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Review Series: Autophagy in Higher Eukaryotes—A Matter of Survival or Death

Neurodegenerative lysosomal disorders

A continuum from development to late age

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Abbreviations: Aβ, amyloid β-peptide; AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; ApoE, apolipoprotein E; APP, amyloid precursor protein; AV, autophagic vacuoles; CMA, chaperone-mediated autophagy; Cat D, cathepsin D; CHMP, charged multivesicular body protein; ClC, chloride channel family; ESCRT, endosomal sorting complex required for transport; FAD, familial Alzheimer's disease; FTD, frontotemporal dementia; Hrs/Vps27p, hepatocyte growth factor-regulated tyrosine kinase substrate; mTOR, mammalian target of rapamycin; MVB, multivesicular body; NCL, neuronal ceroid lipofuscinoses/batten disease; NPC, niemann-pick type C; PS, presenilin; STAM/Hse1p, signal transducing adaptor molecule; Vps, vacuolar protein sorting; Vps4p/SKD1, vacuolar protein sorting 4/suppressor of K(+) transport growth defect 1

Key words: Alzheimer's disease, amyloid, cathepsins, endosomes, autophagy, lysosomes, neuronal cell death, neurodegeneration, brain

Neuronal survival requires continuous lysosomal turnover of cellular constituents delivered by autophagy and endocytosis. Primary lysosomal dysfunction in inherited congenital "lysosomal storage" disorders is well known to cause severe neurodegenerative phenotypes associated with accumulations of lysosomes and autophagic vacuoles (AVs). Recently, the number of inherited adult-onset neurodegenerative diseases caused by proteins that regulate protein sorting and degradation within the endocytic and autophagic pathways has grown considerably. In this Perspective, we classify a group of neurodegenerative diseases across the lifespan as disorders of lysosomal function, which feature extensive autophagic-endocytic-lysosomal neuropathology and may share mechanisms of neurodegeneration related to degradative failure and lysosomal destabilization. We highlight Alzheimer's disease as a disease within this group and discuss how each of the genes and other risk factors promoting this disease contribute to progressive lysosomal dysfunction and neuronal cell death.

Introduction

For more than a century, researchers have known about disorders in which waste products accumulate in cells resulting in the formation of large intracellular vacuoles. Christian de Duve's discovery of lysosomes in 1955 provided a critical biological framework for understanding their pathogenic significance in more than 50 known "storage disorders" characterized by profound developmental disabilities, often including mental retardation and dementia.[1] Substantial progress has since been made toward identifying mutated genes responsible for these inherited developmental disorders.

Early investigators of the aging process also viewed lysosomes as potentially holding secrets to cellular degeneration. They proposed that declining lysosomal degradative efficiency reflected by accumulation of undigested lipid and protein in lipofuscin granules could be a basis for the deterioration in aging cells. Some of the early studies, however, prematurely concluded that lipofuscin accumulation had negligible adverse effects[2] and de Duve's now validated idea that hydrolase release from lysosomes in disease states can induce cell death initially received scant support. For several decades, scientists regarded lysosomes simply as "terminal degradative compartments" which were deployed in pathological states mainly at end stages of degeneration to remove and scavenge cellular debris.

Attention to the lysosomal system has again surged, as a result of the recent understanding of autophagy and endocytosis at the molecular level[3,4] and the emerging appreciation of its involvement in a broad range of diseases.[5] Particular interest in autophagy by neuroscientists has been sparked by the recognition of endosomal-lysosomal-autophagic pathology in major neurodegenerative diseases[6,7] and the identification of increasing numbers of gene mutations that implicate lysosomal system dysfunction directly in adult-onset neurological diseases. In this Perspective, we advance the concept that vesicular compartments that are at the convergence point between the two major pathways to the lysosome, autophagy and endocytosis, are a "hotspot" for genetic mutations causing neurodegenerative disorders not only during development but across the lifespan.[6] We will briefly discuss the cell biology of the autophagy and endocytic pathways and highlight the critical cross-talk between these pathways. Later, we will review emerging genetic data that underscores the role of defects in autophagosome-lysosomal degradation in adult onset neurodegenerative diseases. Finally, we will focus on Alzheimer's disease, where endosomal-autophagic-lysosomal dysfunction is particularly robust, may be instrumental to

