine (polyQ) expansions; HD, coursing with mutant huntingtin (HTT), is the most common disease. Machado-Joseph disease (MJD, or spinocerebellar ataxia type 3 - SCA3), caused by CAG expansion in the ataxin-3 (ATXN3) gene, leading to mutant ATXN3 protein aggregates, is also described since research in animal models for MJD might contribute to management of polyglutamine diseases in general.

Hederagenine and α -hederin, derived from the herb *Hedera helix*, have been shown to increase the autophagic flux through the AMPK pathway in a dose-dependent manner,⁶¹ facilitating the degradation of HTT and ASYN in PC12 cells transfected respectively with polygQ HTT and A53T. The engineered nanoparticle graphene oxide (GO) was able to

increase LC3II with no change in SQSTM1/p62, not interfering with the mTOR pathway, but still activating autophagy via the PI3K/MEK/ERK1/2 pathway and enhancing Htt ubiquitination in a cellular model for HD.⁶²

In yeast and mammalian cell models for HD, genetic manipulation augmenting glycation of HTT was shown to promote intracellular aggregation of the HTT exon-1, whereas the glycation agent methylglyoxal proved to be toxic to HD fruit flies, with diminished life span and eclosion rates. Based on these data, the authors suggested autophagy-lysosomal pathway overactivation as the causative factor, evidencing glycation states as possible drug target.⁶³ In HD-mimicking SH-SY5Y neuroblastoma cells, dopamine was shown to exacerbate mutant HTT-induced oxidative stress, since superoxide anion ($O_2^{\bullet-}$) impaired autophagosome formation, with N-acetyl-L-cysteine (a quinone reductase inducer) and deferoxamine (an iron chelator) showing antioxidant and preventive effects.⁶⁴ Iron can enhance ROS production with neurodegenerative potential, causing lysosomal deficits (iron deposits), autophagic overload and cell death, effects modulated by the second messenger nicotinic acid adenine dinucleotide phosphatase (NAADP) and the GTPase RAB7A, both acting on two-pore channels (TPCN, which release calcium from endolysosomal stores into the cytosol); their inhibition with Ned-19 abolished the aforementioned harmful effects of iron load.

Two studies from the same authors identified autophagy enhancer-67 (AUTEN-67) as a synthetic autophagy stimulator with antiaging and neuroprotective effects. This compound should avoid the side-effects of common autophagy inducers, by acting on the core autophagic process, namely through inhibition of myotubularin-related phosphatase, MTMR14, which hampers the formation of autophagic membranes; it was able to lower A β levels in mice expressing human APP.⁶⁵ Further testing on a *Drosophila* model for HD (transgenic 128Q HTT) MTMR14 improved climbing ability and induced longer lifespan.⁶⁶ In

the same way, the autophagy enhancer-99 (AUTEN-99) decreased neurodegenerative symptoms in *Drosophila* models of HD (expressing human mutant 128Q-HTT) and PD (expressing human mutant Parkin).⁶⁷

Another study reported trehalose as functioning as a chemical chaperone and having antioxidant properties, and further confirmed its autophagy-inducer properties by lowering the levels of p62. It also decreased microglial activation and reversed cytoskeleton disruption favouring astrocytic propagation in cultured striatal cells of HD R6/1 rodents (expressing exon 1 of the HTT gene with 115 CAG repeats) in early postnatal development,⁶⁸ thus adding a neurotrophic function to the already broad spectrum of trehalose. Lactulose and melibiose proved to be good trehalose analogs by lowering ATXN3/Q75 aggregation in a SCA3 ATXN3/Q75-GFP cell model,⁶⁹ which was further confirmed in differentiated SH-SY5Y cells, making these substances a possible therapeutic strategy in polyglutamine aggregation-associated neurodegenerative diseases.

The attempt to modulate mTOR-dependent and -independent pathways simultaneously was shown to be neurotoxic in a MJD mouse model; temserolimus (an inhibitor of mTOR) and lithium (LiCl, an inositol monophosphatase inhibitor), both in individually non-toxic, autophagy-inducing doses, were neurotoxic to wild-type and CMVMJD135 transgenic mice⁷⁰ when administered at the same time, highlighting the need for more research in this field.

Caloric restriction (CR) activated SIRT1 in MJD mice and improved the neuropathology related to motor coordination and imbalance, coursing with increased LC3II and decreased p62 levels (an indicator of improved autophagic flux) in the cerebella. These results evidence both CR and SIRT1 expression as therapeutic strategy. The same authors also studied the effects of resveratrol, a SIRT1 enhancer, which ameliorated motor deficits by restoring SIRT1 mRNA levels in the same transgenic model.⁷¹

Far-infrared radiation (FIR) is a subdivision of the electromagnetic spectrum with biological properties; in a study in MJD78 mice as model for SCA3, FIR decreased mitochondrial fragmentation and improved mitochondrial respiratory function; moreover, FIR treatment of MJD78 cells increased LC3II and Beclin-1 and decreased p62⁷²; importantly, no significant changes were observed in MJD26 cells expressing the normal variant of ATXN3, further linking autophagic impairment with the polyQ expanded ATXN3.

2.4. Autophagy in Amyotrophic lateral sclerosis - ALS - and Frontotemporal dementia - FTD

Mutation of the autophagy receptor SQSTM1/p62, a genetic defect found in ALS and FTD, was associated with defective recognition of LC3II in the LIR interacting region of the phagophore membrane,⁷³ impeding its recruitment into the phagosome.

A repeat expansion in the chromosome-9-open-reading-frame-72 gene (C9orf72) is the most prevalent known genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Loss of function of the C9orf72 protein was linked to less phosphorylation of mTOR and decreased serine/threonine kinase p70S6 (p70S6K1) activity⁷⁴ (its function being here the suppression of autophagy related genes - ATG - expression cascade), thus lifting its inhibitory role on the autophagic process.

However, in a TDP-43 (found in ubiquitinated inclusions) cell culture model treated with berberine (derived from a traditional herb), protein deposition was shown to be not only inhibited but also reverted, in parallel with the down-regulation of mTOR and enhancement of autophagy.⁷⁵ In turn, autophagy inhibition with further treatment with 3-MA (a PI3K inhibitor) halted the recovery process in a PI3K-dependent fashion. A study on a TDP-43-depleted *Drosophila* model elucidated the role of the autophagy-lysosomal pathway in these pathologic states, suggesting that blockage of the final stages of the autophagic pathway

rather than increased autophagic flux *per se* might have a harmful effect, by being able to link TDP-43 loss-of-function to dynactin-1 (which links vesicles to microtubules) down-regulation⁷⁶ and thus bringing autophagosome-lysosome fusion to a halt. Since mTOR inhibition with rapamycin worsened the motor symptoms (decreased larval locomotion), this study unveils the importance of considering both mTOR-dependent and -independent mechanisms, since a single molecule can modulate both pathways, interfering with aggregate formation in a complex system.

