Chapter 12
The Deleterious Duo of Neurodegeneration: Lysosomes and Mitochondria

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Abstract Many studies have demonstrated that the accumulation of aggregate-prone proteins due to defects in cellular quality control systems contributes to the development of neurodegenerative diseases. One form of quality control within neurons is autophagy, an intracellular pathway involved in the breakdown of cytosolic constituents. Lysosomes mediate autophagy, and their dysfunction may contribute to perturbations in cellular homeostasis and affect other organelles such as mitochondria. Mitochondrial malfunction may then further perpetuate lysosomal damage and initiate inflammatory responses. Therefore, lysosomes and mitochondria share a reciprocal relationship where dysfunction in one often affects the function of the other. These consequences of lysosome and mitochondrial impairment complete a deleterious feedback loop that concludes not only in neurodegeneration but also neuroinflammation. Herein, we discuss the primary types of autophagy and their underlying mechanisms, the regulation of lysosomal biogenesis and function, and the link between lysosomal and mitochondrial dysfunction. We conclude this chapter by assessing the role of lysosomal dysfunction in neurodegenerative diseases.

Keywords Alpha-synuclein • TFEB • TFE3 • Autophagy • Lysosome • Synucleinopathies • Lysosomal storage disorders • Gaucher disease • Inflammasome • Reactive oxygen species • Mitophagy • Glucocerebrosidase

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12.1 Autophagy

In 1963, Christian de Duve first described autophagy: a quality control system involved in the degradation of unnecessary or nonfunctional cellular components [1]. Lysosomes, with their acidic pH and lytic hydrolases, mediate autophagy in response to perturbations in cellular homeostasis that may occur as a result of organelle dysfunction, genetic mutations, or nutrient deprivation. Autophagy is particularly important in the context of neurodegeneration, as it has been shown that in the absence of any disease-associated gene products, the loss of autophagy alone is sufficient to cause neural dysfunction and eventually neuronal cell death [2, 3]. Depending on the mechanism by which cellular cargo is transported to the lysosome, autophagy can be classified into three types: microautophagy, macroautophagy, and chaperone-mediated autophagy.

12.1.1 Microautophagy

Little is currently known about microautophagy, especially within mammalian cells; as a result, the majority of our knowledge regarding this autophagic process stems from studies in a few species of yeast: *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Hansenula polymorpha* [4–8]. In mammalian microautophagy, the lysosomal membrane directly invaginates and sequesters cytoplasmic constituents into vesicles intended for degradation [9–11]. This invagination may occur through either a concave retreat of the lysosomal membrane or the lysosomal wrapping mechanism (LWM) [12]. During the LWM, the lysosome elongates from a spherical to tubular shape and extends an arm-like protrusion that envelops soluble cytoplasmic components. The tip of this extension then meets and fuses with the lysosomal membrane, sealing these cellular constituents within a vesicle for breakdown inside the lysosome [13, 14]. Microautophagy, as a form of quality of control, has been implicated in the basal turnover of cellular components, but recent literature suggests that this autophagic process may possess additional functions. Microautophagy could be associated with balancing the influx of membrane components introduced by macroautophagy and may be related to multivesicular body formation as a lysosomal microautophagy-like mechanism involved in selectively delivering cytosolic proteins to late endosomes during biogenesis [11, 15–17]. As highlighted in a review article by Mijalica et al. microautophagy is a field in need of further investigation [10].

12.1.2 Macroautophagy

In contrast to microautophagy, macroautophagy is better understood and is described in detail in Chap. 11. In macroautophagy, cytoplasmic cargo is sequestered within double-membrane vesicles called autophagosomes for transportation to the lysosome (Fig. 12.1c) [18, 19]. Initiation of autophagy starts with the formation of a