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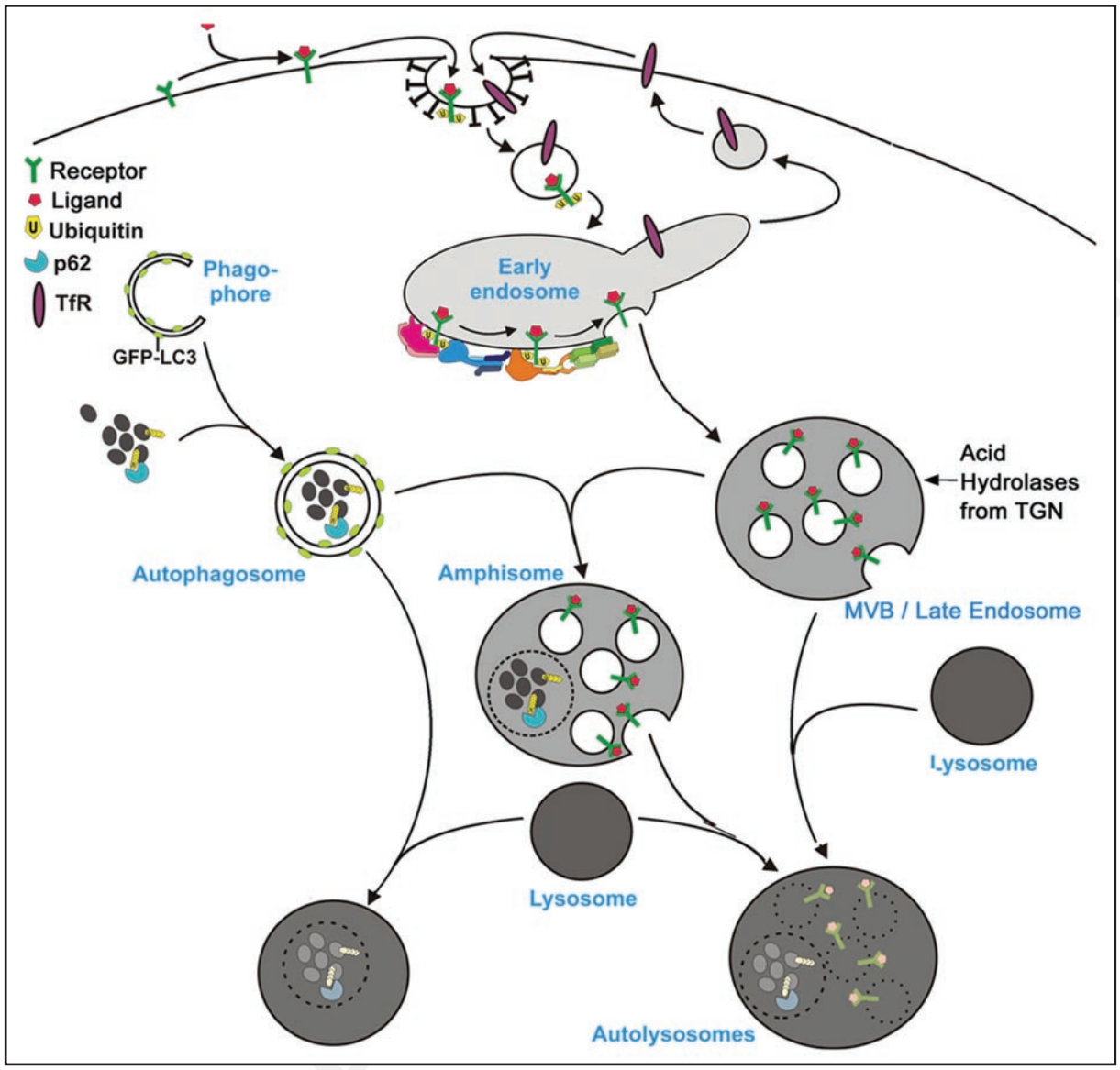

Figure 1. Two major pathways to the lysosome, autophagy and endocytosis. The diagram emphasizes the principal sorting and degradative compartments and interactions with lysosomes. Details are discussed in the text. Figure adapted from Filamorenko et al., 2007 with permission of Rockefeller University Press.

disease progression, and is driven by the genes that cause or promote this disease.