Further evidence of a harmful effect of autophagy was studied in ALS-like superoxide dismutase 1 (SOD1)-G93A transgenic mice through administration of n-butylidenephthalide (n-BP), a known autophagic inhibitor; n-BP prolonged survival more than the clinically approved substance riluzole, enhancing mTOR signalling and decreasing the accumulation of autophagosomes, also inhibiting caspase-3 activity,⁷⁷ an effector caspase involved in apoptosis.

Two studies focusing on aggresome formation in ALS showed it resulted from accumulation of mutant SOD1 through unclear mechanisms. Parkin (also implicated in AD and PD) was shown to cooperate with an E2-enzyme to polyubiquitinate mutated SOD1 at K63, facilitating its autophagic clearance⁷⁸ and suggesting parkin as a potential target in ALS. Moreover, 3-MA worsened and rapamycin improved clearance of remaining aggresomes. The E3-ubiquitin-ligase mahogunin-ring-finger-1 (MGRN1) was shown to be recruited to SOD1 inclusions along with p62 and LAMP2, whereas MGRN1 overexpression favoured autophagic clearance,⁷⁹ proving MGRN1-conducted cellular quality control to be a possible therapeutic option in ALS.

Colchicine and doxorubicin were found to induce the chaperone HSPB8, known to reduce the accumulation of TDP-43 (as well as its 25 kDa fragment, also with pathologic potential) aggregates in ALS and FTD, through cooperation with the co-chaperone BAG3 and

the HSP70/HSC70-CHIP complex, enhancing expression of the main autophagy regulator TFEB, along with increases in p62 and LC3 expression.⁸⁰ A next step should be to find analogs to these drugs with less side-effects. In a FTD model, induced pluripotent stem cells (iPSC)-derived neurons from individuals carrying the tau-A152T variant were rescued from mitochondrial stress and both proteotoxic and excitotoxic effects of hyperphosphorylation of tau upon its CRISPR/Cas9 genetic correction and targeting through pharmacological activation of autophagy with rapamycin.⁸¹

Mutations in the progranulin (PGRN) gene cause frontotemporal lobar degeneration and some degree of inactivation of PGRN was also identified as risk factor for both AD and PD. Haploinsufficient mice for the PGRN gene showed markedly increased PGRN expression in the brain after oral administration of trehalose.⁸² In the same study, common autophagy inhibitors also showed some effect on PGRN expression, particularly Torin1 (a selective mTOR inhibitor that blocks its phosphorylation), suggesting a link between mTOR modulation and PGRN expression. Trehalose however had the strongest effects on PGRN expression in human H4 (neuroglioma), human SH-SY5Y, mouse N2a (neuroblastoma) models and mouse cortical neurons. Since polymorphisms resulting in lower levels of PGRN have been identified as risk factors for AD and PD,⁸³ trehalose might have a broad therapeutic spectrum.

2.5. Autophagy in Prion diseases

Transmissible spongiform encephalopathies (TSE) are caused by misfolding of the scrapie prion protein (PrP^{Sc}), conferring a pathological effect to this glycoprotein, which becomes insensitive to proteases, aggregates and forms amyloidogenic deposits, in a mechanism similar to other neurodegenerative pathologies (e.g. AD, PD). Synthetic prion (PrP₁₀₆₋₁₂₆) induced toxic effects in SK-N-SH neuroblastoma cells, which could be reversed

by treatment with ginsenoside-Rg3, a derivative of the herb ginseng⁸⁴; LC3II levels were increased and more autophagic vacuoles were detected by electron microscopy. The plant derivative hinokitol raised the levels of hypoxia-inducible factor 1 α (HIF-1 α , a transcription factor that promotes survival under low levels of tissue oxygenation), prompting a neuroprotective autophagic flux⁸⁵ (e.g. allowing for increased number of vacuoles observed by electron microscopy) as confirmed with autophagy inhibitors (wortmannin, 3-MA, Atg5 siRNA) that reversed the beneficial effects.

Regulating the PI3K-Akt-mTOR axis could also alleviate PrP₁₀₆₋₁₂₆-induced neural death following transfection with recombinant plasmids expressing histone deacetylase 6 (HDAC6); this enzyme reversed the effects of PrP₁₀₆₋₁₂₆ on raising the levels of phosphorylated mTOR and p70S6K.⁸⁶ Interestingly, HDAC6 increased Akt phosphorylation. A similar effect was shown by inducing parkin overexpression (as described previously in this work, parkin is an E3 ubiquitin ligase, which was shown to be mutated in familial forms of PD); in this case, N2a cells treated with PrP₁₀₆₋₁₂₆ revealed lower levels of soluble parkin, the remaining colocalizing with fluorescein isothiocyanate (FITC)-tagged prion.⁸⁷ Thus, induction of parkin overexpression blocked the translocation of pro-apoptotic Bax to the mitochondrion (which initiates cell death by releasing cytochrome c), enhanced autophagy and thence cell survival.

The FKBP family of peptidyl prolyl isomerases (FK506-binding protein) is involved in several steps of PrP^{Sc} biogenesis; FK506 (a macrolide also named tacrolimus and fujimycin, used as an immunosuppressant) binds to FKBP and inhibits the translocation of cellular prion (PrP^C) to the ER and thus inhibits the misfolding process, which converts it to the scrapie form. N2a cells treated with FK506 induced proteasome-mediated degradation of PrP^{Sc}, also blocking its translocation to the ER and hence lifting the inhibition of the autophagic process.⁸⁸ Moreover, FK506 was shown to act on the V-ATPase catalytic subunit

A (ATP6V1A, a vacuolar proton pump) and promote green fluorescence protein-bound TFEB (which stimulates production of lysosomes) translocation from the cytosol to the nucleus in SH-SY5Y cells,⁸⁹ a mechanism of autophagic enhancement. Another study showed that rapamycin-induced autophagy was toxic to retinal cells treated with PrP₁₀₆₋₁₂₆,⁹⁰ the authors stated however that further studies will be needed to evaluate the applicability of autophagy inhibitors in rescuing PrP^{Sc}-infected cells *in vivo*.