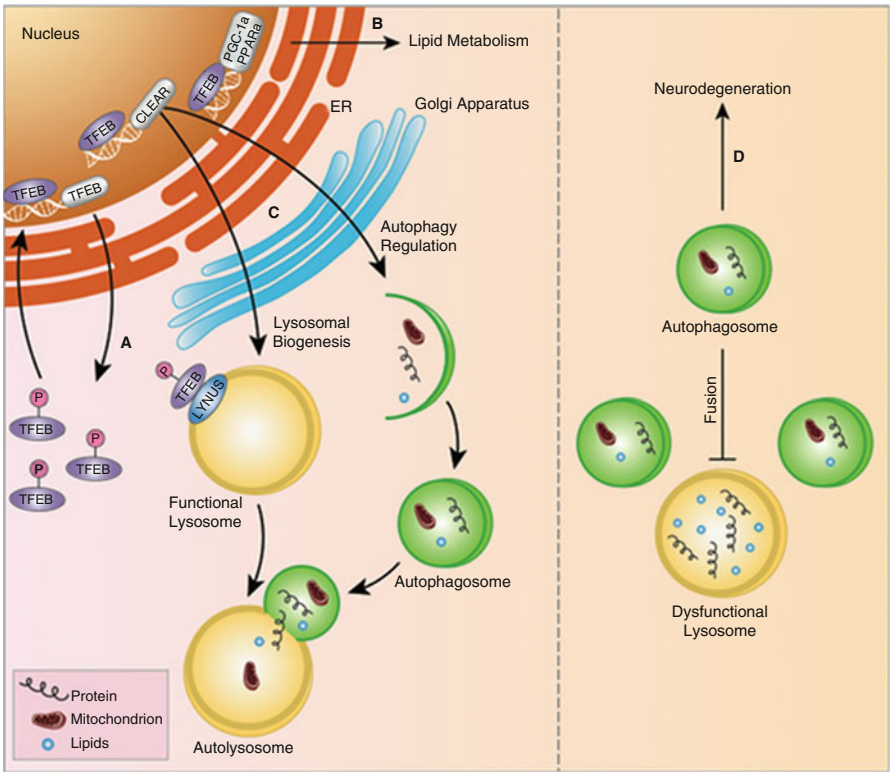

Fig. 12.1 (**a**) TFEB is regulated at both transcriptional and posttranscriptional levels. During nutrient-rich conditions, mTORC1 phosphorylates TFEB, preventing its translocation into the nucleus and sequestering the transcription factor in the cytosol and lysosomal membrane. Under stressful conditions, mTORC1 dissociates from the lysosomal membrane without phosphorylating TFEB, allowing for its translocation to the nucleus. Within the nucleus, TFEB regulates its own transcription as well as genes involved in lipid metabolism and the CLEAR network. (**b**) TFEB contributes to lipid metabolism by activating transcription of PPARα and PGC-1α. These two proteins initiate pathways involved in the degradation of lipids for energy production. (**c**) TFEB activates CLEAR network genes involved in biogenesis of lysosomes and regulation of autophagy, which are both required for proper cellular quality control. (**d**) Defects in the lysosome-autophagy pathway promote accumulation of aggregate-prone proteins such as α-synuclein, huntingtin, and Tau leading to neurodegeneration

double-membrane structure exclusive to macroautophagy termed the phagophore [20–24]. Phagophore assembly is suspected to occur de novo, and within mammalian systems, this process may take place at multiple locations in the cytoplasm [25–29]. The growth of the phagophore is an area of intense debate, as the source of the membrane components used in expansion is currently unknown. Multiple studies have suggested that these building blocks arise from pre-existing membrane compartments, such as those of the mitochondria, endoplasmic reticulum (ER), Golgi apparatus, and plasma membrane. Others speculate that the membrane may originate from a phosphatidylinositol-3-phosphate-enriched portion of the ER named the omegasome [30–36]. Phagophore elongation and expansion involves

sequential recruitment of several molecular complexes and subsequent delivery of membrane components to the growing phagophore (discussed in detail in Chap. 11) [37–42]. Apposition and sealing of the ends of the expanding phagophore membrane concludes autophagosome formation [39].

Once formed, these double-membrane vesicles may fuse with either endosomes or lysosomes resulting in the formation of chimeric organelles called amphisomes and autolysosomes, respectively [43, 44]. Amphisomes are intermediate structures that link the autophagic and endosomal pathways and are capable of further fusion with lysosomes to also produce autolysosomes [45]. Autophagosomes are dependent on microtubules for transport to both endosomes and lysosomes where their merging is facilitated by a variety of proteins including both endosomal sort complex required for transport (ESCRT) and soluble NSF attachment protein receptor (SNARE) proteins as well as Rab7 [46–48]. Of peculiar interest, proper lysosomal function and acidification were found to be important for autophagosome-lysosome and endosome-lysosome fusion as well [49]. Upon fusion of the autophagosome or amphisome with the lysosome, the inner membrane of the autolysosome is quickly degraded. The outer membrane of the autophagosome or amphisome is lost as it fuses with that of the lysosome, and cellular cargo is expelled into the acidic environment of the lysosomal lumen where it is broken down by lytic hydrolases. Lysosomal membrane permeases then release these degradation products back into the cytosol for further use in energy production or biosynthetic pathways [19, 24, 50].

