Brain Disorders Due to Lysosomal Dysfunction

Alessandro Fraldi,[1] Andrés D. Klein,[1] Diego L. Medina,[1] and Carmine Settembre[1,2,3]

[1]Telethon Institute of Genetics and Medicine (TIGEM), 80078 Pozzuoli, Italy

[2]Dulbecco Telethon Institute, 80078 Pozzuoli, Italy

[3]Medical Genetics Unit, Department of Medical and Translational Science, Federico II University, 80131 Naples, Italy; email: fraldi@tigem.it, settembre@tigem.it

Annu. Rev. Neurosci. 2016. 39:277–95

First published online as a Review in Advance on April 18, 2016

The *Annual Review of Neuroscience* is online at neuro.annualreviews.org

This article's doi: 10.1146/annurev-neuro-070815-014031

Keywords

lysosome, autophagy, lysosomal storage disorders, neurodegenerative diseases, α-synuclein, transcription factor EB, TFEB

Abstract

Recent studies of autophagic and lysosomal pathways have significantly changed our understanding of lysosomes; once thought to be simple degradative and recycling centers, lysosomes are now known to be organelles capable of influencing signal transduction, via the mammalian target of rapamycin complex 1 (mTORC1), and regulating gene expression, via transcription factor EB (TFEB) and other transcription factors. These pathways are particularly relevant to maintaining brain homeostasis, as dysfunction of the endolysosomal and autophagic pathways has been associated with common neurodegenerative diseases, such as Alzheimer's, Parkinson's, and Huntington's, and lysosomal storage disorders, a group of inherited disorders characterized by the intralysosomal buildup of partially degraded metabolites. This review focuses on the cellular biology of lysosomes and discusses the possible mechanisms by which disruption of their function contributes to neurodegeneration. We also review and discuss how targeting TFEB and lysosomes may offer innovative therapeutic approaches for treating a wide range of neurological conditions.

Contents

INTRODUCTION

A Journey Through the Lysosomal System

The discovery of lysosomes, through the pioneering work of Christian De Duve and colleagues (De Duve et al. 1955), initiated a new era in cell biology. De Duve described this organelle as an acidic compartment containing several hydrolases. Lysosomes are the main digestive and recycling centers of the cell. Their role is to degrade complex biological molecules (e.g., proteins and lipids) into simpler components (e.g., amino acids and free fatty acids), which can then be recycled to the cytosol and reused either as a source of energy or as building blocks for the biosynthesis of new molecules. Lysosomal degradation is also crucial to preventing the accumulation of toxic or exhausted molecules and damaged organelles in the cell. This latter function is particularly important in nondividing cells, such as neurons, which cannot dilute cytosolic material during mitotic events (Settembre et al. 2013).

Substrates from different locations are delivered to lysosomes through two main pathways: (*a*) Extracellular material reaches the lysosome mainly through endocytosis, which starts at the plasma membrane with the formation of endocytic vesicles carrying cargo molecules; and (*b*) intracellular material is transported to the lysosome through autophagic pathways (**Figure 1**) (Saftig & Klumperman 2009). Three types of autophagy have been identified: microautophagy, chaperone-mediated autophagy, and macroautophagy (Mizushima 2007). During microautophagy, cytosolic proteins are engulfed by the lysosome via the invagination of lysosomal or endosomal membranes. In chaperone-mediated autophagy, cytosolic proteins are transported into the lysosomal lumen through receptor-mediated internalization. However, the most common form of autophagy is macroautophagy, herein referred to simply as autophagy. Autophagy relies on the biogenesis of autophagic vesicles (AVs), which are double membrane–bound organelles originating at endoplasmic reticulum–mitochondria contact sites (Hamasaki et al. 2013). Most of the autophagic cargoes are sequestered by nascent AVs through a receptor-mediated recognition process (Rogov et al. 2014).

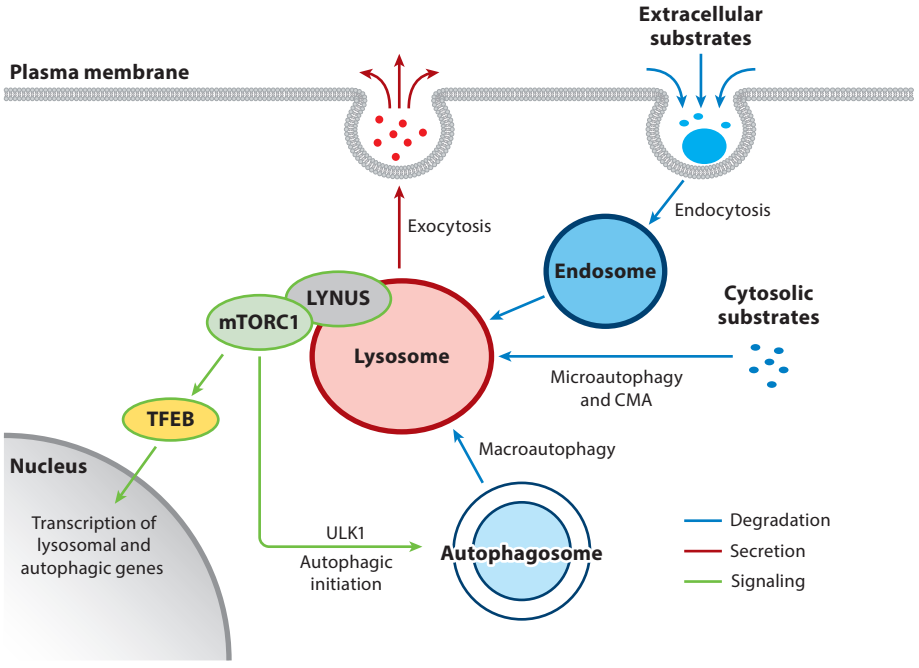

Figure 1

Lysosomal inputs, outputs, and their relationship with key signaling processes. Inputs: Extracellular material is delivered to the lysosomes via endocytosis, and intracellular substrates reach the lysosomes via autophagy, including microautophagy, CMA, and macroautophagy (*blue lines*). Outputs: Lysosomes can fuse with the plasma membrane to drain their contents into the extracellular space and to allow efficient sealing of the plasma membrane in case of injury (*red line*). Signaling: Lysosomes sense nutrient levels and lysosomal stress through the LYNUS machinery on the lysosomal membrane and mTORC1, which can regulate autophagy directly through ULK1 and TFEB phosphorylation (*green lines*). Abbreviations: CMA, chaperone-mediated autophagy; LYNUS, lysosomal nutrient-sensing; mTORC1, mammalian target of rapamycin complex 1; TFEB, transcription factor EB.

Autophagy is particularly relevant in maintaining neuronal health because several potentially harmful proteins (e.g., α-synuclein and huntingtin) are autophagy substrates and accumulate in neurons when autophagy is impaired (Menzies et al. 2015).

Cargoes are delivered to the lysosomes mainly by their fusion with endocytic vesicles and AVs. This requires the coordinated activity of three main classes of proteins: (*a*) motor and adaptor protein complexes that regulate the vectorial movements of vesicles along microtubules (Ravikumar et al. 2005); (*b*) membrane-associated RAB GTPases, such as RAB7, which control organelle trafficking by recruiting multiple effector proteins that mediate the tethering (HOPS proteins) and fusion [soluble *N*-ethylmaleimide-sensitive factor attachment protein (SNAP) receptors (SNAREs)] of target membranes (Kummel & Ungermann 2014); and (*c*) lysosomal membrane proteins (i.e., LAMPs) and ion channels (i.e., MCOLN1, CACNA1A, and P2X4), which facilitate homotropic and heterotropic membrane fusion events (Cao et al. 2015, Eskelinen 2006, Kim et al. 2009, Nishino et al. 2000, Tian et al. 2015).

Once inside the lysosome, substrates are degraded by hydrolases, including sulfatases, glycosidases, peptidases, phosphatases, lipases, and nucleases. These enzymes function at an acidic pH, which is generated in the lumen of the lysosome by the activity of lysosomal ion channels and

transporters (Mindell 2012). The main regulator of lysosomal pH is the proton-pumping V-type adenosine triphosphatase (ATPase), which uses the energy of ATP hydrolysis to pump H^+ into the lumen of the lysosomes. Also contributing to maintaining lysosomal pH are the anion transporter chloride channel 7 (ClC7), the nonselective cation channel mucolipin 1 (TRPML1), the two-pore calcium channels (TCP1 and TCP2), and the potassium channel TMEM175 (Cang et al. 2013, 2015; Graves et al. 2008; Mindell 2012).

Surprisingly, the lysosomal efflux of catabolic products is a mechanism that has been poorly characterized. Very few transporters have been identified: amino acid transporters, such as cystinosin and the PQLC2, LAAT-1, and PAT1 proteins (Kalatzis et al. 2001, Liu et al. 2012, Sagne et al. 2001); sugar exporters, such as sialin and spinster (Rong et al. 2011, Verheijen et al. 1999); and the cholesterol exporter Niemann–Pick disease type C1 (NPC1) protein (Davies et al. 2000). As discussed in the next section, dysfunction in any of the steps required for lysosomal cargo targeting, degradation, and recycling has a detrimental effect on cellular homeostasis and represents the possible cause of many progressive neurodegenerative disorders.