2.6. Autophagy in Peripheral Nervous System disorders

A study in patients with chronic idiopathic axonal polyneuropathy (CIAP) identified increased number of autophagic structures in sural nerve samples as compared to healthy controls.⁹¹ The authors raised the question of whether enhanced autophagy is a consequence or a cause of the neuropathy. *In vitro* studies on adult dorsal root ganglia neurons survival and axonal growth, however, linked autophagy inhibition with reduced cell viability, neurite growth and branching complexity.⁹² Enhanced autophagy in a mouse model of inflammatory peripheral neuropathy was correlated to Schwann cell demyelination, the process continuing nevertheless in *Atg7* knockout models,⁹³ where autophagic activation constituted a failed attempt at regeneration. We highlight the relative lack of studies here in comparison to those on the central nervous system.

In cell culture expressing V30M mutant transthyretin (TTR) aggregates, a model of TTR-related amyloidosis (familial amyloid polyneuropathy or FAP), preservation of the early autophagic steps (conserved levels of LC3 and autophagosomes) occurred along with accumulation of p62. This could be reversed in the gastrointestinal tract from transgenic TTRV30M mice through tauroursodesoxycholic acid (TUDCA), as well as curcumin administration,⁹⁴ boosting autophagic turnover and slowing apoptosis, favouring the use of autophagic enhancers in this condition.

Table 2. Autophagy-enhancing, aggregate-clearing substances in mammalian models of disease			
Agent	Pathology-inducing mechanism	Cleared protein	Reference
Rapamycin (mTOR inhibitor)	Intra-hippocampal injection of amyloid- β ($A\beta_{1-42}$) in rats	amyloid- β ($A\beta_{1-42}$)	24
KT5823 (PKG inhibitor)	Intra-hippocampal injection of $A\beta_{1-42}$ in rats	$A\beta_{1-42}$	28
Rifampicin (antibiotic used in tuberculosis)	APP/OSK mice, expressing human amyloid precursor protein (APP) and the Osaka E693 Δ mutation promoting amyloid oligomerization	$A\beta_{1-42}$, hyperphosphorylated tau	29
	Tau609 mice (transgenic mice expressing 3-repeat and 4-repeat human tau)	Hyperphosphorylated tau	29
L807mts (GSK-3 inhibitor)	5XFAD mice (express five human familial AD gene mutations, including APP)	$A\beta_{1-42}$	30
AUTEN-67	APP/PS1 mice, expressing human APP and presenilin 1	$A\beta_{1-40}$, $A\beta_{1-42}$	65
Dimethyl fumarate (used in multiple sclerosis)	Injection of recombinant vector for α -synuclein (rAAV-6- α -SYN) in the ventral midbrain of rats	α -Synuclein (ASYN)	41
Paeoniflorin (extract of <i>Paeonialactiflora</i>)	Injection of 6-hydroxydopamine (6-OHDA) in the striatum of rats	ASYN	48
Metformin (oral antidiabetic)	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine plus probenecid (MPTP/p)-treated mice	ASYN	52
Trehalose (natural disaccharide)	Injection of recombinant vector for ASYN (rAAV-1/2- α -SYN-A53T) in the SNpc of rats	ASYN	43
	R6/1 mutant mice, expressing exon 1 of the human huntingtin (HTT) gene with 115 CAG repeats	Mutant HTT	68

3. Conclusions

In harmony with previous reviews related to this subject,⁹⁵⁻⁹⁷ we further sustain that autophagic pathways have an unquestionable role on both the development of pathology and its management. Although its key actors seem to be in part identified, how this role is played is still unclear, especially in neurodegenerative diseases, where therapeutic strategies are still dismaying and curative options hardly exist.

Looking at the results as a whole, we are drawn to conclude that enhancing autophagy in neurodegenerative processes is a valid and promising therapeutic option (see Table 2 for an overview of the substances that were able to clear protein aggregates in mammalian models of disease); however, a harmful effect of this action is not to be firmly ruled out. This discrepancy in some studies could have various reasons, from which we hypothesise three:

- Although the majority of neurodegenerative diseases course with accumulation of protean aggregates, which overwhelm the clearance response and exhibit overlapping pathologic processes, the responsible molecule or the underlying deficiencies vary;
- Acting on prodromal or symptomatic phases surely has different outcomes, and few studies make such distinction; since such diseases course with long prodromal states⁹⁸ but show mostly irreversible damages, the focus is increasingly on preventive action rather than symptomatic relief (which is already the aim of most available drugs);
- We further raise the question of the validity of the models being used for human disease and how representative they are for neurodegenerative diseases.⁹⁹

In spite of the continuously growing amount of research in this field, we emphasise the need to better understand the basic pathways of autophagic clearance and its place among the general pathways of nutrient utilisation and cell survival; owing to the complex interrelationships between survival and death pathways, the effects of autophagy and

autophagy-enhanced apoptosis are still hard to differentiate *in vivo* and this constitutes an important puzzle holding back the development of aggregate clearance strategies.

Neurodegenerative diseases are mainly multifactorial in the way they develop in a particular individual, but growing knowledge of genetic risk factors could allow for prevention already in the prodromal phase, in the context of a more personalized medicine in the future.^{100,101} We emphasize also the protective effect of healthy habits in general, here in particularly through modification of caloric intake in both quantity and quality, as aging and protein aggregation are in close relation.^{102,103}

The future of autophagy in neurodegeneration control therefore depends on clearly understanding whether enhancing it is beneficial (for example “bad” autophagy in ALS and “good” in AD?), how its modulation affects overall cellular functions (autophagy as both rescue and unwanted death pathway?) and the process itself, allowing for more directed molecular targeting.

Method

This review summarizes the work done on the intersection of the autophagic and neurodegenerative processes from a molecular therapeutic point-of-view. In order to bring some degree of systematization, the main body of results was filtered as follows: PubMed-indexed articles published between 2016/01/01 and 2017/03/24 (includes E-pubs ahead of print) where full text in English was available; autophagy- and neurodegeneration-related results were crossed (590 from 6354 and 13105 respectively), reviews were removed (177); from these were excluded articles not focusing on autophagy or neurodegenerative processes, articles aiming at defining study models or disease markers rather than therapeutic possibilities, guidelines and publications other than original research articles; among the 284 resulting articles, those present in this review were deemed the most relevant by the authors, not including those where the actual results, despite having relevant implications on other areas, have no clear influence on the management of neurodegenerative diseases.

Search terms were written as “autophag*” and “neurodegenerat*” in order to include all directly related terms as well as MeSH terms, automatically selected by PubMed.