Major Pathways to the Lysosome

Lysosomes are components of two major degradative and processing pathways, autophagy and endocytosis, which are briefly reviewed below (Fig. 1).

Autophagy[8] refers to at least three processes by which intracellular constituents enter lysosomes for degradation: chaperone-mediated autophagy (CMA), microautophagy and macroautophagy.[9] In CMA, cytosolic proteins containing a KFERQ motif are selectively targeted to the lysosomal lumen for degradation. In microautophagy, small quantities of cytoplasm non-selectively enter lysosomes when the lysosomal membrane invaginates and pinches off small vesicles for digestion within the lumen. Finally, macroautophagy, mediates large-scale degradation of cytoplasmic constituents. During macroautophagy, an elongated "isolation" membrane, created from a pre-autophagosomal structure (PAS) or "phagophore", sequesters a region of cytoplasm to form a double-membrane-limited autophagosome. Two ubiquitin-like protein conjugation pathways are known to coordinate this process.[10] Sequestered material within autophagosomes is digested when lysosomes or late endosomes fuse with the outer membrane of the autophagosome.[11] Autophagy is constitutively active in neurons and required for survival.[12] Induction of autophagy is generally controlled by the mTOR kinase (mammalian Target of Rapamycin), which is regulated by growth factors (especially insulin) and nutrient levels. mTOR–independent induction of autophagy involving Beclin 1 and the class III phosphatidylinositol 3-kinase hVps34 can also occur.

During endocytosis,[13] extracellular materials (e.g., growth factors, lipoproteins) and membrane proteins are internalized into clathrin-coated pits and directed to early endosomes by the small GTPase

Rab5 and several Rab5 effector proteins. After internalization, many cell surface proteins and lipids are returned to the plasma membrane via recycling endosomes. Other early endosome cargos reach late endosomes either by budding off transport vesicles or by directly maturing to late endosomes. During this maturation process mediated by the replacement of Rab5 by Rab7, a multivesicular body (MVB) is created by the inward budding of the surface membrane to form a collection of internal vesicles.[14] Cathepsins and other acid hydrolases are delivered to MVB/late endosomes when shuttle vesicles from the trans-Golgi network (TGN) or lysosomes fuse with these compartments.[15] The acidic pH within the vesicles (3.5–6.0) is maintained by an ATP-dependent proton pump, the vacuolar ATPase,[16] and is modulated by inward chloride and cation currents, and low molecular weight activators and inhibitors.[17]

The sorting of cargoes into intraluminal vesicles of the MVB/late endosome involves ubiquitination of the cargoes and the actions of three separate protein complexes called ESCRT-I, ESCRT-II and ESCRT-III (endosomal sorting complex required for transport). Ubiquitinated endocytosed integral membrane proteins, upon recognition by Hrs/Vps27p and STAM/Hse1p are transferred to the ESCRT-I complex.[18] The ESCRT-II complex mediates the subsequent delivery of cargo to the MVB/late endosome. The inward membrane invagination and fission of vesicles containing ESCRT-II is mediated mainly by ESCRT-III. Ubiquitin, the crucial signal for efficient sorting into the MVB,[19] is then removed proteolytically and Vps4/SKD1, an AAA-ATPase, dissociates the ESCRT apparatus from the MVB.