The Lysosome as a Signaling Organelle

The static view of the lysosome as a simple digestive vacuole has changed significantly during the past decade. It is now recognized that lysosomes have a broader function and are involved in several other fundamental processes, including regulating signaling and energy metabolism (**Figure 1**) (Settembre et al. 2013). In fact, the mammalian target of rapamycin complex 1 (mTORC1) kinase, a master controller of cell and organism growth, is activated on the lysosomal surface (Sancak et al. 2008, 2010). The docking of mTORC1 to the lysosomal membrane is regulated by the lysosomal nutrient-sensing (LYNUS) machinery, which is composed of a growing list of proteins that cooperate to sense amino acid levels in the lumen of lysosomes (**Figure 1**). Multiple lysosomal transmembrane and membrane-associated proteins, including the lysosomal V-ATPase, belong to the LYNUS machinery. Pharmacological inhibition of V-ATPase strongly suppresses mTORC1 activity, further underlining the central role of the lysosome as a regulator of mTORC1 activity (Zoncu et al. 2011).

Recently, mTORC2 has also been found to be associated with the lysosomal membrane (Arias et al. 2015). The Akt kinase and the phosphatase PHLPP1 can also associate with lysosomal membranes to regulate mTORC2 activity (Arias et al. 2015). Although the function of lysosomes in the PHLPP1–Akt–mTORC2 axis remains unknown, these findings suggest that lysosomes may play a broad role as regulators of intracellular signaling.

Lysosome Biogenesis

Lysosome biogenesis relies on both endocytic and biosynthetic pathways. Newly synthesized lysosomal proteins reach lysosomes directly (e.g., through mannose-6-phosphate-dependent trafficking from the *trans*-Golgi network) or indirectly (from the plasma membrane through endocytosis) (for further details, see Saftig & Klumperman 2009). The observation that lysosomal genes tend to be coexpressed in different tissues and cell types under varying conditions suggests that lysosome biogenesis is a transcriptionally regulated process. This hypothesis led to the discovery of the coordinated lysosomal expression and regulation (CLEAR) network and its master regulator transcription factor EB (TFEB), a member of the microphthalmia-associated transcription factor (MITF) subfamily of transcription factors. The activation of the CLEAR network by TFEB enhances the expression of lysosomal genes, which increases the number of lysosomes and promotes the degradation of lysosomal substrates (Palmieri et al. 2011, Sardiello et al. 2009). TFEB

also activates the transcription of genes encoding proteins involved in a wide variety of functions related to lysosomal cellular clearance, such as lysosomal biogenesis, autophagy, exocytosis, and phagocytosis (Palmieri et al. 2011, Settembre et al. 2011).

The identification of the CLEAR network and of TFEB indicates that cellular clearance regulation is a dynamic process that can be modulated according to cellular requirements. Indeed, in normal feeding conditions TFEB resides in the cytoplasm, but in conditions known to promote cellular catabolism, such as nutrient deprivation, TFEB translocates to the nucleus, where it activates the CLEAR network (Settembre et al. 2011).

The cytosol-to-nucleus shuttling of TFEB is modulated by phosphorylation, which involves multiple kinases. The main regulator of TFEB nuclear translocation is mTORC1, which phosphorylates at least two critical serine residues (Ser211 and Ser142) of TFEB. When phosphorylated, TFEB is retained in the cytoplasm through its interaction with members of the 14-3-3 protein family. The activity of mTORC1 is regulated by the lysosome; thus, these observations suggest the existence of a lysosome-to-nucleus signaling mechanism that regulates lysosome biogenesis and function through mTORC1 and TFEB. Cellular conditions that lead to mTORC1 inactivation, such as nutrient starvation or lysosomal inhibition, induce TFEB nuclear translocation and de novo lysosome and autophagosome biogenesis (Martina et al. 2012, Roczniak-Ferguson et al. 2012, Settembre et al. 2012). Interestingly, other members of the basic helix-loop-helix family of transcription factors, such as MITF and TFE3, seem to be regulated by similar mechanisms (Martina et al. 2012, Roczniak-Ferguson et al. 2012).

Recently, a calcium-activated serine–threonine phosphatase, calcineurin, was identified as a TFEB phosphatase. Calcineurin dephosphorylates TFEB at the key residues Ser211 and Ser142, which are involved in determining the subcellular localization of TFEB. Calcineurin activity is induced locally by lysosomal calcium release through the lysosomal calcium channel MCOLN1, indicating that lysosomes can regulate TFEB activity in multiple ways (Medina et al. 2015).

An additional mechanism through which lysosomal homeostasis can be maintained is the autophagic lysosomal reformation process. During growth factor–induced autophagy, the increased frequency of AV–lysosome fusion induces autolysosome accumulation and lysosomal consumption. This depletion is counterbalanced by the de novo formation of lysosomes from autolysosomes through a membrane tubulation process mediated by transient and autophagy-mediated mTORC1 reactivation (Yu et al. 2010).

LYSOSOMAL STORAGE DISORDERS

Overview

Lysosomal storage disorders (LSDs) are inherited diseases characterized by the progressive accumulation of undigested macromolecules within the cell due to lysosomal dysfunction (**Figure 2**). More than 50 different types of LSDs have been described, occurring owing to mutations in genes encoding for lysosomal soluble hydrolases, membrane proteins, or lysosomal accessory proteins that result in a specific lysosomal catabolic pathway being blocked (Futerman & van Meer 2004).

Nearly two-thirds of patients with LSDs exhibit at least some neurological involvement (Boustany 2013). The clinical presentations are heterogeneous, as systemic and neurological signs arise at different ages and progress at different rates. Usually, disturbances in vision are among the earliest neurological signs. Actually, the first case of an LSD was reported by Warren Tay, a British ophthalmologist, in 1881 (Fernandes Filho & Shapiro 2004). As these diseases progress, children usually experience seizures, hearing loss, intellectual disability, and neuromotor regression. Common, late-onset (adult) manifestations include depression, dementia, and psychosis, in addition

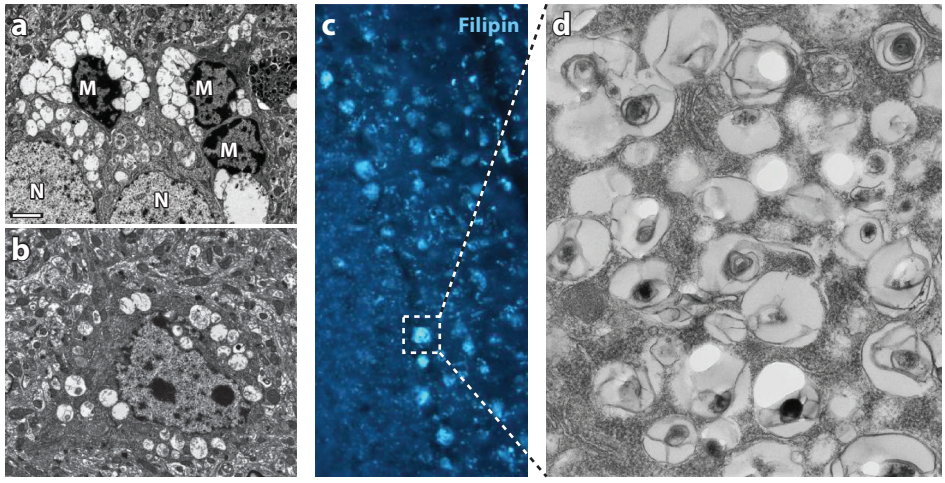

Figure 2

Buildup of storage material in brains from mice with lysosomal storage disorders. (*a*) Transmission electron microscopy of microglial cells (M) juxtaposed with hippocampal neurons (N) in the brain of a mouse model of multiple sulfatase deficiency (MSD). Note the massive lysosomal storage in the cytoplasm of microglial cells compared with neurons. The scale bar indicates 3 μm. (*b*) Lysosomal vacuolization in Purkinje cells from an MSD mouse model. (*c*) Cerebellar cholesterol accumulation, visualized by filipin staining, in a mouse model of Niemann–Pick type C (NPC) disease. (*d*) Ultrastructure of storage bodies in Purkinje cells from the NPC mouse model.

to other neurological deficits (Jardim et al. 2010, Pastores & Maegawa 2013). The neurological manifestations largely result in neuronal dysfunction or even death (Bellettato & Scarpa 2010). Common morphological neuronal alterations include neuroaxonal dystrophies (with axonal spheroids), meganeurites, and ectopic dendritogenesis (March et al. 1997, Micsenyi et al. 2009, Siegel & Walkley 1994).

In addition to morphological changes, individual LSDs show specific temporal and spatial neuropathological alterations, with neurodegeneration and inflammation affecting regions of early vulnerability. The proposed reasons for neuronal-specific susceptibilities are (*a*) the buildup of metabolites, which may exert differential signaling effects on each neuronal subtype; (*b*) different neuronal populations, which require varying proportions of macromolecules; and (*c*) other intrinsic differential neuronal vulnerabilities to storage (Platt et al. 2012).

An unresolved question in the field is how endosomal and lysosomal alterations lead to the distinctive cell dysfunction and pathology that is characteristic of each disease. We review two distinct pathogenetic processes that have been proposed to contribute to neurodegeneration in LSDs.