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Footnotes

The authors declare no conflict of interest.

References

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- ¹ I-Hsun Li, Kuo-Hsing Ma, Tzu-Jen Kao, Yang-Yi Lin, Shao-Ju Weng, Ting-Yin Yen, et al. Involvement of autophagy upregulation in 3,4-methylenedioxymethamphetamine ('ecstasy')-induced serotonergic neurotoxicity, *NeuroToxicology*, Volume 52, January 2016, P. 114-126
- ² Pla A, Pascual M, Guerri C., Autophagy Constitutes a Protective Mechanism against Ethanol Toxicity in Mouse Astrocytes and Neurons, Amédée T, ed. *PLoS ONE*. 2016;11(4):e0153097
- ³ Li, T., You, H., Mo, X. et al., GOLPH3 Mediated Golgi Stress Response in Modulating N2A Cell Death upon Oxygen-Glucose Deprivation and Reoxygenation Injury, *Mol Neurobiol* (2016) 53: 1377
- ⁴Ju-Xian Song, Yue-Ru Sun, Ivana Peluso, Yu Zeng, Xing Yu, Jia-Hong Lu, et al., A novel curcumin analog binds to and activates TFEB in vitro and in vivo independent of MTOR inhibition, *Autophagy* Vol. 12 , Iss. 8, 2016
- ⁵ Thiranut Jaronwitchawan, Nipha Chaicharoenaudomrung, Jirapat Namkaew, Parinya Noisa, Curcumin attenuates paraquat-induced cell death in human neuroblastoma cells through modulating oxidative stress and autophagy, *Neuroscience Letters*, Volume 636, 1 January 2017, Pages 40-47, ISSN 0304-3940
- ⁶ Isei Tanida, Takashi Ueno, Eiki Kominami, LC3 and Autophagy, *Autophagosome and Phagosome* Volume 445 of the series *Methods in Molecular Biology* pp 77-88
- ⁷ Geir Bjørkøy, Trond Lamark, Serhiy Pankiv, Aud Øvervatn, Andreas Brech, Terje Johansen, Chapter 12 Monitoring Autophagic Degradation of p62/SQSTM1, *Methods in Enzymology*, Academic Press, 2009, Volume 452, Pages 181-197
- ⁸ Cinzia Fabrizi, Elena Pompili, Stefania De Vito, Francesca Somma, Angela Catizone, Giulia Ricci, et al., Impairment of the autophagic flux in astrocytes intoxicated by trimethyltin, *NeuroToxicology*, Volume 52, January 2016, Pages 12-22, ISSN 0161-813X

- ⁹ Fabrizi, C., Pompili, E., Somma, F., De Vito, S., Ciraci, V., Artico, M., et al. (2017) Lithium limits trimethyltin-induced cytotoxicity and proinflammatory response in microglia without affecting the concurrent autophagy impairment, *J. Appl. Toxicol.*, 37: 207–213
- ¹⁰ Tony Frugier, Juliet M. Taylor, Catriona McLean, Nicole Bye, Philip M. Beart, Rodney J. Devenish, et al., Evidence for the recruitment of autophagic vesicles in human brain after stroke, *Neurochemistry International*, Volume 96, June 2016, Pages 62-68, ISSN 0197-0186
- ¹¹ TaeHee Kim, Suresh L. Mehta, Balarama Kaimal, Kirsten Lyons, Robert J. Dempsey and Raghu Vemuganti, Poststroke Induction of α -Synuclein Mediates Ischemic Brain Damage, *Journal of Neuroscience* 29 June 2016, 36 (26) 7055-7065
- ¹² Bo Fang, Xiao-Qian Li, Na-Ren Bao, Wen-Fei Tan, Feng-Shou Chen, Xiao-Li Pi, et al., Role of autophagy in the bimodal stage after spinal cord ischemia reperfusion injury in rats, *Neuroscience*, Volume 328, 22 July 2016, Pages 107-116, ISSN 0306-4522
- ¹³ Min Wang, Yu-Jiao Li, Yi Ding, Hui-Nan Zhang, Ting Sun, Kun Zhang, Silibinin Prevents Autophagic Cell Death upon Oxidative Stress in Cortical Neurons and Cerebral Ischemia-Reperfusion Injury, *Neurobiol* (2016) 53: 932
- ¹⁴ Eeva-Liisa Eskelinen, Roles of LAMP-1 and LAMP-2 in lysosome biogenesis and autophagy, *Molecular Aspects of Medicine*, Volume 27, Issues 5–6, October–December 2006, Pages 495-502
- ¹⁵ Rami, A., Benz, A.P., Niquet, J. et al., Axonal Accumulation of Lysosomal-Associated Membrane Protein 1 (LAMP1) Accompanying Alterations of Autophagy Dynamics in the Rat Hippocampus Upon Seizure-Induced Injury, *Neurochem Res* (2016) 41: 53
- ¹⁶ A Caccamo, E Ferreira, C Branca and S Oddo, p62 improves AD-like pathology by increasing autophagy, *Molecular Psychiatry* advance online publication 30 August 2016

- ¹⁷ Yajing Peng, Mi Jin Kim, Rikki Hullinger, Kenneth J. O’Riordan, Corinna Burger, Mariana Pehar, Luigi Puglielli; Improved proteostasis in the secretory pathway rescues Alzheimer’s disease in the mouse. *Brain* 2016; 139 (3): 937-952
- ¹⁸ Wenhui Fan, Yan Long, Yujie Lai, Xuefeng Wang, Guojun Chen, Binglin Zhu, NPAS4 Facilitates the Autophagic Clearance of Endogenous Tau in Rat Cortical Neurons, *Mol Neurosci* (2016) 58: 40
- ¹⁹ Khoosheh Khayati, Henri Antikainen, Edward M. Bonder, Gregory F. Weber, Warren D. Kruger, Hieronim Jakubowski, et al., The amino acid metabolite homocysteine activates mTORC1 to inhibit autophagy and form abnormal proteins in human neurons and mice, *FASEB J* February 2017 31:598-609
- ²⁰ Martínez-Pizarro A, Desviat LR, Ugarte M, Pérez B, Richard E. Endoplasmic Reticulum Stress and Autophagy in Homocystinuria Patients with Remethylation Defects. Jin D-Y, ed. *PLoS ONE*. 2016;11(3):e0150357
- ²¹ Zhang Y, Liu C, Wang J, et al. MiR-299-5p regulates apoptosis through autophagy in neurons and ameliorates cognitive capacity in APP^{swe}/PS1^{dE9} mice. *Scientific Reports*. 2016;6:24566
- ²² Minzhen Deng, Liping Huang, Baile Ning, Nanbu Wang, Qinxin Zhang, Caixia Zhu, et al., β -asarone improves learning and memory and reduces Acetyl Cholinesterase and Beta-amyloid 42 levels in APP/PS1 transgenic mice by regulating Beclin-1-dependent autophagy, *Brain Research*, Volume 1652, 1 December 2016, Pages 188-194, ISSN 0006-8993
- ²³ Zhongfeng Xue, Yalei Guo, Yongqi Fang, Moderate activation of autophagy regulates the intracellular calcium ion concentration and mitochondrial membrane potential in beta-amyloid-treated PC12 cells, *Neuroscience Letters*, Volume 618, 8 April 2016, Pages 50-57, ISSN 0304-3940