12.1.3 Chaperone-Mediated Autophagy

Not all forms of autophagy require the formation of vesicles for transporting cargo to the lysosomal lumen. In chaperone-mediated autophagy (CMA), cellular cargo is individually targeted and directly transported across the lysosomal membrane into the lumen for degradation. This catabolic process, currently only described in mammals, was the first subtype of autophagy for which selectivity was demonstrated. Selectivity occurs through multiple steps [51–53]. The first is the recognition of substrate proteins containing a specific KFERQ pentapeptide motif by heat-shock cognate protein of 70 kDa (hsc70). All known CMA substrate proteins possess this pentapeptide motif, and several studies have demonstrated that it is both necessary and sufficient for lysosomal targeting [51, 54–57]. Recognition of protein substrates by hsc70 is regulated by the accessibility of the pentapeptide motif, as this structure may be concealed by protein folding, protein-protein interactions, and binding to subcellular membranes. Several studies have also demonstrated that posttranslational modifications of KFERQ-like motifs, which are peptide motifs that contain four out of the five required amino acid residues typically found in a KFERQ motif, regulate substrate binding as well. Since the KFERQ motif depends on its charge for proper interaction with hsc70, phosphorylation or acetylation of KFERQ-like motifs may compensate for changes in charge caused by the absence of one of the required amino acid residues typically included in a KFERQ motif. In this manner, a CMA-targeting motif may be formed upon modification of the KFERQ-like motif, allowing for association with hsc70 [52, 56, 58, 59].

Once protein substrates are bound to hsc70, this substrate/chaperone complex is delivered to the lysosomal membrane where it interacts with the cytosolic tail of lysosome-associated membrane protein type 2A (LAMP-2A), a single-span membrane protein [60]. LAMP-2A exists as a monomer at the lysosomal membrane, but, upon binding of its protein substrate, forms a multiprotein complex composed of free LAMP-2A monomers and other proteins [61]. Protein substrates may bind the monomeric form of LAMP-2A in their folded conformations; however, in order to be translocated into the lysosomal lumen, these proteins must first be unfolded. This unfolding process occurs before the assembly of the multiprotein translocation complex and may be mediated by hsc70 and its associated co-chaperones [53, 62, 63]. While numerous factors, such as the protein density and fluidity of the lysosomal membrane, influence assembly of the multiprotein complex, very few proteins have been identified as regulators of this molecular machinery [64]. Recently, work by Bandyopadhyay and colleagues have demonstrated that both GFAP and EF1α modulate the assembly and disassembly of this translocation complex in a GTP-dependent manner [65]. Furthermore, two chaperones, hsc70 and heat-shock protein 90 (hsp90), have also been implicated in this process. These chaperones function not only in the assembly and disassembly of the translocation complex but also contribute to the stabilization of LAMP-2A during multimerization [51, 61, 66].

Translocation of the unfolded protein into the lysosomal lumen occurs one by one through the LAMP-2A multiprotein complex and is dependent on the presence of an intralysosomal isoform of hsc70 (lys-hsc70) [63, 67]. This isoform of hsc70 is located in the lysosomal lumen and may enter the lysosome via fusion with hsc70-containing late endosomes [53, 66]. To date, the exact mechanism by which lys-hsc70 contributes to substrate translocation is unknown; however, it has been proposed that this protein may "pull" the substrate through the LAMP-2A translocation complex or passively "hold" the substrate, preventing its release back into the cytosol [53]. Depending on cellular conditions and cell type, the population of lysosomes containing lys-hsc70 fluctuates between 20 % and 80 %, and therefore, not all lysosomes are capable of CMA [68]. Upon entry of the protein substrate into the lysosomal lumen, substrate degradation occurs by resident hydrolases, and this process is accompanied by the dissociation of the LAMP-2A translocation complex. These resulting monomers of LAMP-2A are then free to further bind substrates and initiate new cycles of CMA [61].

12.2 Transcription Factor EB (TFEB)

12.2.1 The Lysosome

While investigating the mechanism of action of insulin within liver cells, Christian de Duve serendipitously stumbled upon a nonspecific acid phosphatase that possessed a phantasmic enzymatic activity. Tantalized by the "vanishing acts" that this acid phosphatase performed, de Duve abandoned his work on insulin and pursued this accidental finding instead. In 1955, after years of investigating this unexpected

observation, de Duve described the lysosome. Roughly 20 years later, he was awarded the Nobel Prize in Physiology and Medicine for this discovery [69, 70]. Traditionally, lysosomes, Greek for "digestive body" and formed from the combination of the words *lysis and soma*, have been considered static cellular organelles that are not influenced by environmental cues. They are primarily implicated in the catabolism of macromolecules obtained from multiple cellular processes such as endocytosis, autophagy, and phagocytosis [10, 52, 71–74]. However, the new concept of lysosomal adaptation has subsequently broadened our perspective from merely its static role in cellular clearance [75]. Recent studies by Ballabio and colleagues have demonstrated that a majority of the 96 lysosomal genes involved in lysosomal biogenesis and function coordinately express and are influenced by environmental factors, both extracellular and intracellular, through a basic helix-loop-helix transcription factor known as transcription factor EB (TFEB), the master regulator of the coordinate lysosomal expression and regulation (CLEAR), which encompasses these lysosomal genes [76, 77]. Lysosomes have now emerged as a critical player involved in nutrient sensing, signaling, and metabolism, in addition to their established duty in cellular macromolecule degradation. At the heart of this adaptive and dynamic lysosome model is the activity of TFEB, the master regulator of lysosomal biogenesis and function that modulates the interplay between lysosome-mediated cellular processes and environmental influences.