Cell-Autonomous Dysfunction: Defective Autophagy

Basal levels of autophagy are essential for neuronal survival and function (Hara et al. 2006, Komatsu et al. 2006). Autophagy prevents toxic proteins from reaching harmful concentrations in neurons and degrades exhausted organelles, such as mitochondria. Genetic inhibition of autophagy in neurons consistently induces the progressive accumulation of cytosolic polyubiquitinated proteins and dysfunctional mitochondria, ultimately leading to neurodegeneration in mouse models (Hara et al. 2006, Komatsu et al. 2006). Purkinje cells lacking autophagy show axonal dystrophic swelling that precedes cell death (Komatsu et al. 2007). Similarly, neurons in different LSD mouse models

show the progressive accumulation of polyubiquitinated proteins and dysfunctional mitochondria as prominent features, suggesting that autophagic dysfunction may mediate neurodegeneration in LSDs (Lieberman et al. 2012, Settembre et al. 2008). Multiple independent experiments have suggested that the fusion between the lysosome and autophagosome is delayed when the lysosome is filled with storage material. The cytoplasm of neurons isolated from different LSD models is consistently full of undigested autophagosomes, dysfunctional mitochondria, and protein aggregates, which stain positively for the autophagy receptor P62 (SQSTM1) (de Pablo-Latorre et al. 2012, Lieberman et al. 2012).

The molecular mechanisms by which lysosomal storage impairs autophagy flux require further investigation. One plausible explanation for the block in AV–lysosome fusion observed in LSDs could be that lysosomal storage inhibits the process of autophagic lysosome reformation during autophagy. Notably, fibroblasts derived from patients with Scheie syndrome (cell line GM01256), Fabry's disease (GM00636), and aspartylglucosaminuria (GM02056) show impaired mTOR reactivation and defective de novo lysosome reformation upon prolonged serum starvation. This, in turn, may lead to the accumulation of hybrid vesicles, which are not able to fuse with newly formed AVs (Yu et al. 2010). This condition may ultimately block the autophagy flux.

In addition, experimental evidence obtained from LSD cells has suggested that lysosomal storage induces a secondary accumulation of cholesterol in the endolysosomal membrane of LSD cells, thereby reducing the ability of lysosomes to fuse efficiently with both endocytic vesicles and AVs. Mechanistically, cholesterol accumulation impairs the function of the SNAREs, which are key components of the cellular membrane fusion machinery (Fraldi et al. 2010). Reducing membrane cholesterol levels in LSD cells restores normal SNARE function and, in turn, membrane fusion. These results support a model in which cholesterol abnormalities determine lysosomal dysfunction and the endocytic traffic jam in LSDs by impairing the membrane fusion machinery (Fraldi et al. 2010).

The accumulation of lipids, either as primary or as secondary storage products, in LSDs may also contribute to autophagy inhibition by disturbing lysosomal calcium homeostasis. Recent evidence has indicated that calcium regulates many important lysosomal functions, including the fusion between autophagosomes and lysosomes. Alterations in cellular calcium homeostasis have been described in different LSDs and, in some cases, have been proposed to occur as a consequence of dysfunction in the activity of lysosomal calcium channel TRPML1, the mutation of which causes mucolipidosis type IV (ML-IV). TRPML1 is involved in the maturation of the endolysosomal compartment and in AV–lysosome fusion (Curcio-Morelli et al. 2010, Medina et al. 2015, Vergarajauregui et al. 2008, Wang et al. 2015). In addition, overexpression of TRPML1 increases LC3-positive autophagosomes and autophagic flux, a process that can be inhibited by the calcium chelator BAPTA (Medina et al. 2015). This evidence suggests that TRPML1 may promote vesicle fusion through lysosomal calcium release (Medina et al. 2015, Wang et al. 2015). In NPC1, an LSD characterized by the accumulation of a broad range of lipids (Butler et al. 1993, te Vruchte et al. 2004), the accumulation of sphingomyelin dramatically inhibits TRPML1 activity in lysosomes, and TRPML1 overexpression or activation rescues trafficking defects and ameliorates lysosomal storage in NPC1 cells (Shen et al. 2012, Wang et al. 2015). Sphingomyelin accumulates in various LSDs, suggesting that TRPML1 impairment may be part of the pathogenic mechanism of various LSDs (Chen et al. 2010, Lloyd-Evans et al. 2008, Schulze & Sandhoff 2011, Xu et al. 2012).

Non-Cell-Autonomous Mechanisms of Neurodegeneration

Neuronal survival and function strictly depend on the homeostasis of the central nervous system (CNS), which, in turn, relies on the critical activities of other CNS-resident cell populations, such

as astrocytes and microglia. Astrocytes are the main neuronal cell type responsible for maintaining brain homeostasis. They play a critical part in neurotransmitter trafficking and recycling, nutrient and ion metabolism, and protection against oxidative stress. Consistent with such a variety of fundamental functions exerted by astrocytes to support neurons, astrocyte impairment has been found to contribute to neuronal dysfunction in several neurodegenerative diseases, such as amyotrophic lateral sclerosis, Alzheimer's disease (AD), and Huntington's disease (HD). Several studies have demonstrated that astrocytes are integral components of the neuropathology of LSDs, suggesting that modulation of astrocyte function may impact the course of the disease (Rama Rao & Kielian 2016). For example, a mouse model of multiple sulfatase deficiency (MSD), in which *Sumf1* was selectively deleted in astrocytes to induce lysosomal storage in astrocytes alone, showed progressive cortical neurodegeneration despite undetectable lysosomal vacuolization in the cytoplasm of neurons (Di Malta et al. 2012). Ex vivo neuronal astrocyte coculture experiments have demonstrated that astrocytes affected by lysosomal storage have reduced glutamate buffering capacity, which could, in turn, induce neuronal excitotoxicity (Di Malta et al. 2012). Consistent with this observation, cultured NPC astrocytes show increased membrane permeability due to overexpression of connexin 43 hemichannels, which probably allows the release of glutamate (Saez et al. 2013). However, the detailed molecular cascade leading from lysosomal storage to altered glutamate buffering remains unknown. It is possible that the endocytic trafficking of glutamate receptors may also be impaired by defects in the endolysosomal system.

An additional mechanism by which lysosomal storage in astrocytes could induce neurodegeneration is through the buildup of lipid droplets. In both *Drosophila* and mouse models of neurodegenerative disorders, the accumulation of lipid droplets in glial cells is sufficient to trigger neurodegeneration (Liu et al. 2015). Emerging data suggest that lysosomes have a crucial function in regulating lipid droplet homeostasis. The inhibition of lysosomal function, such as that observed in LSDs, leads to the accumulation of lipid droplets owing to defective degradation (Singh et al. 2009). The influence of astrocyte dysfunction on neuronal loss is particularly relevant for some neuronal populations, such as cortical neurons. In fact, behavioral studies in MSD mice have attributed specific aspects of the neurobehavioral phenotype only to astrocyte dysfunction (Di Malta et al. 2012).

Microglia are the resident immune cells in the brain and share many, if not all, of the properties of macrophages in other tissues. Electron microscopy analysis of brain slices isolated from LSD mouse models show prominent vacuolization in macrophages and microglial cells (**Figure 2**). Expansion and activation of the microglial cell population have been observed throughout the brain in multiple LSD models characterized by neurodegeneration. This is associated with a generalized neuroinflammatory phenotype, which is characterized by increased levels of cytokines, such as tumor necrosis factor-α, macrophage inflammatory protein-1α, and interleukins, and of reactive oxygen species, which can further exacerbate neuronal stress (Di Malta et al. 2012, Fraldi et al. 2007, Vitner et al. 2015, Wu & Proia 2004).

Microglial activation in LSDs may represent an attempt by the organism to remove undigested material or damaged neurons. Therefore, the massive storage observed in microglia may derive not only from endogenous buildup but also from their phagocytic activity. In an additional scenario, microglial activation could be the consequence of neuronal dysfunction (Enquist et al. 2007, Lopez et al. 2011, Yu et al. 2011). This activation could be the consequence of defective neuronal apoptosis due to impaired autophagy that diverts neurons toward different types of neuronal death, such as necrosis or necroptosis, which are associated with chronic microglial activation. A notable example is Gaucher's disease, in which defective caspase-8 activity leads to the activation of RIPK-1 and -3 in neurons and microglia, which probably lead respectively, to necroptosis and neuroinflammation (Vitner et al. 2014). However, blocking neuroinflammation in different mouse models of LSDs

seems to ameliorate neurodegeneration only partially, suggesting that microglial activation does not play a major part in the CNS phenotype (Luzi et al. 2009, Macauley et al. 2014, Williams et al. 2014).