-
- ²⁴ Singh, A.K., Kashyap, M.P., Tripathi, V.K. et al., Neuroprotection Through Rapamycin-Induced Activation of Autophagy and PI3K/Akt1/mTOR/CREB Signaling Against Amyloid- β -Induced Oxidative Stress, Synaptic/Neurotransmission Dysfunction, and Neurodegeneration in Adult Rats, *Mol Neurobiol* (2016)
- ²⁵ Deng, H. & Mi, M., Resveratrol Attenuates A β 25–35 Caused Neurotoxicity by Inducing Autophagy Through the TyrRS-PARP1-SIRT1 Signaling Pathway, *Neurochem Res* (2016) 41: 2367
- ²⁶ D. F. Silva, A. R. Esteves, C. R. Oliveira, S. M. Cardoso, Mitochondrial Metabolism Power SIRT2-Dependent Deficient Traffic Causing Alzheimer's-Disease Related Pathology, *Mol Neurobiol* (2016)
- ²⁷ Alexandre V. Ivachtchenko, Yan Lavrovsky, and Yan A. Ivanenkov, AVN-211, Novel and Highly Selective 5-HT₆ Receptor Small Molecule Antagonist, for the Treatment of Alzheimer's Disease, *Molecular Pharmaceutics* 2016 13 (3), 945-963
- ²⁸ Shariatpanahi, M., Khodagholi, F., Ashabi, G., Bonakdar Yazdi, B., Hassani, S., Azami, K., et al. The involvement of protein kinase G inhibitor in regulation of apoptosis and autophagy markers in spatial memory deficit induced by A β . *Fundam Clin Pharmacol*, 30: 364–375
- ²⁹ Tomohiro Umeda, Kenjiro Ono, Ayumi Sakai, Minato Yamashita, Mineyuki Mizuguchi, William L. Klein, et al. Rifampicin is a candidate preventive medicine against amyloid- β and tau oligomers. *Brain* 2016; 139 (5): 1568-1586
- ³⁰ Licht-Murava A, Paz R, Vaks L, Avrahami L, Plotkin B, Eisenstein M, et al. A unique type of GSK-3 inhibitor brings new opportunities to the clinic, *Sci. Signal.* 15 Nov 2016: Vol. 9, Issue 454, pp. ra110
- ³¹ Gao, J., Deng, Y., Yin, C., Liu, Y., Zhang, W., Shi, J. and Gong, Q. (2017), Icariside II, a novel phosphodiesterase 5 inhibitor, protects against H₂O₂-induced PC12 cells death by inhibiting mitochondria-mediated autophagy. *J. Cell. Mol. Med.*, 21: 375–386

-
- ³² Nguyen T. Tien, Ilker Karaca, Irfan Y. Tamboli and Jochen Walter J, Trehalose Alters Subcellular Trafficking and the Metabolism of the Alzheimer-associated Amyloid Precursor Protein, *Biol Chem.* 2016 May 13;291(20):10528-40
- ³³ Oriol Busquets, Miren Ettcheto, Mercè Pallàs, Carlos Beas-Zarate, Ester Verdaguer, Carme Auladell, et al. Long-term exposition to a high fat diet favors the appearance of β -amyloid depositions in the brain of C57BL/6J mice. A potential model of sporadic Alzheimer's disease, *Mech Ageing Dev*, EPUB 15 November 2016, ISSN 0047-6374
- ³⁴ Dong W., Wang R., Ma LN. et al., Influence of age-related learning and memory capacity of mice: different effects of a high and low caloric diet, *Aging Clin Exp Res* (2016) 28: 303
- ³⁵ Thangthaeng, N., Poulouse, S.M., Gomes, S.M. et al. Tart cherry supplementation improves working memory, hippocampal inflammation, and autophagy in aged rats. *AGE* (2016) 38: 393
- ³⁶ Braidly, N., Mohamed Essa, M., Poljak, A., Selvaraju, S., Al-Adawi, S., Manivasagam, T., et al. Consumption of pomegranates improves synaptic function in a transgenic mice model of Alzheimer's disease. *Oncotarget*, 7(40), 64589-64604
- ³⁷ Cecarini, V., Bonfili, L., Cuccioloni, M. et al., Effects of Ghrelin on the Proteolytic Pathways of Alzheimer's Disease Neuronal Cells, *Mol Neurobiol* (2016) 53: 3168
- ³⁸ Kou, X., Liu, X., Chen, X., Li, J., Yang, X., Fan, J., Yang, Y., & Chen, N. (2016). Ampelopsin attenuates brain aging of D-gal-induced rats through miR-34a-mediated SIRT1/mTOR signal pathway. *Oncotarget*, 7(46), 74484-74495
- ³⁹ Fernandes HJR, Hartfield EM, Christian HC, et al. ER Stress and Autophagic Perturbations Lead to Elevated Extracellular α -Synuclein in GBA-N370S Parkinson's iPSC-Derived Dopamine Neurons. *Stem Cell Reports*. 2016;6(3):342-356
- ⁴⁰ Amira Zarrouk, Thomas Nury, El-Mostafa Karym, Anne Vejux, Randa Sghaier, Catherine Gondcaille, et al. Attenuation of 7-ketocholesterol-induced overproduction of reactive oxygen

species, apoptosis, and autophagy by dimethyl fumarate on 158 N murine oligodendrocytes, *J Steroid Biochem Mol Biol*, EPUB 24 February 2016, ISSN 0960-0760

⁴¹ Lastres-Becker I, García-Yagüe AJ, Scannevin RH, et al. Repurposing the NRF2 Activator Dimethyl Fumarate as Therapy Against Synucleinopathy in Parkinson's Disease. *Antioxidants & Redox Signaling*. 2016;25(2):61-77