12.2.2 Regulation of TFEB

TFEB is located within the cytoplasm and on the surface of the cholesterol sparse lysosomal membrane, where it is regulated through an "inside-out" signaling model initiated by the level of accumulated amino acids in the acidic lumen of the lysosome (see Fig. 12.1a). These amino acid levels are sensed by the lysosome nutrient signaling (LYNUS) machinery, which propagates a signal through a protein complex known as Ragulator to RAG GTPases that subsequently recruit mTORC1 to the lysosomal surface [78–85]. The subcellular localization of TFEB depends largely on the mTORC1-mediated phosphorylation of TFEB at two crucial serine residues: Ser142 and Ser211 [77, 84, 86, 87]. Under favorable conditions, mTORC1 phosphorylates TFEB, sequestering the transcription factor in the cytoplasm and lysosomal surface. However, during adverse cellular circumstances—starvation, stress, and lysosome dysfunction or inhibition—mTORC1 dissociates from the LYNUS complex without phosphorylating TFEB, allowing for TFEB nuclear translocation [76, 77, 81, 84, 86, 87]. Within the nucleus, TFEB activates transcription of the CLEAR network genes pertinent in the lysosomal-autophagy pathway, as well as those involved in lipid metabolism such as peroxisome proliferator-activated receptor α (PPARα), peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α), and their respective target genes. Furthermore, once inside the nucleus TFEB also positively regulates its own function by binding to the CLEAR motif within the promotor region of its associated gene, thus initiating its

own transcription. Therefore, the activity of TFEB is regulated both at the transcriptional and posttranscriptional levels (see Fig. 12.1a), and presents a mechanism for which environmental influences, both extracellular and intracellular, can be transmitted from lysosome to nucleus [75–77].

12.2.3 Lipid Catabolism

TFEB is also implicated in lipid catabolism due to the intertwined features of autophagy and lipid metabolism. Through an autophagic process known as macrolipophagy, lipid droplets are transported to lysosomes via autophagosomes, where they are degraded into free fatty acids and glycerol [88, 89]. Mouse liver cells overexpressing TFEB exhibited upregulation of genes implicated in lipid catabolic processes, such as lipophagy and fatty acid oxidation, as well as downregulation of those involved in lipid biogenesis [75]. TFEB exerts its transcriptional control of lipid metabolism by inducing two key modulators of energy metabolism: PPARα and PGC-1α (see Fig. 12.1b) [75–90]. Cells stressed by starvation undergo TFEB-activated transcription of both PPARα and PGC-1α, which subsequently initiates a metabolic response where energy is produced through the breakdown of lipid reserves [75]. Furthermore, studies performed on *Atg7* knockout mice, whose autophagic pathways are suppressed, demonstrate that TFEB mediates lipid metabolism through an autophagy-dependent manner [75]. Therefore, TFEB weaves together the lysosomal-autophagic pathway with lipid metabolism. It is becoming increasingly clear that the lysosome is not only a cellular garbage disposal, but it also serves as an intricate player involved in nutrient sensing, signaling, and metabolism.

12.2.4 TFEB, Autophagy, and Neurodegeneration

In many neurodegenerative proteopathies, the lysosomal-autophagy pathway is disrupted and the pathogenic formation of misfolded protein aggregates occurs (see Fig. 12.1c) [91–93]. Therefore, as the master regulator of the lysosomal-autophagy pathway, the role of TFEB in neurodegeneration is an area of intensive investigation. Studies utilizing a rat model of Parkinson's disease generated by overexpressing human alpha-synuclein (α-syn) in the midbrain demonstrated that elevated α-syn levels induced TFEB retention in the cytoplasm, leading to lysosomal dysfunction, α-syn accumulation in autophagosomes, and a progressive increase in α-syn oligomers [94]. Since α-syn is structurally and functionally similar to 14-3-3 proteins, a group of proteins known to interact with and trap phosphorylated TFEB within the cytoplasm, a possible pathogenic mechanism, has been postulated where aggregates of α-syn bind to phosphorylated TFEB, preventing its nuclear translocation and eventually leading to impairment of autophagic processes [86, 87, 94–96].