A BROADER ROLE FOR THE LYSOSOME IN NEURODEGENERATIVE DISORDERS

Other Neurological Diseases with Lysosomal Involvement

Lysosomal dysfunction has been associated with neuropathology not only in LSDs but also in many other neurodegenerative diseases, including common, late-onset forms of neurodegeneration [Parkinson's disease (PD), HD, and AD] (Nixon et al. 2008). Several factors contribute to the failure of lysosomal function in these neurodegenerative conditions. These can be classified into two major groups: aging and genetic factors (**Figure 3**). Age-associated changes affect either lysosomal hydrolase activities or key lysosomal membrane components (e.g., cholesterol or

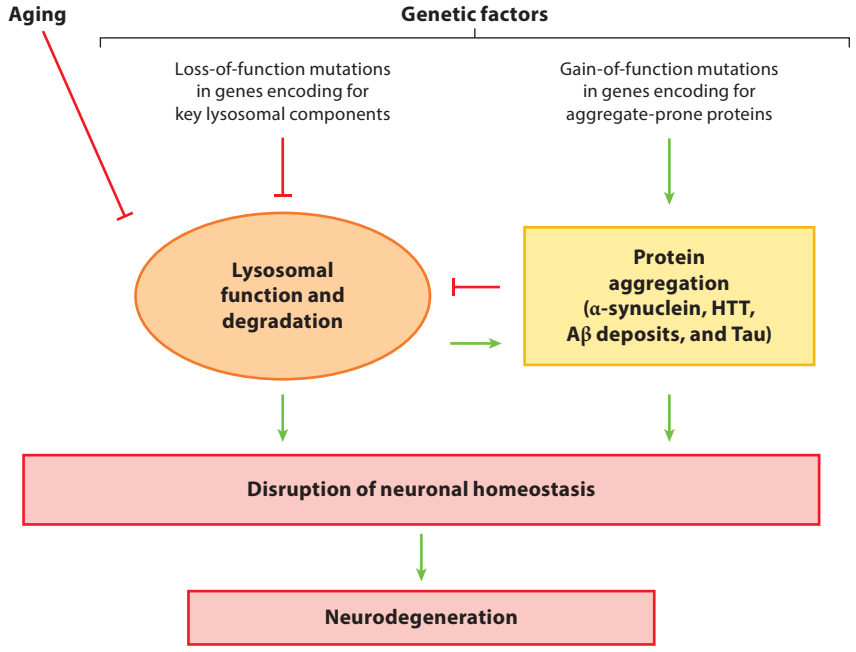

Figure 3

Central role of lysosomes in neurodegenerative diseases. Neuronal cell death caused by defective cellular clearance can result from at least two interconnected mechanisms. First, loss-of-function mutations in lysosomal genes [i.e., *TRPML1* (mucolipin 1), *NPC1* (Niemann–Pick type C1), *GBA1* (β-glucosidase-1, acid), and many others] affect cellular degradation and recycling processes. Second, gain-of-function mutations of aggregate-prone proteins [i.e., α-synuclein, HTT (huntingtin), amyloid-β (Aβ) deposits, and Tau] may lead to enhanced protein aggregation. Interestingly, lysosomal dysfunction can lead to enhanced protein aggregation or the reverse. The consequences of both types of alterations can increase the levels of neurotoxic compounds that may disrupt neuronal homeostasis [i.e., autophagy, calcium signaling, protein homeostasis (proteostasis), and others], ultimately leading to neurodegeneration. Finally, researchers have observed a reduced lysosomal autophagic function during aging that may also contribute to neuronal dyshomeostasis and cell death.

LAMP-2A) and have been reported to limit rates of protein degradation (Cuervo & Dice 2000). Genetic factors involve mutations in genes encoding either for key lysosomal components or for proteins involved in transport to the lysosome, mitophagy, or other autophagy-related functions. However, in some cases, lysosomal activity can be affected by indirect pathways, such as the generation of protein aggregates caused by gain-of-function mutations.

Amyloid-β (Aβ) deposits are a major pathological hallmark of AD. It has been reported that the functionality of endolysosomal recycling trafficking is critical to determining the so-called amyloidogenic process by which the amyloid precursor protein is sequentially cleaved to generate Aβ peptides (Das et al. 2013, Rajendran & Annaert 2012). Therefore, any detrimental effect on endolysosomal transport results in alterations of Aβ production. A subset of AD patients carry mutations in presenilin-1 (*PSEN1*) that show lysosomal and autophagic dysfunction (Lee et al. 2010). Lysosome dysfunction in these patients can be explained by two different mechanisms, one involving defects in the lysosomal acidification machinery and the other in lysosomal Ca^{2+} homeostasis (Coen et al. 2012, Lee et al. 2015).

HD is an inherited brain condition caused by mutations in huntingtin (*HTT*), a gene encoding the aggregate-prone protein HTT. When mutated, the HTT protein forms abnormal, toxic polyglutamine expansions (Walker 2007). Expanded HTT may affect the efficiency of autophagy by inhibiting cargo recognition by autophagosomes (Jeong et al. 2009), by inhibiting autophagosome biogenesis and transport, or both (Rui et al. 2015, Wong & Holzbaur 2014). Notably, the pharmacological induction of autophagy in HD mouse and fly models ameliorates the phenotype of the disease (Ravikumar et al. 2004, Yamamoto et al. 2006).

PD is the second most common neurodegenerative disorder, affecting approximately 1.6% of the population older than 60 years (Bertram & Tanzi 2005). α-Synuclein is an aggregate-prone protein that forms intraneuronal inclusions called Lewy bodies. α-Synuclein accumulation and aggregation play a central role in the pathophysiology of PD and in a subset of neurodegenerative conditions known as synucleinopathies (Spillantini et al. 1997). Genetic forms of PD are often directly associated with mutations in the gene encoding α-synuclein (*SNCA*), which lead to the abnormal aggregation of the protein (Bras et al. 2015). Recent findings have shown the involvement of lysosomal dysfunction in these processes, although the mechanisms that mediate α-synuclein aggregation and neurotoxicity remain unclear. The aggregated forms of α-synuclein can, in fact, bind the lysosome, thus disrupting its activity (Cuervo et al. 2004, Freeman et al. 2013, Martinez-Vicente et al. 2008, Winslow et al. 2010). In addition, α-synuclein toxicity has been reported to be associated with a progressive decline in markers of lysosome function and to be accompanied by cytoplasmic retention of TFEB, highlighting the importance of lysosomal and autophagic dysfunction in PD (Decressac et al. 2013). Mechanistically, the sequestration of TFEB in the cytoplasm may be due to the accumulation of α-synuclein, which shares structural and functional homology with 14-3-3 proteins and can bind similar targets (Ostrerova et al. 1999, Perez et al. 2002). Similarly, the polyglutamine-expanded androgen receptor (polyQ-AR), which is associated with X-linked spinal and bulbar muscular atrophy, interferes with TFEB transactivation, which accounts for the autophagic flux defects present in the disease's motor neuron–like cells (Cortes et al. 2014).

A significant number of patients with PD show mutations in lysosomal genes, which represent risks and predisposing factors for disease pathogenesis (Shachar et al. 2011). Several familial forms of PD present heterozygous mutations in the gene encoding for the lysosomal enzyme β-glucocerebrosidase (*GBA*), and homozygous mutations cause Gaucher's disease, a neurovisceral degenerative LSD (Sidransky et al. 2009). Lower levels of GBA in lysosomes lead to the increased accumulation of glucosylceramide, which stabilizes soluble oligomeric α-synuclein intermediates that, in turn, are converted into amyloid fibrils (Mazzulli et al. 2011). Furthermore, the accumulation of α-synuclein also blocks the trafficking of newly synthesized GBA to the lysosome and,

thus, further amplifies glucosylceramide accumulation. These data have revealed the existence of a bidirectional pathogenic loop mediated by α-synuclein and GBA (Mazzulli et al. 2011). In hereditary forms of parkinsonism, mutations in ATPase type 13A2 (*ATP13A2*), a component of the lysosomal acidification machinery, are associated with lysosomal dysfunction, defective clearance of autophagosomes, and accumulation of α-synuclein (Ramirez et al. 2006). Interestingly, mutated forms of *ATP13A2* have been recently found in patients with neuronal ceroid lipofuscinosis, a severe neurodegenerative LSD (Bras et al. 2012). Mutations in the *LRRK2* gene cause autosomal-dominant forms of PD (Zimprich et al. 2004). Although the link between *LRRK2* mutations and lysosomal pathways has not been completely elucidated, the expression of LRRK2 mutants in in vitro and in vivo models induced lysosomal stress characterized by the accumulation of multivesicular bodies and abnormal autophagosomes (reviewed in Li et al. 2014). Mutations in *VPS35* (vacuolar protein sorting 35), which encodes for an endosomal protein involved in the retrograde transport between endosomes and the *trans*-Golgi network, have been found in some PD patients (Zimprich et al. 2011). Finally, a significant number of hereditary forms of PD show mutations in the *PINK* (PTEN-induced putative kinase) or *PARKIN* (PD protein) genes associated with the accumulation of dysfunctional mitochondria owing to defective mitophagy (Geisler et al. 2010).

In addition to the more common, late-onset forms of neurodegeneration described above, lysosomal autophagic dysfunction is involved in the pathogenesis of other neuropathologies. Impaired autophagic flux and an excessive number of autophagic vacuoles may contribute to the development of amyotrophic lateral sclerosis (Song et al. 2012). Moreover, mutations in either the small integral membrane protein of lysosome/late endosome (SIMPLE) or endosomal-localized protein RAB7 cause autosomal-dominant forms of one of the most common neuromuscular disorders, Charcot–Marie–Tooth disease, by, respectively, dysregulating endosomal trafficking (Lee et al. 2012) or altering endosomal-mediated signaling (BasuRay et al. 2013). Mutations in the dynein and dynactin motor proteins cause motor neuron diseases characterized by defective AV–lysosome fusion.

The Emerging Role of α-Synuclein in Lysosomal-Mediated Neurodegeneration

How lysosomal failure impacts pathogenic cascades in neurodegenerative diseases downstream is the object of intense work. Lysosomal autophagic pathways are crucial to maintaining normal neuronal homeostasis and function. Therefore, any decline in their functions may contribute to neuropathogenic processes (Harris & Rubinsztein 2012, Wong & Cuervo 2010).