⁴² Redmann M, Wani WY, Volpicelli-Daley L, Darley-Usmar V, Zhang J. Trehalose does not improve neuronal survival on exposure to alpha-synuclein pre-formed fibrils. *Redox Biology*. 2017;11:429-437

⁴³ Qing He, James B. Koprach, Ying Wang, Wen-bo Yu, Bao-guo Xiao, Jonathan M. Brotchie et al., Treatment with Trehalose Prevents Behavioral and Neurochemical Deficits Produced in an AAV α -Synuclein Rat Model of Parkinson's Disease, *Mol Neurobiol*. 2016 May; 53(4):2258-68

⁴⁴ Miyara, M., Kotake, Y., Tokunaga, W., Sanoh, S. and Ohta, S. (2016), Mild MPP+ exposure impairs autophagic degradation through a novel lysosomal acidity-independent mechanism. *J. Neurochem.*, 139: 294–308

⁴⁵ Ghosh A, Tyson T, George S, Hildebrandt EN, Steiner JA, Madaj Z, et al., Mitochondrial pyruvate carrier regulates autophagy, inflammation, and neurodegeneration in experimental models of Parkinson's disease, *Science Translational Medicine* 07 Dec 2016: Vol. 8, Issue 368, pp. 368ra174

⁴⁶ Dzamko N, Gysbers A, Perera G, et al. Toll-like receptor 2 is increased in neurons in Parkinson's disease brain and may contribute to alpha-synuclein pathology. *Acta Neuropathologica*. 2017;133(2):303-319

⁴⁷ Zhong, J., Li, X., Wan, L., Chen, Z., Zhong, S., Xiao, S., & Yan, Z. (2016). Knockdown of NogoA prevents MPP+-induced neurotoxicity in PC12 cells via the mTOR/STAT3 signaling pathway. *Molecular Medicine Reports*, 13, 1427-1433

-
- ⁴⁸ Xiao-Su Gu, Fen Wang, Cai-Yi Zhang, Cheng-Jie Mao, Jing Yang, Ya-Ping Yang, et al., Neuroprotective Effects of Paeoniflorin on 6-OHDA-Lesioned Rat Model of Parkinson's Disease *Neurochem Res* (2016) 41: 2923
- ⁴⁹ Sheng Zhang, Xue-Hong, GuiLi-Ping, HuangMin-Zhen, DengRuo-Ming, FangXue-Hong Ke, Neuroprotective Effects of β -Asarone Against 6-Hydroxy Dopamine-Induced Parkinsonism via JNK/Bcl-2/Beclin-1 Pathway, *Mol Neurobiol* (2016) 53: 83
- ⁵⁰ Lingyun Li, Luyan Gao, Yunzhen Song, Zheng-Hong Qin, Zhongqin Liang, Activated cathepsin L is associated with the switch from autophagy to apoptotic death of SH-SY5Y cells exposed to 6-hydroxydopamine, *Biochem Biophys Res Commun*, Volume 470, Issue 3, 12 February 2016, Pages 579-585, ISSN 0006-291X
- ⁵¹ Jaishree Jalewa, Mohit Kumar Sharma, Simon Gengler, Christian Hölscher, A novel GLP-1/GIP dual receptor agonist protects from 6-OHDA lesion in a rat model of Parkinson's disease, *Neuropharmacology*, Volume 117, 1 May 2017, Pages 238-248, ISSN 0028-3908
- ⁵² Lu M, Su C, Qiao C, Bian Y, Ding J, Hu G. Metformin Prevents Dopaminergic Neuron Death in MPTP/P-Induced Mouse Model of Parkinson's Disease via Autophagy and Mitochondrial ROS Clearance. *International Journal of Neuropsychopharmacology*. 2016;19(9):pyw047
- ⁵³ Ana R. Esteves, Daniela M. Arduíno, Diana F. Silva, Sofia D. Viana, Frederico C. Pereira, Sandra M. Cardoso, Mitochondrial Metabolism Regulates Microtubule Acetylation and Autophagy Through Sirtuin-2: Impact for Parkinson's Disease, *Mol Neurobiol* (2017)
- ⁵⁴ De Oliveira RM, Vicente Miranda H, Francelle L, et al. The mechanism of sirtuin 2-mediated exacerbation of alpha-synuclein toxicity in models of Parkinson disease. Bates G, ed. *PLoS Biology*. 2017;15(3):e2000374

-
- ⁵⁵ Liu, J., Chen, M., Wang, X., Wang, Y., Duan, C., Gao, G., et al. Piperine induces autophagy by enhancing protein phosphatase 2A activity in a rotenone-induced Parkinson's disease model. *Oncotarget*, 7(38), 60823-60843
- ⁵⁶ Sarah Jinna, Robert E. Drolet, Paige E. Cramer, Andus Hon-Kit Wong, Dawn M. Toolan, et al., TMEM175 deficiency impairs lysosomal and mitochondrial function and increases α -synuclein aggregation, *Proc Natl Acad Sci USA*. 2017, 114(9):2389-2394
- ⁵⁷ Alexey V. Berezhnov, Marc P. M. Soutar, Evgeniya I. Fedotova, Maria S. Frolova, Helene Plun-Favreau, Valery P. Zinchenko, et al. Intracellular pH Modulates Autophagy and Mitophagy, April 15, 2016 *J Biol Chem* 291, 8701-8708
- ⁵⁸ Koentjoro B, Park J-S, Sue CM. Nix restores mitophagy and mitochondrial function to protect against PINK1/Parkin-related Parkinson's disease. *Scientific Reports*. 2017;7:44373
- ⁵⁹ Gentry EG, Henderson BW, Arrant AE, et al., Rho Kinase Inhibition as a Therapeutic for Progressive Supranuclear Palsy and Corticobasal Degeneration, *The Journal of Neuroscience*. 2016;36(4):1316-1323
- ⁶⁰ Feng-Tao Liu, Yu-Jie Yang, Jian-Jun Wu, Shan Li, Yi-Lin Tang, Jue Zhao, et al. Fasudil, a Rho kinase inhibitor, promotes the autophagic degradation of A53T α -synuclein by activating the JNK 1/Bcl-2/beclin 1 pathway, *Brain Research*, Volume 1632, 1 February 2016, Pages 9-18, ISSN 0006-8993
- ⁶¹ An-Guo Wu, Wu Zeng, Vincent Kam-Wai Wong, Yi-Zhun Zhu, Amy C.Y. Lo, Liang Liu, et al. Hederagenin and α -hederin promote degradation of proteins in neurodegenerative diseases and improve motor deficits in MPTP-mice, *Pharmacological Research*, Volume 115, January 2017, Pages 25-44, ISSN 1043-6618
- ⁶² Peipei Jin, Pengfei Wei, Yunjiao Zhang, Jun Lin, Rui Sha, Yi Hu, et al. Autophagy-mediated clearance of ubiquitinated mutant huntingtin by graphene oxide, *Nanoscale*, 2016,8, 18740-18750