Lysosomes Dynamically Interact with Related Degradative Compartments

Although often referred to as "terminal" degradative compartments, lysosomes imaged in living cells continuously fuse or interact transiently ("kiss and run") with other compartments in the autophagic and endocytic pathways (autophagolysosomes, late endosomes and amphisomes) and even with the plasma membrane under certain conditions.[20] Amphisomes, a hybrid compartment formed by the fusion of autophagosomes with early or MVB/late endosomes, represent a critical intersection of the endocytic and autophagy pathways. Isolated amphisomes contain markers for early endosomes and late endosomes.[21] Autophagy stimulation increases fusion between MVBs and autophagosomes to form amphisomes, which potentially contain hydrolases and a protein pump[22] and are capable of proteolysis.[23] Amphisomes are clearly important in neurons, where a considerable proportion of endocytosed cargo is directed to the autophagic pathway prior to being degraded by lysosomes.[12] Recently, it has been shown that functional multivesicular bodies in the endocytic pathway are vital for the autophagic clearance of ubiquitinated proteins, including ones that accumulate in amyotrophic lateral sclerosis (ALS) and Huntington's disease.[23] These observations suggest that "late" sorting/degradative steps in the autophagic and endocytic pathways share certain regulatory mechanisms and that perturbing one of this group of interacting compartments potentially can alter the function of the others. It is easy to see, therefore, how a defective protein residing in one of these compartments may have pleiotropic pathogenic effects in both autophagy and endo/lysosomal function in neurodegenerative disorders. In the lysosomal storage disorder Niemann-Pick Type C (NPC), for example, a mutation in a resident late endosomal protein alters early, as well as late, endosome function and in $Npc1^{-/-}$ mice, also disturbs autophagy.[24]

The Lysosomal System: A Genetic "Hotspot" for Neurodegenerative Diseases Across the Lifespan

The lysosomal system, broadly defined to include compartments with which lysosomes interact, is a convergence point for a surprising number of genetic mutations that cause neurodegenerative diseases across the age spectrum. Primary lysosomal dysfunction in diseases of the developing nervous system is well known; however, the frequency with which primary defects in resident proteins of lysosomes or interacting compartments may lead to adult onset neurodegenerative diseases is only now being recognized. Unlike the degradative problem in some protein misfolding diseases (e.g., CAG repeat disorders), where elimination of a single mutant protein may be affected, juvenile/adult onset disorders with a primary defect in lysosomal function impair the turnover of most, or all, long-lived proteins and organelles normally eliminated by autophagy. Impairment is reflected in the accumulation of waste products within a particular degradative compartment, as in many lysosomal storage disorders, along with the accumulation of autophagic or endocytic compartments themselves (e.g., AV, lysosome, MVB/late endosome). With severe degradative impairment, onset is commonly developmental and systemic and early death may, therefore, preclude or overshadow full-blown neurodegenerative disease. Forestalling early death in several animal models of these developmental lysosomal disorders has, in fact, enabled a prominent neurodegenerative phenotype to emerge in adult animals.[25,26] Neurodegenerative phenotypes seem more likely to develop from milder forms of inherited lysosomal dysfunction, and their emergence may require additional aging-related lysosomal impairments.

We propose, therefore, that lysosomal disorders are a continuum of diseases across the lifespan. Some examples along this continuum (Table 1) are discussed below. In each of these disorders, robust AV accumulation is associated with extensive, though not necessarily exclusive, neurodegeneration of cortex and hippocampus and commonly involves molecular defects in the degradative clearance of AVs.

A dozen or more developmental disorders with prominent neurodegenerative phenotypes are caused by defects in functionally dissimilar enzymes, which disrupt the internal environment of the lysosome.[5] A picture is emerging that many of these disorders may also impair turnover by autophagy.[27] The lysosomal protease cathepsin D (Cat D) is ubiquitously expressed, yet loss-of-function mutations of Cat D cause a relatively selective neurodevelopmental disorder. Depending on the Cat D mutation and presumably the degree of Cat D insufficiency, the disorder manifests as congenital neuronal ceroid lipofuscinoses/Batten disease (NCL) with severe mental retardation or as a juvenile neurodegenerative disorder associated with dementia in humans, sheep and bulldogs.[28,29] In mice completely lacking Cat D, autophagosome/autolysosome-like structures progressively accumulate with aging in neurons and dystrophic neurites,[30] similar to the pattern seen in ClN-2, a late infantile form of NCL.[31] A similar neurological pattern is also observed in mice after the deletion of both cathepsins B and L[32] (Fig. 2) or by pharmacologically inhibiting cysteine proteases.[33] Loss of the

Table 1 **Neurodegenerative disorders associated with genes affecting lysosomal system function**