Increasing attention has been paid to α-synuclein as a critical molecule that potentially mediates lysosomal dysfunction–driven neurodegenerative processes. Lysosomal autophagic pathways play a key role in α-synuclein clearance (Lee et al. 2004, Mak et al. 2010). Therefore, impaired lysosomal degradation may lead to α-synuclein aggregation in the absence of α-synuclein gain-of-function mutations as well, such as those occurring in PD. Consistent with this hypothesis, α-synuclein has been reported to accumulate in several LSDs (Shachar et al. 2011), suggesting that LSD patients may, at some point, develop α-synuclein-associated neuropathology by mechanisms that, in principle, may be similar to those occurring in PD and related synucleinopathies. Interestingly, α-synuclein has been recently identified as a key chaperone, assisting synaptic vesicle recycling and transmission at presynaptic terminals. Specifically, α-synuclein ensures the proper function of synaptic SNARE proteins, which represent the key component of cellular fusion machinery at nerve terminals (Burgoyne & Morgan 2011, Burre et al. 2010). Therefore, α-synuclein accumulation may contribute to neurodegeneration by causing deficits in α-synuclein chaperone activity at the level of synapses, with consequent presynaptic failure. This may represent a new mechanism of neuronal degeneration not only in PD but also in LSDs.

Synaptic integrity strictly depends on maintaining efficient protein homeostasis (proteostasis) at the presynaptic terminals (Kramer & Schulz-Schaeffer 2007, Lundblad et al. 2012, Nemani et al. 2010, Scheff et al. 2007). Because lysosomes play a key role in controlling proteostasis, it is conceivable that lysosomal autophagic pathways are key players in controlling protein homeostatic processes at presynaptic terminals. Therefore, a failure of lysosomal function may disrupt the maintenance of proteostasis at synapses and contribute to neurodegenerative processes. Moreover, impaired trafficking caused by lysosomal dysfunction may cause traffic jams at presynaptic levels. Disruptions in lysosomal proteolysis by either inhibiting cathepsins or suppressing lysosomal acidification have been consistently shown to slow the axonal transport of autolysosomes, late endosomes, and lysosomes and to cause their selective accumulation within dystrophic axonal swellings (Lee et al. 2011). Altogether, these observations raise the intriguing hypothesis that lysosomal dysfunction may contribute to neurodegeneration by exerting a disruptive action at the synaptic level through the deregulation of the proteostasis of key synaptic components (e.g., SNARE) and/or through the impairment of the axonal synaptic traffic.

TARGETING LYSOSOMAL BIOGENESIS AND FUNCTION AS INNOVATIVE THERAPY TO TREAT MULTIPLE NEURODEGENERATIVE DISORDERS

Given the central role of lysosomes in the pathogenesis of multiple neurodegenerative disorders, the identification of a single approach able to enhance lysosomal function may represent a therapeutic approach for treating multiple neurological disorders. Attempts to develop treatments for LSDs have focused on single diseases rather than on designing a general approach. The following strategies have been investigated or are under development: (*a*) gene therapy or enzyme replacement therapies to restore the function of the missing proteins; (*b*) substrate reduction therapies that aim to reduce the amount of storage material produced; (*c*) small molecules or chaperones to rescue misfolded or unstable enzymes; (*d*) cellular therapies to restore injured cells; (*e*) inhibition of pathways that cause cell death, so that damaged cells can continue to provide at least some function; and (*f*) stimulation of bypass pathways to compensate for the loss of lysosomal proteins (Klein & Futerman 2013).

TFEB-targeted therapies belong to the strategies focused on stimulating compensatory pathways. TFEB-mediated effects on the activation of the lysosomal autophagic pathway may represent a completely new approach to treating LSDs and neurodegenerative conditions. In glia-differentiated neuronal stem cells derived from mouse models of two severe types of LSDs, MSD and mucopolysaccharidosis type IIIA, TFEB overexpression induced clearance of lysosomal storage due to upregulation of lysosomal exocytosis, and it rescued cellular vacuolization (Medina et al. 2011). TFEB increased the pool of lysosomes in proximity to the plasma membrane and promoted their fusion with it by activating TRPML1-mediated lysosomal calcium release (Medina et al. 2011). TFEB overexpression consistently induces lysosomal storage clearance in most of the LSDs, with the exception of ML-IV, which is characterized by mutations in *TRPML1* (Medina et al. 2011). Similarly, lysosomal exocytosis is impaired in ML-IV (LaPlante et al. 2006, Medina et al. 2011), and *TRPML1* overexpression or pharmacological activation of the channel induces lysosomal exocytosis (Dong et al. 2009, Samie et al. 2013) and clearance of cholesterol accumulation in cellular models of NPC (Wang et al. 2015). Thus, upon proper cellular stimulation, TRPML1 mediates lysosomal calcium release to trigger lysosomal exocytosis. The *TRPML1* gene is a transcriptional target of TFEB (Palmieri et al. 2011), and *TRPML1* overexpression induces TFEB nuclear translocation, suggesting the presence of a positive feedback loop regulating TFEB-mediated functions (Medina et al. 2011, 2015).

Furthermore, similar approaches have shown promising results in murine models of common neurodegenerative diseases, such as PD, HD, and AD (Decressac et al. 2013, Polito et al. 2014, Tsunemi et al. 2012), highlighting the role of lysosomes in the pathogenesis of these disorders. Specifically, in an inducible cellular model of HD, TFEB overexpression was able to reduce pathogenic HTT accumulation (Sardiello et al. 2009). Subsequently, Tsunemi et al. (2012) showed that the beneficial effects of genetic overexpression of PGC-1α in HD transgenic mice were mediated, at least in part, by the transcriptional activation of TFEB. Complementary approaches to enhance autophagy, including TFEB overexpression, have shown that it is possible to protect nigral neurons from α-synuclein toxicity; conversely, the inhibition of autophagy exacerbated α-synuclein toxicity in PD mouse models (Decressac & Bjorklund 2013, Decressac et al. 2013). Moreover, viral-mediated TFEB overexpression in AD mouse models specifically cleared hyperphosphorylated and misfolded Tau species while leaving normal Tau intact (Polito et al. 2014). TFEB-mediated effects reduce neurofibrillary tangle pathology and rescue behavioral synaptic deficits and neurodegeneration (Polito et al. 2014). Knowledge of the signaling pathways involved in TFEB regulation may allow for the development of innovative, pharmacologically mediated modulation of lysosomal function via activation of TFEB or its downstream target, an attractive therapeutic strategy for promoting cellular clearance in LSDs (Settembre et al. 2013). Recent data have supported this new approach by demonstrating that a well-known candidate molecule for LSDs, genistein, partially reduces the accumulation of glycosaminoglycans by inducing TFEB nuclear translocation (Moskot et al. 2014). In addition, the chemical activation of TFEB by hydroxypropyl-β-cyclodextrin improved the clearance of toxic proteins and clinical symptoms in mouse models of AD and PD (Bar-On et al. 2006, Kilpatrick et al. 2015, Song et al. 2014, Yao et al. 2012).

CONCLUSIONS

In this review, we have highlighted the current understanding of the lysosomal–autophagosomal system for brain physiology. Lysosomal dysfunction may cause devastating consequences owing to its central role in cellular detoxification (the degradation and recycling of macromolecules and organelles) and in signaling via TFEB, mTOR, and others. Indeed, the most common brain disorders and LSDs are associated with improper lysosomal function leading to vesicular trafficking alterations, defective calcium signaling, impaired autophagic flux, astrocytosis, and microglial activation, which together contribute, in varying degrees, to neuronal dysfunction and to a characteristic pathology. The observed vesicular trafficking alterations may dysregulate the proteostasis of key synaptic components, thus contributing to neuronal dysfunction. Calcium imbalance triggers abnormal signaling. Cholesterol accumulation impairs the vesicular membrane fusion machinery. Defective autophagy induces the progressive accumulation of polyubiquitinated proteins and dysfunctional organelles, ultimately leading to neurodegeneration. Further studies should be conducted to understand whether alterations in lysosome-to-nucleus signaling and in the lysosomal regulation of mTORC1 play any part in the pathogenesis of LSDs and other neurodegenerative disorders.

Most therapeutic strategies for brain disorders have been disease oriented. But we have described several examples in which targeting the lysosome through TFEB or downstream effectors (such as the lysosomal calcium channel TRPML1) or with drugs (such as genistein) has improved the neurological symptoms of brain disorders in animal models. These data point to the CLEAR network as a novel, attractive target for treating a wide variety of diseases of the brain.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The authors thank M. Decressac and G. Diez-Roux for critically reading the manuscript. They acknowledge the support of the Mariani Foundation (to A.F.), the Telethon Foundation (to A.F. and C.S.), a Marie Curie Career Integration Grant (to C.S.), and grants from STAR/Fondazione San Paolo, the Italian Ministry of Research, and the National MPS society (to C.S.). We apologize to authors whose work could not be cited owing to space constraints.