-
- ⁶³ Vicente Miranda H, Gomes MA, Branco-Santos J, et al., Glycation potentiates neurodegeneration in models of Huntington's disease. *Scientific Reports*. 2016;6:36798
- ⁶⁴ Chiara Vidoni, Andrea Castiglioni, Christian Seca, Eleonora Secomandi, Mariarosa A.B. Melone, Ciro Isidoro, Dopamine exacerbates mutant Huntingtin toxicity via oxidative-mediated inhibition of autophagy in SH-SY5Y neuroblastoma cells: Beneficial effects of anti-oxidant therapeutics, *Neurochemistry International*, Volume 101, December 2016, Pages 132-143, ISSN 0197-0186
- ⁶⁵ Papp D, Kovács T, Billes V, et al. AUTEN-67, an autophagy-enhancing drug candidate with potent antiaging and neuroprotective effects. *Autophagy*. 2016;12(2):273-286
- ⁶⁶ Billes V, Kovács T, Hotzi B, Manzóger A, Tagscherer K, Komlós M et al., AUTEN-67 (Autophagy Enhancer-67) Hampers the Progression of Neurodegenerative Symptoms in a *Drosophila* model of Huntington's Disease, *Journal of Huntington's Disease*, vol. 5, no. 2, pp. 133-147, 2016
- ⁶⁷ Kovács T, Billes V, Komlós M, et al. The small molecule AUTEN-99 (autophagy enhancer-99) prevents the progression of neurodegenerative symptoms. *Scientific Reports*. 2017;7:42014
- ⁶⁸ Perucho J, Gómez A, Muñoz MP, de Yébenes JG, Mena MÁ, Casarejos MJ, Trehalose rescues glial cell dysfunction in striatal cultures from HD R6/1 mice at early postnatal development, *Mol Cell Neurosci*. 2016 Jul;74:128-45
- ⁶⁹ Lin CH, Wu YR, Yang JM, Chen WL, Chao CY, Chen IC et al., Novel Lactulose and Melibiose Targeting Autophagy to Reduce PolyQ Aggregation in Cell Models of Spinocerebellar Ataxia 3, *CNS Neurol Disord Drug Targets*. 2016;15(3):351-9
- ⁷⁰ S. Duarte-Silva, A. Silva-Fernandes, A. Neves-Carvalho, C. Soares-Cunha, A. Teixeira-Castro, P. Maciel, Combined therapy with m-TOR-dependent and -independent autophagy

inducers causes neurotoxicity in a mouse model of Machado–Joseph disease, *Neuroscience*, Volume 313, 28 January 2016, Pages 162-173, ISSN 0306-4522

⁷¹ Cunha-Santos J, Duarte-Neves J, Carmona V, Guarente L, Pereira de Almeida L, Cavadas C. Caloric restriction blocks neuropathology and motor deficits in Machado–Joseph disease mouse models through SIRT1 pathway. *Nature Communications*. 2016;7:11445

⁷² Jui-Chih Chang, Shey-Lin Wu, Fredrik Hoel, Yu-Shan Cheng, Ko-Hung Liu, Mingli Hsieh, Far-infrared radiation protects viability in a cell model of Spinocerebellar Ataxia by preventing polyQ protein accumulation and improving mitochondrial function, *Scientific Reports*, 6:30436

⁷³ Goode A, Butler K, Long J, et al., Defective recognition of LC3B by mutant SQSTM1/p62 implicates impairment of autophagy as a pathogenic mechanism in ALS-FTLD, *Autophagy*. 2016;12(7):1094-1104

⁷⁴ Ugolino J, Ji YJ, Conchina K, et al. Loss of C9orf72 Enhances Autophagic Activity via Deregulated mTOR and TFEB Signaling. Cox GA, ed. *PLoS Genetics*. 2016;12(11):e1006443

⁷⁵ Chang C-F, Lee Y-C, Lee K-H, et al. Therapeutic effect of berberine on TDP-43-related pathogenesis in FTLN and ALS. *Journal of Biomedical Science*. 2016;23:72

⁷⁶ Xia Q, Wang H, Hao Z, et al. TDP-43 loss of function increases TFEB activity and blocks autophagosome–lysosome fusion. *The EMBO Journal*. 2016;35(2):121-142

⁷⁷ Kuo-Wei Hsueh, Tzyy-Wen Chiou, Shu-Fen Chiang, Toru Yamashita, Koji Abe, Cesar V. Borlongan, et al. Autophagic down-regulation in motor neurons remarkably prolongs the survival of ALS mice, *Neuropharmacology*, Volume 108, September 2016, Pages 152-160, ISSN 0028-3908

⁷⁸ Cheryl Yung, Di Sha, Lian Li, Lih-Shen Chin, Parkin Protects Against Misfolded SOD1 Toxicity by Promoting Its Aggresome Formation and Autophagic Clearance, *Molecular Neurobiology* November 2016, Volume 53, Issue 9, pp 6270–6287

⁷⁹ Deepak Chhangani, Fumito Endo, Ayeman Amanullah, Arun Upadhyay, Seiji Watanabe, Ribhav Mishra, et al. Mahogunin ring finger 1 confers cytoprotection against mutant SOD1 aggresomes and is defective in an ALS mouse model, *Neurobiology of Disease*, Volume 86, February 2016, Pages 16-28, ISSN 0969-9961

⁸⁰ Valeria Crippa, Vito G. D’Agostino, Riccardo Cristofani, Paola Rusmini, Maria E. Cicardi, Elio Messi et al., Transcriptional induction of the heat shock protein B8 mediates the clearance of misfolded proteins responsible for motor neuron diseases, *Sci. Rep.* 6, 22827

⁸¹ M. Catarina Silva, Chialin Cheng, Waltraud Mair, Sandra Almeida, Helen Fong, M. Helal U. Biswas et al., Human iPSC-Derived Neuronal Model of Tau-A152T Frontotemporal Dementia Reveals Tau-Mediated Mechanisms of Neuronal Vulnerability, *Stem Cell Reports*, Vol. 7, 325–340, September 13, 2016