Disease	Defective molecules	Cellular location of defective molecules	Endo/lysosomal phenotype	Ref
Alzheimer's disease (AD)	APP, PS	Broad distribution includes endosomes, autophagosomes	Degradative steps of autophagy impaired AV, autolysosome, lysosomal dense body accumulation	6, 99
Parkinson disease (PD)	α-synuclein, ATP, BA2	Synaptic vesicle, lysosomes	Degradative steps of autophagy impaired Accumulation of autophagosome like structure	48–50
Huntington disease (HD)	Htt	Glucocerebrosidase-lysosomes Nucleus, cytoplasm	Late sorting/degradative steps of autophagy impaired Autophagosome-like vacuole accumulation	23, 57
Down syndrome	APP duplication	endosome, plasma membrane, lysosome	Upregulated endocytosis Enlarged early/late endosome	114
Frontotemporal dementia (FTD-3 subtype)	CHMP2B	Endosome	Impaired endosome maturation Accumulation of large dysmorphic endosomes and autophagosomes	115
Amyotrophic lateral sclerosis (ALS)	Rab5, CHMP2B	Early endosomes, late endosomes	Impaired early endosome dysfunction, late sorting/ degradative steps of autophagy AV pathology	23
Charcot-Marie Tooth disease type 2B	Rab7	Late endosome	Defective endosome maturation	116
Niemann-Pick disease type C (NPC)	NPC1/2	Late endosome	Early/late endosome dysfunction and impaired autophagy leading to accumulations of sphingolipids and cholesterol	44, 117
Neuronal ceroid lipofuscinoses/Batten disease (NCL)	CLN-1&2	Late endosome Lysosome	Impaired lysosomal degradation Autophagosome/autolysosome-like structures accumulation	30, 97
Creutzfeldt-Jakob disease	PrP	Cell surface, endosome	Impaired function of lysosomal enzymes and marked AV accumulation	118
Autosomal dominant hereditary spastic paraplegia (ADHSP)	Spastin	Endosomes	Impaired multivesicular body fusion with lysosomes	119
Chediak-Higashi syndrome (CHS)	Lyst	Endosome, MVB	Giant lysosomes accumulation and fail to form phagolysosomes	26
Inclusion body myositis (IBM)		Late endosome, AV, lysosome	Impaired lysosomal degradation and accumulation of autophagy-related "rimmed" vacuoles	120
Osteopetrosis	ClC-7	Lysosome	Defective lysosome acidification mainly affects bone resorption	34

H(+)-ATPase a3 subunit, or chloride channel interacting protein ClC-7, impairs lysosome acidification and thus impedes proteolysis causing selective defects in bone resorption (osteopetrosis) and mental retardation in humans and mice.[34] Rescuing the bone phenotype in the mouse model by transgenic expression of ClC-7 in osteoclasts increases lifespan and elicits a mainly neurodegenerative phenotype with extensive autophagic-lysosomal pathology.[25] Deletion of ClC-3 or ClC-6 produces prominent neurodegenerative phenotypes resembling NCL.[35,36]

Niemann-Pick type C disease (NPC) arises from defective cholesterol trafficking within the endocytic pathway[37] caused by mutations in either of two functionally related genes, *NPC1* and *NPC2*.[37] Located primarily in late endosomes/lysosomes, NPC1 influences the trafficking of NPC2, a protein that resides in late endosomes and the trans-Golgi network.[38,39] Cells with dysfunctional NPC1 or NPC2 accumulate unesterified cholesterol in late endosomes, reflecting a failure of cholesterol to efficiently exit this compartment.[38,40] Juvenile and adult-onset cases experience prominent cognitive decline accompanied by cortical and subcortical neuron loss,[41] lysosomal storage in neurons, and extensive neuroaxonal dystrophy with spheroids containing degenerating organelles and neurofilaments.[42] In NPC1-null mice, robust AV accumulation precedes neuron death.[43,44] Notably, NPC is one of a very few disorders in which hallmark pathologies of Alzheimer disease including neurofibrillary tangles and Aβ42 within enlarged endosomes develop in the absence of tau mutations or β-amyloid deposition.[45]

Frontotemporal dementia linked to chromosome 3 (FTD-3), a late-age onset primary progressive dementia, is one of three genetically distinct FTD subgroups. Recently, mutations of Vps2B/CHMP2B, an ESCRT-III member, were identified as a genetic basis for a form of dementia, which accounts for a significant proportion of all FTD cases[46] and also for amyotrophic lateral sclerosis (ALS) in a subpopulation of patients. Depleting ESCRT subunits or overexpressing CHMP2B mutant proteins inhibits autophagic degradation of ubiquitinated proteins, leading to their aggregation[47] and cell death.[23]