LITERATURE CITED

Arias E, Koga H, Diaz A, Mocholi E, Patel B, Cuervo AM. 2015. Lysosomal mTORC2/PHLPP1/Akt regulate chaperone-mediated autophagy. *Mol. Cell* 59:270–84

Bar-On P, Rockenstein E, Adame A, Ho G, Hashimoto M, Masliah E. 2006. Effects of the cholesterol-lowering compound methyl-β-cyclodextrin in models of α-synucleinopathy. *J. Neurochem.* 98:1032–45

BasuRay S, Mukherjee S, Romero EG, Seaman MN, Wandinger-Ness A. 2013. Rab7 mutants associated with Charcot-Marie-Tooth disease cause delayed growth factor receptor transport and altered endosomal and nuclear signaling. *J. Biol. Chem.* 288:1135–49

Bellettato CM, Scarpa M. 2010. Pathophysiology of neuropathic lysosomal storage disorders. *J. Inherit. Metab. Dis.* 33:347–62

Bertram L, Tanzi RE. 2005. The genetic epidemiology of neurodegenerative disease. *J. Clin. Investig.* 115:1449–57

Boustany RM. 2013. Lysosomal storage diseases—the horizon expands. *Nat. Rev. Neurol.* 9:583–98

Bras J, Guerreiro R, Hardy J. 2015. SnapShot: genetics of Parkinson's disease. *Cell* 160:570–70

Bras J, Verloes A, Schneider SA, Mole SE, Guerreiro RJ. 2012. Mutation of the parkinsonism gene ATP13A2 causes neuronal ceroid-lipofuscinosis. *Hum. Mol. Genet.* 21:2646–50

Burgoyne RD, Morgan A. 2011. Chaperoning the SNAREs: a role in preventing neurodegeneration? *Nat. Cell Biol.* 13:8–9

Burre J, Sharma M, Tsetsenis T, Buchman V, Etherton MR, Sudhof TC. 2010. α-Synuclein promotes SNARE-complex assembly in vivo and in vitro. *Science* 329:1663–67

Butler JD, Vanier MT, Pentchev PG. 1993. Niemann-Pick C disease: Cystine and lipids accumulate in the murine model of this lysosomal cholesterol lipidosis. *Biochem. Biophys. Res. Commun.* 196:154–59

Cang C, Aranda K, Seo YJ, Gasnier B, Ren D. 2015. TMEM175 is an organelle K^+ channel regulating lysosomal function. *Cell* 162:1101–12

Cang C, Zhou Y, Navarro B, Seo YJ, Aranda K, et al. 2013. mTOR regulates lysosomal ATP-sensitive two-pore Na^+ channels to adapt to metabolic state. *Cell* 152:778–90

Cao Q, Zhong XZ, Zou Y, Murrell-Lagnado R, Zhu MX, Dong XP. 2015. Calcium release through P2X4 activates calmodulin to promote endolysosomal membrane fusion. *J. Cell Biol.* 209:879–94

Chen FW, Li C, Ioannou YA. 2010. Cyclodextrin induces calcium-dependent lysosomal exocytosis. *PLOS ONE* 5:e15054

Coen K, Flannagan RS, Baron S, Carraro-Lacroix LR, Wang D, et al. 2012. Lysosomal calcium homeostasis defects, not proton pump defects, cause endo-lysosomal dysfunction in PSEN-deficient cells. *J. Cell Biol.* 198:23–35

Cortes CJ, Miranda HC, Frankowski H, Batlevi Y, Young JE, et al. 2014. Polyglutamine-expanded androgen receptor interferes with TFEB to elicit autophagy defects in SBMA. *Nat. Neurosci.* 17:1180–89

Cuervo AM, Dice JF. 2000. When lysosomes get old. *Exp. Gerontol.* 35:119–31

Cuervo AM, Stefanis L, Fredenburg R, Lansbury PT, Sulzer D. 2004. Impaired degradation of mutant α-synuclein by chaperone-mediated autophagy. *Science* 305:1292–95

Curcio-Morelli C, Charles FA, Micsenyi MC, Cao Y, Venugopal B, et al. 2010. Macroautophagy is defective in mucolipin-1-deficient mouse neurons. *Neurobiol. Dis.* 40:370–77

Das U, Scott DA, Ganguly A, Koo EH, Tang Y, Roy S. 2013. Activity-induced convergence of APP and BACE-1 in acidic microdomains via an endocytosis-dependent pathway. *Neuron* 79:447–60

Davies JP, Chen FW, Ioannou YA. 2000. Transmembrane molecular pump activity of Niemann-Pick C1 protein. *Science* 290:2295–98

De Duve C, Pressman BC, Gianetto R, Wattiaux R, Appelmans F. 1955. Tissue fractionation studies. 6. Intracellular distribution patterns of enzymes in rat-liver tissue. *Biochem. J.* 60:604–17

de Pablo-Latorre R, Saide A, Polishhuck EV, Nusco E, Fraldi A, Ballabio A. 2012. Impaired parkin-mediated mitochondrial targeting to autophagosomes differentially contributes to tissue pathology in lysosomal storage diseases. *Hum. Mol. Genet.* 21:1770–81

Decressac M, Bjorklund A. 2013. TFEB: pathogenic role and therapeutic target in Parkinson disease. *Autophagy* 9:1244–46

Decressac M, Mattsson B, Weikop P, Lundblad M, Jakobsson J, Bjorklund A. 2013. TFEB-mediated autophagy rescues midbrain dopamine neurons from α-synuclein toxicity. *PNAS* 110:E1817–26

Di Malta C, Fryer JD, Settembre C, Ballabio A. 2012. Astrocyte dysfunction triggers neurodegeneration in a lysosomal storage disorder. *PNAS* 109:E2334–42

Dong XP, Wang X, Shen D, Chen S, Liu M, et al. 2009. Activating mutations of the TRPML1 channel revealed by proline-scanning mutagenesis. *J. Biol. Chem.* 284:32040–52

Enquist IB, Lo Bianco C, Ooka A, Nilsson E, Mansson JE, et al. 2007. Murine models of acute neuronopathic Gaucher disease. *PNAS* 104:17483–88

Eskelinen EL. 2006. Roles of LAMP-1 and LAMP-2 in lysosome biogenesis and autophagy. *Mol. Asp. Med.* 27:495–502

Fernandes Filho JA, Shapiro BE. 2004. Tay-Sachs disease. *Arch. Neurol.* 61:1466–68

Fraldi A, Annunziata F, Lombardi A, Kaiser HJ, Medina DL, et al. 2010. Lysosomal fusion and SNARE function are impaired by cholesterol accumulation in lysosomal storage disorders. *EMBO J.* 29:3607–20

Fraldi A, Hemsley K, Crawley A, Lombardi A, Lau A, et al. 2007. Functional correction of CNS lesions in an MPS-IIIA mouse model by intracerebral AAV-mediated delivery of sulfamidase and *SUMF1* genes. *Hum. Mol. Genet.* 16:2693–702

Freeman D, Cedillos R, Choyke S, Lukic Z, McGuire K, et al. 2013. α-Synuclein induces lysosomal rupture and cathepsin dependent reactive oxygen species following endocytosis. *PLOS ONE* 8:e62143

Futerman AH, van Meer G. 2004. The cell biology of lysosomal storage disorders. *Nat. Rev. Mol. Cell Biol.* 5:554–65

Geisler S, Holmstrom KM, Treis A, Skujat D, Weber SS, et al. 2010. The PINK1/Parkin-mediated mitophagy is compromised by PD-associated mutations. *Autophagy* 6:871–78

Graves AR, Curran PK, Smith CL, Mindell JA. 2008. The Cl^-/H^+ antiporter ClC-7 is the primary chloride permeation pathway in lysosomes. *Nature* 453:788–92

Hamasaki M, Furuta N, Matsuda A, Nezu A, Yamamoto A, et al. 2013. Autophagosomes form at ER–mitochondria contact sites. *Nature* 495:389–93

Hara T, Nakamura K, Matsui M, Yamamoto A, Nakahara Y, et al. 2006. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. *Nature* 441:885–89

Harris H, Rubinsztein DC. 2012. Control of autophagy as a therapy for neurodegenerative disease. *Nat. Rev. Neurol.* 8:108–17

Jardim LB, Villanueva MM, de Souza CF, Netto CB. 2010. Clinical aspects of neuropathic lysosomal storage disorders. *J. Inherit. Metab. Dis.* 33:315–29

Jeong H, Then F, Melia TJ Jr., Mazzulli JR, Cui L, et al. 2009. Acetylation targets mutant huntingtin to autophagosomes for degradation. *Cell* 137:60–72

Kalatzis V, Cherqui S, Antignac C, Gasnier B. 2001. Cystinosin, the protein defective in cystinosis, is a H^+-driven lysosomal cystine transporter. *EMBO J.* 20:5940–49

Kilpatrick K, Zeng Y, Hancock T, Segatori L. 2015. Genetic and chemical activation of TFEB mediates clearance of aggregated α-synuclein. *PLOS ONE* 10:e0120819

Kim HJ, Soyombo AA, Tjon-Kon-Sang S, So I, Muallem S. 2009. The Ca^{2+} channel TRPML3 regulates membrane trafficking and autophagy. *Traffic* 10:1157–67

Klein AD, Futerman AH. 2013. Lysosomal storage disorders: old diseases, present and future challenges. *Pediatr. Endocrinol. Rev.* 11(Suppl. 1):59–63

Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, et al. 2006. Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature* 441:880–84