⁸² Holler CJ, Taylor G, McEachin ZT, et al. Trehalose upregulates progranulin expression in human and mouse models of GRN haploinsufficiency: a novel therapeutic lead to treat frontotemporal dementia. *Molecular Neurodegeneration*. 2016;11:46

⁸³ Yongdui Chen, Siqi Li, Liling Su, Jinghao Sheng, Wen Lv, Guangdi Chen, Zhengping Xu, Association of *progranulin* polymorphism rs5848 with neurodegenerative diseases: a meta-analysis, *Journal of Neurology*, April 2015, Volume 262, Issue 4, pp 814–822

⁸⁴ Moon, J., Lee, J., Lee, Y., & Park, S. (2016). Autophagy flux induced by ginsenoside-Rg3 attenuates human prion protein-mediated neurotoxicity and mitochondrial dysfunction. *Oncotarget*, 7(52), 85697-85708

- ⁸⁵ Moon J-H, Lee J-H, Lee Y-J, Park S-Y. Hinokitiol protects primary neuron cells against prion peptide-induced toxicity via autophagy flux regulated by hypoxia inducing factor-1. *Oncotarget*. 2016;7(21):29944-29957
- ⁸⁶ Ting Zhu, Deming Zhao, Zhiqi Song, Zhen Yuan, Chaosi Li, Yunsheng Wang, et al. HDAC6 alleviates prion peptide-mediated neuronal death via modulating PI3K-Akt-mTOR pathway, *Neurobiology of Aging*, Volume 37, January 2016, Pages 91-102, ISSN 0197-4580
- ⁸⁷ Sher Hayat Khan, Deming Zhao, Syed Zahid Ali Shah, Mohammad Farooque Hassan, Ting Zhu, Zhiqi Song, et al., Parkin Overexpression Ameliorates PrP106–126-Induced Neurotoxicity via Enhanced Autophagy in N2a Cells, *Cell Mol Neurobiol* (2016)
- ⁸⁸ Stocki P, Sawicki M, Mays CE, et al. Inhibition of the FKBP family of peptidyl prolyl isomerases induces abortive translocation and degradation of the cellular prion protein. Glick BS, ed. *Molecular Biology of the Cell*. 2016;27(5):757-767
- ⁸⁹ Dongyoung Kim, Hui-Yun Hwang, Jin Young Kim, Ju Yeon Lee, Jong Shin Yoo, György Marko-Varga, et al. FK506, an Immunosuppressive Drug, Induces Autophagy by Binding to the V-ATPase Catalytic Subunit A in Neuronal Cells, *Journal of Proteome Research* 2017 16 (1), 55-64
- ⁹⁰ Moon J-H, Lee J-H, Nazim UM, et al. Human prion protein-induced autophagy flux governs neuron cell damage in primary neuron cells. *Oncotarget*. 2016;7(21):29989-30002
- ⁹¹ Samuelsson K, Osman AAM, Angeria M, Risling M, Mohseni S, Press R. Study of Autophagy and Microangiopathy in Sural Nerves of Patients with Chronic Idiopathic Axonal Polyneuropathy. Nógrádi A, ed. *PLoS ONE*. 2016;11(9):e0163427
- ⁹² Clarke, J.-P. and Mearow, K., Autophagy inhibition in endogenous and nutrient-deprived conditions reduces dorsal root ganglia neuron survival and neurite growth in vitro. *Journal of Neuroscience Research*, 94: 653–670

- ⁹³ Jang, S. Y., Shin, Y. K., Park, S. Y., Park, J. Y., Lee, H. J., Yoo, Y. H., Kim, et al. Autophagic myelin destruction by schwann cells during wallerian degeneration and segmental demyelination. *Glia*, 64: 730–742
- ⁹⁴ Cristina A. Teixeira, Maria do Rosário Almeida, Maria João Saraiva, Impairment of autophagy by TTR V30M aggregates: in vivo reversal by TUDCA and curcumin, *Clinical Science* Aug 08, 2016, 130 (18) 1665-1675
- ⁹⁵ Frake RA, Ricketts T, Menzies FM, Rubinsztein DC. Autophagy and neurodegeneration. *The Journal of Clinical Investigation*. 2015;125(1):65-74
- ⁹⁶ Tor Erik Rusten & Harald Stenmark, p62, an autophagy hero or culprit, *Nature Cell Biology* 12, 207 - 209 (2010)
- ⁹⁷ Dale E. Bredesen, Rammohan V. Rao, Patrick Mehlen, Cell death in the nervous system, *Nature*. 2006 October 19; 443(7113): 796–802
- ⁹⁸ Ya-Chin Chang, Chia-Wei Lin, Chen-Ming Hsu, Guey-Jen Lee-Chen, Ming-Tsan Su, Long-Sun Ro, et al. Targeting the prodromal stage of spinocerebellar ataxia type 17 mice: G-CSF in the prevention of motor deficits via upregulating chaperone and autophagy levels, *Brain Research*, Volume 1639, 15 May 2016, Pages 132-148, ISSN 0006-8993
- ⁹⁹ Jose Felix Moruno Manchon, Ndidi-Ese Uzor, Steven Finkbeiner, and Andrey S. Tsvetkov, SPHK1/sphingosine kinase 1-mediated autophagy differs between neurons and SH-SY5Y neuroblastoma cells, *Autophagy* Vol. 12 , Iss. 8,2016
- ¹⁰⁰ Seung-Yong Yoon, Dong-Hou Kim, Alzheimer's disease genes and autophagy, *Brain Research*, Volume 1649, Part B, 15 October 2016, Pages 201-209, ISSN 0006-8993
- ¹⁰¹ Valdés P, Schneider BL. Gene Therapy: A Promising Approach for Neuroprotection in Parkinson's Disease? *Frontiers in Neuroanatomy*. 2016;10:123

¹⁰² Maria Xilouri, Leonidas Stefanis, Chaperone mediated autophagy in aging: Starve to prosper, Ageing Research Reviews, Volume 32, December 2016, Pages 13-21, ISSN 1568-1637

¹⁰³ Dong-Kyu Kim, Hee-Sun Lim, Ichiro Kawasaki, Yhong-Hee Shim, Nishant N. Vaikath, Omar M. A. El-Agnaf, et al. Anti-aging treatments slow propagation of synucleinopathy by restoring lysosomal function, Autophagy Vol. 12 , Iss. 10,2016