Overexpression of TFEB, whether by genetic or pharmacological means, has been shown to mediate the clearance of α-syn and halt the progression of Parkinsonian neurodegeneration in both rat nigral dopaminergic neurons and human neuroglioma cells through an autophagy-dependent pathway [94, 97]. Recent research has also demonstrated that overexpression of TFEB is neuroprotective in both Huntington's and Alzheimer's diseases as well. In a Huntington's disease mouse model, TFEB-mediated induction of PGC-1α was shown to rescue neurotoxicity via cellular clearance of huntingtin protein aggregation and reduction of oxidative stress [98]. Furthermore, TFEB overexpression in a mouse model of Alzheimer's disease showed that TFEB is capable of allaying phosphorylated Tau and neurofibrillary tangle-associated neuropathology by enhancing the clearance of both hyperphosphorylated and misfolded Tau proteins [99].

12.2.5 Transcription Factor E3

Recently, a second member of the MiTF/TFE family was found to be a CLEAR network regulator. Like TFEB, translocation of transcription factor E3 (TFE3) from the cytosol to the nucleus is dependent upon interaction with Rag GTPases, mTORC1-dependent phosphorylation status, and nutrient availability [100]. Under conditions of starvation, TFE3 translocates from the cytosol to the nucleus where it binds to the CLEAR motif of promoters of genes belonging to the CLEAR network. Research indicates that TFE3-induced autophagy and lysosomal biogenesis are independent of TFEB. Relative protein abundance of both transcription factors, which has been shown to be different in various cell types, is speculated to be the decisive factor in taking on the role of master regulator. Just like TFEB, overexpression of TFE3 can promote lysosomal exocytosis and clearage of lysosomal substrate storage in several lysosomal storage disorders [100]. Together, these results suggest that TFEB and TFE3, both master regulators of the lysosomal-autophagy pathway, may be promising therapeutic targets for the development of a broad-spectrum neuroprotective drug.

12.3 Lysosomal and Mitochondrial Dysfunction: Are They Connected?

Mitochondria are eukaryotic organelles involved in a variety of cellular processes ranging from energy production to regulation of cellular calcium concentration and apoptosis. Mitochondria consist of a double-membrane structure that separates the inner-membrane space from the mitochondrial matrix [101]. Located in the inner mitochondrial membrane is the electron transport chain where oxidative phosphorylation occurs. This is also the primary site of both cellular energy and reactive oxygen species (ROS) production [102–105] (see Chap. 1). Growing evidence

demonstrates that lysosomes and mitochondria share a mutual relationship, where dysfunction in one organelle often impairs the function of the other. Lysosomal damage not only directly disturbs the lysosomal-autophagy pathway but can also cause lysosomal membrane permeabilization (LMP) resulting in the release of lysosomal luminal contents into the cytosol [74, 106–108]. Whether by insufficient turnover of damaged mitochondria or via direct interaction with lysosomal cathepsins released as a result of LMP, the consequences of lysosomal dysfunction impair mitochondrial function and lead to the loss of mitochondrial membrane potential, increased ROS production, decreased generation of ATP, and eventually the discharge of mitochondrial components into the cytosol via mitochondrial membrane permeabilization [109–112]. Mitochondrial dysfunction may also occur through mutations in gene-encoding proteins involved in mitochondrial quality control and homeostasis such as PTEN-induced putative kinase 1 (*PINK1*/*PARK6*), the E3 ubiquitin ligase parkin (*PARK2*), and DJ-1 (*PARK7*). Mutations in these genes have been implicated in familial forms of Parkinson disease [101, 113–117] (see Chap. 11). While it is unclear whether lysosomal dysfunction precedes mitochondrial damage or vice versa, disruption of the intricate balance between the functions of these two organelles establishes a deleterious feedback loop [107]. A failure to degrade defective mitochondria by the lysosome results in the accumulation of dysfunctional mitochondria and the subsequent leakage of ROS. In turn, these oxidative species may perpetuate lysosome dysfunction and subsequently enhance mitochondrial stress culminating in inflammation and cell death [107, 111, 114, 118]. Therefore, aberrant quality control of both lysosomes and mitochondria has profound consequences on the pathogenesis of a multitude of diseases, especially for those distinguished by neurodegeneration [112, 119–121].