Komatsu M, Wang QJ, Holstein GR, Friedrich VL Jr., Iwata J, et al. 2007. Essential role for autophagy protein Atg7 in the maintenance of axonal homeostasis and the prevention of axonal degeneration. *PNAS* 104:14489–94

Kramer ML, Schulz-Schaeffer WJ. 2007. Presynaptic α-synuclein aggregates, not Lewy bodies, cause neurodegeneration in dementia with Lewy bodies. *J. Neurosci.* 27:1405–10

Kummel D, Ungermann C. 2014. Principles of membrane tethering and fusion in endosome and lysosome biogenesis. *Curr. Opin. Cell Biol.* 29:61–66

LaPlante JM, Sun M, Falardeau J, Dai D, Brown EM, et al. 2006. Lysosomal exocytosis is impaired in mucolipidosis type IV. *Mol. Genet. Metab.* 89:339–48

Lee HJ, Khoshaghideh F, Patel S, Lee SJ. 2004. Clearance of α-synuclein oligomeric intermediates via the lysosomal degradation pathway. *J. Neurosci.* 24:1888–96

Lee JH, McBrayer MK, Wolfe DM, Haslett LJ, Kumar A, et al. 2015. Presenilin 1 maintains lysosomal Ca^{2+} homeostasis via TRPML1 by regulating vATPase-mediated lysosome acidification. *Cell Rep.* 12:1430–44

Lee JH, Yu WH, Kumar A, Lee S, Mohan PS, et al. 2010. Lysosomal proteolysis and autophagy require presenilin 1 and are disrupted by Alzheimer-related PS1 mutations. *Cell* 141:1146–58

Lee S, Sato Y, Nixon RA. 2011. Lysosomal proteolysis inhibition selectively disrupts axonal transport of degradative organelles and causes an Alzheimer's-like axonal dystrophy. *J. Neurosci.* 31:7817–30

Lee SM, Chin LS, Li L. 2012. Charcot-Marie-Tooth disease-linked protein SIMPLE functions with the ESCRT machinery in endosomal trafficking. *J. Cell Biol.* 199:799–816

Li JQ, Tan L, Yu JT. 2014. The role of the *LRRK2* gene in Parkinsonism. *Mol. Neurodegener.* 9:47

Lieberman AP, Puertollano R, Raben N, Slaugenhaupt S, Walkley SU, Ballabio A. 2012. Autophagy in lysosomal storage disorders. *Autophagy* 8:719–30

Liu B, Du H, Rutkowski R, Gartner A, Wang X. 2012. LAAT-1 is the lysosomal lysine/arginine transporter that maintains amino acid homeostasis. *Science* 337:351–54

Liu L, Zhang K, Sandoval H, Yamamoto S, Jaiswal M, et al. 2015. Glial lipid droplets and ROS induced by mitochondrial defects promote neurodegeneration. *Cell* 160:177–90

Lloyd-Evans E, Morgan AJ, He X, Smith DA, Elliot-Smith E, et al. 2008. Niemann-Pick disease type C1 is a sphingosine storage disease that causes deregulation of lysosomal calcium. *Nat. Med.* 14:1247–55

Lopez ME, Klein AD, Dimbil UJ, Scott MP. 2011. Anatomically defined neuron-based rescue of neurodegenerative Niemann-Pick type C disorder. *J. Neurosci.* 31:4367–78

Lundblad M, Decressac M, Mattsson B, Bjorklund A. 2012. Impaired neurotransmission caused by overexpression of α-synuclein in nigral dopamine neurons. *PNAS* 109:3213–19

Luzi P, Abraham RM, Rafi MA, Curtis M, Hooper DC, Wenger DA. 2009. Effects of treatments on inflammatory and apoptotic markers in the CNS of mice with globoid cell leukodystrophy. *Brain Res.* 1300:146–58

Macauley SL, Wong AM, Shyng C, Augner DP, Dearborn JT, et al. 2014. An anti-neuroinflammatory that targets dysregulated glia enhances the efficacy of CNS-directed gene therapy in murine infantile neuronal ceroid lipofuscinosis. *J. Neurosci.* 34:13077–82

Mak SK, McCormack AL, Manning-Bog AB, Cuervo AM, Di Monte DA. 2010. Lysosomal degradation of α-synuclein in vivo. *J. Biol. Chem.* 285:13621–29

March PA, Thrall MA, Brown DE, Mitchell TW, Lowenthal AC, Walkley SU. 1997. GABAergic neuroaxonal dystrophy and other cytopathological alterations in feline Niemann-Pick disease type C. *Acta Neuropathol.* 94:164–72

Martina JA, Chen Y, Gucek M, Puertollano R. 2012. MTORC1 functions as a transcriptional regulator of autophagy by preventing nuclear transport of TFEB. *Autophagy* 8:903–14

Martinez-Vicente M, Talloczy Z, Kaushik S, Massey AC, Mazzulli J, et al. 2008. Dopamine-modified α-synuclein blocks chaperone-mediated autophagy. *J. Clin. Investig.* 118:777–88

Mazzulli JR, Xu YH, Sun Y, Knight AL, McLean PJ, et al. 2011. Gaucher disease glucocerebrosidase and α-synuclein form a bidirectional pathogenic loop in synucleinopathies. *Cell* 146:37–52

Medina DL, Di Paola S, Peluso I, Armani A, De Stefani D, et al. 2015. Lysosomal calcium signalling regulates autophagy through calcineurin and TFEB. *Nat. Cell Biol.* 17:288–99

Medina DL, Fraldi A, Bouche V, Annunziata F, Mansueto G, et al. 2011. Transcriptional activation of lysosomal exocytosis promotes cellular clearance. *Dev. Cell* 21:421–30

Menzies FM, Fleming A, Rubinsztein DC. 2015. Compromised autophagy and neurodegenerative diseases. *Nat. Rev. Neurosci.* 16:345–57

Micsenyi MC, Dobrenis K, Stephney G, Pickel J, Vanier MT, et al. 2009. Neuropathology of the *Mcoln1*$^{-/-}$ knockout mouse model of mucolipidosis type IV. *J. Neuropathol. Exp. Neurol.* 68:125–35

Mindell JA. 2012. Lysosomal acidification mechanisms. *Annu. Rev. Physiol.* 74:69–86

Mizushima N. 2007. Autophagy: process and function. *Genes Dev.* 21:2861–73

Moskot M, Montefusco S, Jakobkiewicz-Banecka J, Mozolewski P, Wegrzyn A, et al. 2014. The phytoestrogen genistein modulates lysosomal metabolism and transcription factor EB (TFEB) activation. *J. Biol. Chem.* 289:17054–69

Nemani VM, Lu W, Berge V, Nakamura K, Onoa B, et al. 2010. Increased expression of α-synuclein reduces neurotransmitter release by inhibiting synaptic vesicle reclustering after endocytosis. *Neuron* 65:66–79

Nishino I, Fu J, Tanji K, Yamada T, Shimojo S, et al. 2000. Primary LAMP-2 deficiency causes X-linked vacuolar cardiomyopathy and myopathy (Danon disease). *Nature* 406:906–10

Nixon RA, Yang DS, Lee JH. 2008. Neurodegenerative lysosomal disorders: a continuum from development to late age. *Autophagy* 4:590–99

Ostrerova N, Petrucelli L, Farrer M, Mehta N, Choi P, et al. 1999. α-Synuclein shares physical and functional homology with 14-3-3 proteins. *J. Neurosci.* 19:5782–91

Palmieri M, Impey S, Kang H, di Ronza A, Pelz C, et al. 2011. Characterization of the CLEAR network reveals an integrated control of cellular clearance pathways. *Hum. Mol. Genet.* 20:3852–66

Pastores GM, Maegawa GH. 2013. Clinical neurogenetics: neuropathic lysosomal storage disorders. *Neurol. Clin.* 31:1051–71

Perez RG, Waymire JC, Lin E, Liu JJ, Guo F, Zigmond MJ. 2002. A role for α-synuclein in the regulation of dopamine biosynthesis. *J. Neurosci.* 22:3090–99

Platt FM, Boland B, van der Spoel AC. 2012. Lysosomal storage disorders: the cellular impact of lysosomal dysfunction. *J. Cell Biol.* 199:723–34

Polito VA, Li H, Martini-Stoica H, Wang B, Yang L, et al. 2014. Selective clearance of aberrant tau proteins and rescue of neurotoxicity by transcription factor EB. *EMBO Mol. Med.* 6:1142–60

Rajendran L, Annaert W. 2012. Membrane trafficking pathways in Alzheimer's disease. *Traffic* 13:759–70

Rama Rao KV, Kielian T. 2016. Astrocytes and lysosomal storage diseases. *Neuroscience* 323:195–206

Ramirez A, Heimbach A, Grundemann J, Stiller B, Hampshire D, et al. 2006. Hereditary parkinsonism with dementia is caused by mutations in *ATP13A2*, encoding a lysosomal type 5 P-type ATPase. *Nat. Genet.* 38:1184–91

Ravikumar B, Acevedo-Arozena A, Imarisio S, Berger Z, Vacher C, et al. 2005. Dynein mutations impair autophagic clearance of aggregate-prone proteins. *Nat. Genet.* 37:771–76

Ravikumar B, Vacher C, Berger Z, Davies JE, Luo S, et al. 2004. Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. *Nat. Genet.* 36:585–95