12.3.1 Mitophagy

Neurons are peculiarly susceptible to the subtle sequela of lysosomal and mitochondrial dysfunction due to their reduced capability for glycolysis and reliance on oxidative phosphorylation for energy production [122, 123]. In order to maintain lysosomal and mitochondrial function and homeostasis, the cell employs specialized turnover pathways that target specific organelles (see also Chap. 11). Lysosomes mediate mitochondria-specific autophagy, often referred to as mitophagy, within cells, and this catabolic mechanism is regulated by two key proteins: PINK1 and parkin. In viable mitochondria, PINK1 is continually expressed and recruited to the outer mitochondrial membrane (OMM) where this serine/threonine kinase is imported into the mitochondrial matrix in a mitochondrial membrane potential-dependent manner. Once in the mitochondrial matrix, PINK1 is immediately degraded by proteases, thus regulating its expression [101, 114, 116]. However, upon the accumulation of aberrant proteins or the loss of mitochondrial membrane potential following mitochondrial insult, PINK1 regulation is impaired, allowing for accumulation of PINK1 on the OMM and its activation via autophosphorylation

[124, 125]. Activated PINK1 recruits parkin to the OMM and phosphorylates not only the ubiquitin-like domain of parkin but also ubiquitin itself. Phosphorylation of both protein species is required for full activation of the E3 ubiquitin ligase function of parkin [126–128]. Once relegated to the mitochondrial surface, parkin ubiquitinates various OMM proteins involved in mitochondrial maintenance, and this polyubiquitination labels the damaged mitochondria for turnover [129–131]. Cells with defective lysosomes, and therefore impaired mitophagy, are unable to effectively breakdown defective mitochondria resulting in their accumulation. These damaged mitochondria may leak reactive oxygen species (ROS), such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2), from complex I and III of the respiratory chain. If left unchecked, these oxidative species may ultimately cause neuronal death by not only further perpetuating organelle damage but also by activating the NLRP3 inflammasome and therefore initiating inflammatory responses [111, 112].

12.3.2 Oxidative Stress

ROS are important mediators of the downstream consequences stemming from the combined effects of lysosomal and mitochondrial dysfunction. The mitochondrial respiratory chain is the principal producer of ROS, primarily in the forms of O_2^- and H_2O_2, within the cell and during abnormal lysosomal and mitochondrial function, and contributes to the elevation of oxidative stress [112]. Lysosomes are particularly vulnerable to ROS-induced damage, as these oxidative species peroxidize lysosomal membrane lipids resulting in destabilization of the membrane and, potentially, even LMP. Due to their ability to inherit cargo during fusion with autophagosomes, lysosomes may acquire large amounts of iron during the degradation of macromolecules. This accumulation of iron within lysosomes has been speculated to contribute to lysosomal susceptibility to oxidative damage [107, 132]. Simultaneously, elevated oxidative stress may damage mitochondria as well. Reactive oxygen species may peroxidize lipids in the mitochondrial membrane resulting in loss of membrane potential as well as fragmented mitochondrial morphology [112, 116, 133]. Under high levels of oxidative stress, mitochondrial membrane permeabilization may also occur and release cytochrome c into the cytosol, therefore activating apoptosis [134]. Mitochondrial DNA (mtDNA) neighbors the electron transport chain within the inner mitochondrial membrane and is prone to oxidation and release during mitochondrial damage. Once released into the cytosol, oxidized mtDNA and local ROS trigger assembly and activation of the NLRP3 inflammasome [111, 134–136]. Beyond these organelles, ROS may also regulate the activity of proteins through posttranslational modification. One example is DJ-1, a neuroprotective protein encoded by *PARK7* that has been implicated in familial forms of Parkinson's disease when mutated. DJ-1 is dependent on localization to the mitochondria for proper neuroprotective activity, and this translocation is redox regulated by oxidation of the Cys106 residue into cysteine sulfinic acid by ROS. After translocation to the mitochondria, DJ-1 protects cells from oxidative

stress-induced death by modulating mitophagy and, together with other cellular processes, assumes a role in antioxidant response [137, 138].

12.3.3 Inflammasome Activation

Inflammasomes are intracellular multiprotein complexes that initiate an inflammatory response in response to pathogens and intracellular insults. The nucleotide-binding oligomerization domain-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome is the best characterized and is closely associated with lysosomal and mitochondrial dysfunction [139]. This protein complex is formed at the interface of the mitochondria and endoplasmic reticulum in an area known as the mitochondria-associated endoplasmic reticulum membrane. The NLRP3 inflammasome is chiefly composed of three components: a NOD-like receptor (NLRP3), the adaptor protein ASC, and caspase-1. Once assembled and activated, the NLRP3 inflammasome cleaves proIL-1β and proIL-18 into their bioactive forms, where these proinflammatory messenger molecules may subsequently modulate immune and inflammatory pathways [110, 111, 139–141]. Growing evidence identifies both ROS and oxidized mtDNA as activators of the NLRP3 inflammasome. Cells with impaired mitophagy, and consequently prolonged clearance of defective mitochondria, may spontaneously secrete ROS and oxidized mtDNA into the cytosol, resulting in consistent activation of the NLRP3 inflammasome [12, 36]. Recently, elegant work by Shi and colleagues has demonstrated that autophagy may function as a negative regulator of inflammasome activation. Their data suggests that inflammasome activation concomitantly induces autophagosome formation by initiating nucleotide exchange on the G protein RalB. Inflammasomes subsequently undergo ubiquitination and are transported by adaptor proteins p62 and LC3 to autophagosomes for elimination. These results suggest that autophagy may modulate the intensity of inflammation by directly degrading active inflammasomes and therefore may result in uncontrolled inflammation during lysosomal and mitochondrial dysfunction [142]. Taken together, the inflammasome represents a link between lysosomal and mitochondrial dysfunction and inflammation, which contributes to the pathogenesis of not only neurodegenerative but also autoinflammatory diseases [143, 144].

12.4 Lysosomal Storage Disorders and Neurodegeneration

Lysosomal storage disorders (LSDs) are rare inborn metabolic diseases in which lysosomal function is severely compromised due to mutations in gene-encoding enzymes resident in lysosomes involved in the breakdown of specific substrates. The subsequent accumulation of substrate within lysosomes has a variety of consequences such as lysosomal enlargement, altered lysosomal pH, and diminished

activity of lysosomal enzymes. Over 50 different LSDs have been described, and mutations in LSD-associated genes in patients, as well as carriers, have been linked to neurodegeneration, more particularly synucleinopathies [145]. Among LSD-associated genes, the molecular link between mutations in the glucocerebrosidase gene (*GBA1*) and Parkinson's disease (PD) is the most established [146].

12.4.1 GBA1 and Synucleinopathies

Pathological mutations in both alleles of the *GBA1* gene cause Gaucher disease (GD), the most common LSD. This disorder is characterized by lysosomal accumulation of the substrate glucosylceramide (GC), due to a deficiency in the lysosome-resident glucocerebrosidase enzyme (GCase) [147]. The cells most affected in GD patients are macrophages, which are involved in breakdown of senescent cells with GC-rich membranes such as erythrocytes. "Gaucher cells," which are the macrophages that have lysosomes engorged with substrate, can infiltrate the spleen, liver, and bone marrow, resulting in inflammation and organomegaly [148]. GD has been historically classified into non-neuronopathic type 1, acute neuronopathic type 2, and chronic neuronopathic type 3. Today, clinicians acknowledge a broad range of clinical manifestations associated with GD and subsequently can have difficulty classifying patients into specific GD subtypes [147]. Over the last 6 years, large cohort studies have established that the presence of mutations in the *GBA1* gene is a risk factor for the development of synucleinopathies including PD [146], dementia with Lewy bodies (DLB) (Fig. 12.2) [149], and, most recently, multiple system atrophy (MSA) [150]. All three synucleinopathies are characterized by the presence of inclusions of aggregated α-syn, a 14 kDa protein that is speculated to be involved in the regulation of synaptic vesicle dynamics and neurotransmitter release [151, 152]. In PD and DLB, the α-syn-positive Lewy bodies and neurites are mainly located in neurons of the substantia nigra, cerebral cortex, and hippocampus, while in MSA, the α-syn inclusions are located in glial oligodendrocytes [146, 149, 150]. The molecular link between mutations in the *GBA1* gene and PD was established by molecular analyses of the *GBA1* gene on a large pan-ethnic cohort comprising 5,691 patients with PD and 4,898 controls. This study revealed a strong association between *GBA1* mutations and the development of PD with an odds ratio of 5.43 and earlier onset of PD symptoms in patients with *GBA1* mutations [146]. These results have been replicated in multiple large cohorts with different ethnic backgrounds [114, 153, 154]. Today, *GBA1* mutations are widely considered the most common genetic risk factor for PD. However, it is important to keep in mind that most patients with GD and mutant *GBA1* carriers never develop synucleinopathies. These observations suggest that *GBA1* mutations and subsequent dysfunctional GCase enzyme are not a direct cause of synucleinopathy development; other cellular processes affecting organelle homeostasis, such as ER-stress and lysosomal and mitochondrial function, might play a more central role in synucleinopathy pathogenesis. The presence of dysfunctional GCase could exacerbate organelle dysfunction and subsequent α-syn accumulation.