Roczniak-Ferguson A, Petit CS, Froehlich F, Qian S, Ky J, et al. 2012. The transcription factor TFEB links mTORC1 signaling to transcriptional control of lysosome homeostasis. *Sci. Signal.* 5:ra42

Rogov V, Dotsch V, Johansen T, Kirkin V. 2014. Interactions between autophagy receptors and ubiquitin-like proteins form the molecular basis for selective autophagy. *Mol. Cell* 53:167–78

Rong Y, McPhee CK, Deng S, Huang L, Chen L, et al. 2011. Spinster is required for autophagic lysosome reformation and mTOR reactivation following starvation. *PNAS* 108:7826–31

Rui YN, Xu Z, Patel B, Chen Z, Chen D, et al. 2015. Huntingtin functions as a scaffold for selective macroautophagy. *Nat. Cell Biol.* 17:262–75

Saez PJ, Orellana JA, Vega-Riveros N, Figueroa VA, Hernandez DE, et al. 2013. Disruption in connexin-based communication is associated with intracellular Ca^{2+} signal alterations in astrocytes from Niemann-Pick type C mice. *PLOS ONE* 8:e71361

Saftig P, Klumperman J. 2009. Lysosome biogenesis and lysosomal membrane proteins: trafficking meets function. *Nat. Rev. Mol. Cell Biol.* 10:623–35
Sagne C, Agulhon C, Ravassard P, Darmon M, Hamon M, et al. 2001. Identification and characterization of a lysosomal transporter for small neutral amino acids. *PNAS* 98:7206–11
Samie M, Wang X, Zhang X, Goschka A, Li X, et al. 2013. A TRP channel in the lysosome regulates large particle phagocytosis via focal exocytosis. *Dev. Cell* 26:511–24
Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM. 2010. Ragulator–Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. *Cell* 141:290–303
Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, et al. 2008. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science* 320:1496–501
Sardiello M, Palmieri M, di Ronza A, Medina DL, Valenza M, et al. 2009. A gene network regulating lysosomal biogenesis and function. *Science* 325:473–77
Scheff SW, Price DA, Schmitt FA, DeKosky ST, Mufson EJ. 2007. Synaptic alterations in CA1 in mild Alzheimer disease and mild cognitive impairment. *Neurology* 68:1501–8
Schulze H, Sandhoff K. 2011. Lysosomal lipid storage diseases. *Cold Spring Harb. Perspect. Biol.* 3:a004804
Settembre C, Di Malta C, Polito VA, Garcia Arencibia M, Vetrini F, et al. 2011. TFEB links autophagy to lysosomal biogenesis. *Science* 332:1429–33
Settembre C, Fraldi A, Jahreiss L, Spampanato C, Venturi C, et al. 2008. A block of autophagy in lysosomal storage disorders. *Hum. Mol. Genet.* 17:119–29
Settembre C, Fraldi A, Medina DL, Ballabio A. 2013. Signals from the lysosome: a control centre for cellular clearance and energy metabolism. *Nat. Rev. Mol. Cell Biol.* 14:283–96
Settembre C, Zoncu R, Medina DL, Vetrini F, Erdin S, et al. 2012. A lysosome-to-nucleus signalling mechanism senses and regulates the lysosome via mTOR and TFEB. *EMBO J.* 31:1095–108
Shachar T, Lo Bianco C, Recchia A, Wiessner C, Raas-Rothschild A, Futerman AH. 2011. Lysosomal storage disorders and Parkinson's disease: Gaucher disease and beyond. *Mov. Disord.* 26:1593–604
Shen D, Wang X, Li X, Zhang X, Yao Z, et al. 2012. Lipid storage disorders block lysosomal trafficking by inhibiting a TRP channel and lysosomal calcium release. *Nat. Commun.* 3:731
Sidransky E, Nalls MA, Aasly JO, Aharon-Peretz J, Annesi G, et al. 2009. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. *N. Engl. J. Med.* 361:1651–61
Siegel DA, Walkley SU. 1994. Growth of ectopic dendrites on cortical pyramidal neurons in neuronal storage diseases correlates with abnormal accumulation of GM2 ganglioside. *J. Neurochem.* 62:1852–62
Singh R, Kaushik S, Wang Y, Xiang Y, Novak I, et al. 2009. Autophagy regulates lipid metabolism. *Nature* 458:1131–35
Song CY, Guo JF, Liu Y, Tang BS. 2012. Autophagy and its comprehensive impact on ALS. *Int. J. Neurosci.* 122:695–703
Song W, Wang F, Lotfi P, Sardiello M, Segatori L. 2014. 2-Hydroxypropyl-β-cyclodextrin promotes transcription factor EB-mediated activation of autophagy: implications for therapy. *J. Biol. Chem.* 289:10211–22
Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. 1997. α-Synuclein in Lewy bodies. *Nature* 388:839–40
te Vruchte D, Lloyd-Evans E, Veldman RJ, Neville DC, Dwek RA, et al. 2004. Accumulation of glycosphingolipids in Niemann-Pick C disease disrupts endosomal transport. *J. Biol. Chem.* 279:26167–75
Tian X, Gala U, Zhang Y, Shang W, Nagarkar Jaiswal S, et al. 2015. A voltage-gated calcium channel regulates lysosomal fusion with endosomes and autophagosomes and is required for neuronal homeostasis. *PLOS Biol.* 13:e1002103
Tsunemi T, Ashe TD, Morrison BE, Soriano KR, Au J, et al. 2012. PGC-1α rescues Huntington's disease proteotoxicity by preventing oxidative stress and promoting TFEB function. *Sci. Transl. Med.* 4:142ra97
Vergarajauregui S, Connelly PS, Daniels MP, Puertollano R. 2008. Autophagic dysfunction in mucolipidosis type IV patients. *Hum. Mol. Genet.* 17:2723–37
Verheijen FW, Verbeek E, Aula N, Beerens CE, Havelaar AC, et al. 1999. A new gene, encoding an anion transporter, is mutated in sialic acid storage diseases. *Nat. Genet.* 23:462–65
Vitner EB, Futerman AH, Platt N. 2015. Innate immune responses in the brain of sphingolipid lysosomal storage diseases. *Biol. Chem.* 396:659–67

Vitner EB, Salomon R, Farfel-Becker T, Meshcheriakova A, Ali M, et al. 2014. RIPK3 as a potential therapeutic target for Gaucher's disease. *Nat. Med.* 20:204–8

Walker FO. 2007. Huntington's disease. *Lancet* 369:218–28

Wang W, Gao Q, Yang M, Zhang X, Yu L, et al. 2015. Up-regulation of lysosomal TRPML1 channels is essential for lysosomal adaptation to nutrient starvation. *PNAS* 112:E1373–81

Williams IM, Wallom KL, Smith DA, Al Eisa N, Smith C, Platt FM. 2014. Improved neuroprotection using miglustat, curcumin and ibuprofen as a triple combination therapy in Niemann–Pick disease type C1 mice. *Neurobiol. Dis.* 67:9–17

Winslow AR, Chen CW, Corrochano S, Acevedo-Arozena A, Gordon DE, et al. 2010. α-Synuclein impairs macroautophagy: implications for Parkinson's disease. *J. Cell Biol.* 190:1023–37

Wong E, Cuervo AM. 2010. Autophagy gone awry in neurodegenerative diseases. *Nat. Neurosci.* 13:805–11

Wong YC, Holzbaur EL. 2014. The regulation of autophagosome dynamics by huntingtin and HAP1 is disrupted by expression of mutant huntingtin, leading to defective cargo degradation. *J. Neurosci.* 34:1293–305

Wu YP, Proia RL. 2004. Deletion of macrophage-inflammatory protein 1 α retards neurodegeneration in Sandhoff disease mice. *PNAS* 101:8425–30

Xu M, Liu K, Swaroop M, Porter FD, Sidhu R, et al. 2012. δ-Tocopherol reduces lipid accumulation in Niemann-Pick type C1 and Wolman cholesterol storage disorders. *J. Biol. Chem.* 287:39349–60

Yamamoto A, Cremona ML, Rothman JE. 2006. Autophagy-mediated clearance of huntingtin aggregates triggered by the insulin-signaling pathway. *J. Cell Biol.* 172:719–31

Yao J, Ho D, Calingasan NY, Pipalia NH, Lin MT, Beal MF. 2012. Neuroprotection by cyclodextrin in cell and mouse models of Alzheimer disease. *J. Exp. Med.* 209:2501–13

Yu L, McPhee CK, Zheng L, Mardones GA, Rong Y, et al. 2010. Termination of autophagy and reformation of lysosomes regulated by mTOR. *Nature* 465:942–46

Yu T, Shakkottai VG, Chung C, Lieberman AP. 2011. Temporal and cell-specific deletion establishes that neuronal *Npc1* deficiency is sufficient to mediate neurodegeneration. *Hum. Mol. Genet.* 20:4440–51

Zimprich A, Benet-Pages A, Struhal W, Graf E, Eck SH, et al. 2011. A mutation in VPS35, encoding a subunit of the retromer complex, causes late-onset Parkinson disease. *Am. J. Hum. Genet.* 89:168–75

Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, et al. 2004. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron* 44:601–7

Zoncu R, Bar-Peled L, Efeyan A, Wang S, Sancak Y, Sabatini DM. 2011. mTORC1 senses lysosomal amino acids through an inside-out mechanism that requires the vacuolar H^+-ATPase. *Science* 334:678–83