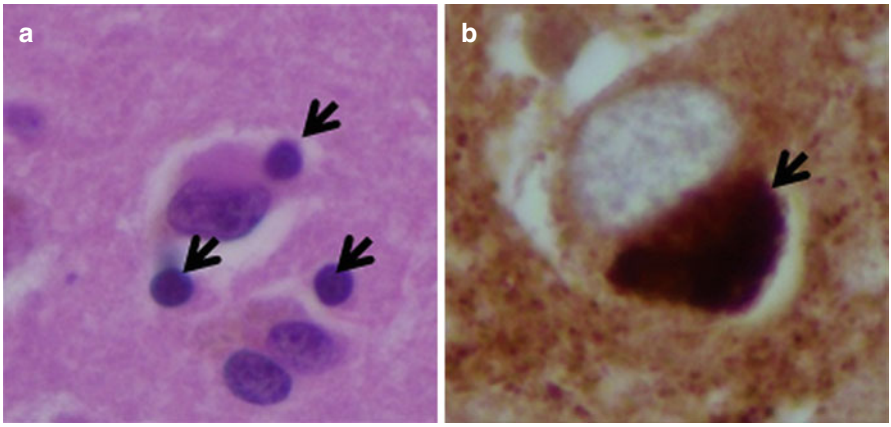

Fig. 12.2 Histology of hippocampal Lewy bodies (*arrows*) from a patient with GD and DLB. (**a**) Hematoxylin-eosin stain and (**b**) anti-α-syn immunostaining of hippocampal tissue from the same individual

12.4.2 GCase and α-syn Homeostasis

Initially, the mechanistic link between dysfunctional GCase enzyme and α-syn aggregation focused on gain-of-function or loss-of function hypotheses, where the former supports the direct involvement of dysfunctional GCase enzyme in the aggregation of α-syn, and the latter supports the role of lysosomal GC substrate accumulation in α-syn aggregation [155]. Currently, in vitro and in vivo research supports a reciprocal relationship between GCase and α-syn where downregulation of GCase protein expression or enzyme activity results in accumulation of α-syn. Increases in α-syn protein expression results in reduced GCase protein expression and enzyme activity (reviewed by [114, 156]). Furthermore, three independent studies support the observation of reduced GCase activity and protein expression in postmortem brains of sporadic PD and DLB patients without *GBA1* mutations, reinforcing the reciprocal relationship in relevant human samples [157–159]. The molecular mechanism of the reciprocal relationship is not fully understood although there is some evidence that an increase in α-syn protein levels inhibits ER-to-Golgi trafficking of GCase, which subsequently results in downregulation of GCase translocation to lysosomes. Less GCase in lysosomes can lead to lysosomal GC substrate accumulation and subsequent lysosomal dysfunction, which in turn may stimulate accumulation and oligomerization of α-syn throughout the cell. Buildup of α-syn aggregates could, in turn, inhibit ER-to-Golgi trafficking of GCase resulting in further decrease of this enzyme within lysosomes [160]. This reciprocal positive feedback loop could eventually lead to neurodegeneration. Evidence for this hypothesis came from a neuronopathic GD type 2 mouse model lacking GCase. Here, autophagy and proteosomal impairment lead to accumulation of fragmented mitochondria and α-syn in cultured neurons and astrocytes of the midbrain [161]. Although this

gba$^{-/-}$ model is not reflective of PD, it suggested that the lack of GCase expression promotes α-syn accumulation through impairment of cellular turnover pathways [101]. Novel insights into maintenance of α-syn homeostasis by manipulating GCase enzyme levels are promising for the development of new treatments for synucleinopathies. Although GCase enzyme replacement therapy does not improve PD symptoms, as the recombinant enzyme does not cross the blood-brain barrier [162], molecular inhibitors of glucosylceramide synthase for GC substrate reduction therapy and molecular chaperones for enhancing GCase translocation to the lysosomes can cross the blood-brain barrier and therefore show potential as therapeutics [163–166]. Recent research indicates associations similar to that found between mutations in *GBA1*, and the development of synucleinopathies can be expanded to other LSD-associated genes. Large molecular cohort studies suggest that mutations in the sphingomyelin phosphodiesterase (SMPD1) and α-N-acetylglucosaminidase (NAGLU) genes, which are associated with Niemann-Pick disease A and B and mucopolysaccharidosis type III B, respectively, may be implicated in the development PD [167, 168]. These observations suggest that mutations in other lysosomal-resident enzymes might be classified as risk factors for the development of synucleinopathies.

12.5 Conclusion

When first described by Christian de Duve, and for many years after, lysosomes were often considered static organelles primarily involved in the degradation of cellular constituents. However, recent insights into lysosomal function and regulation have demonstrated otherwise. In fact, lysosomes are now considered dynamic organelles capable of not only cellular cleanup but also nutrient sensing and lipid catabolism. As mediators of autophagy, lysosomes also play an important role in the development of neurodegenerative diseases. Lysosomal dysfunction leads to not only the accumulation of aggregate-prone proteins but also impairs other organelles such as mitochondria. Together, dysfunction of this deleterious duo might drive a destructive feedback loop that culminates in the neuropathology often found in Parkinson's, Alzheimer's, and Huntington's diseases. The association between LSDs and neurodegenerative diseases such as PD, LBD, and MSA further highlight the importance of proper lysosomal function in neuronal health. Further investigations exploring the relationship between lysosomal and mitochondrial dysfunction hold promise for the discovery of new potential drug targets.

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