Death of dopaminergic neurons in Parkinson disease is associated with the accumulation of inclusions (Lewy bodies) containing α-synuclein, a cytosolic protein with synaptic functions. Reflecting a multi-factorial disease process, the cell death pattern has features of apoptosis and necrosis and includes the accumulation of autophagosome-like structures.[48] While macroautophagy and proteasome

pathways are involved in α-synuclein turnover,[49,50] the rate-limiting degradative mechanism in neuronal cells is chaperone-mediated autophagy[51] which is impaired by mutations of α-synuclein that cause familial Parkinson disease. Due to an abnormally tight binding to the surface receptor, LAMP 2a, the mutant α-synuclein protein enters lysosomes inefficiently and importantly, also blocks uptake and degradation of other substrates.[9] Overexpressed α-synuclein may also impair autophagosome-lysosome fusion when macroautophagy is recruited to compensate for the CMA defect.[52] Notably, mutations of ATP13A2, a lysosomal ATPase highly expressed in brain, cause a form of hereditary Parkinsonism with dementia.[53]

Additional neurodegenerative diseases involving primary defects in lysosome-related compartments are listed in Table 1 and others deserve brief mention as conditions involving secondary defects in lysosomal system function that may contribute to disease development. In prion diseases such as scrapie, misfolding of the prion protein PrP^{c} causes the pathogenic β-helical PrP^{sc} to form in the endocytic pathway[54] and accumulate in MVB/late endosome.[55] Overloading this system seems to impair lysosomal enzyme function and lead to significant AV accumulation.[56] In Huntington disease, appreciable AV accumulation[57] may be related not only to effects of aggregates of mutant huntingtin[58] but also to interactions of this mutant protein with proteins modulating clathrin-mediated endocytosis and MVB vesiculation.[59] In these proteopathies where the lysosomal degradative system itself may be relatively competent, inducing autophagy to speed degradation of mutant proteins could, therefore, have therapeutic effects, as suggested by studies in several Huntington's disease models.[60]

Alzheimer's Disease: A Disorder with Particularly Extensive Autophagic-Endosomal-Lysosomal Dysfunction

The magnitude of AV accumulation within dystrophic neurites distinguishes Alzheimer's disease from other late-onset neurodegenerative disorders associated with lysosomal dysfunction. Dystrophic neurites in the AD brain are best known for containing abnormal aggregates of the microtubule associated protein, tau, but they are, in fact, mainly filled with AVs, which range from autophagosomes[61] to autolysosomes, and lysosomal dense bodies[62] (Fig. 2). "Neuritic dystrophy", the focal swelling of regions along axons or dendrites, is a common response to neuronal injury but in most neuropathic states and in aging, various forms of dystrophic neurites contain mixtures of neurofilaments, tubular profiles, mitochondria and vacuoles in different proportions[63] similar to organelle mixtures accumulating after experimental blockade of axonal transport.[64] By contrast, autophagic-lysosomal compartments are the most prevalent organelles by far in dystrophic neurites of the AD brain. Moreover, the extent of neuritic dystrophy and the characteristically large size of these neuritic distensions in AD[65] are not typical of most CNS neurodegenerative diseases.[66] This enormous storage of autophagy-related compartments has been relatively unappreciated despite its potentially important implications for β-amyloidogenesis[67] and neuronal survival in AD.[7]

The autophagic pathology in AD is consistent with a defect in AV clearance rather than solely with an increased autophagy induction. Both immature AVs and hydrolase-containing dense lysosomes accumulate in dystrophic neurites, suggesting that fusion between these compartments is inefficient. Cathepsin-positive AVs in neurites are frequently double-membrane-limited and contain LC3, an Atg protein normally degraded rapidly after autophagosome-lysosome fusion, suggesting defective protein degradation within autolysosomes. Impaired maturation of AVs to lysosomes is also supported by recent evidence that neuronal autophagy is quite active but very efficient: few immature AVs collect even when autophagy in cortical neurons in culture is markedly induced with rapamycin or serum starvation.[68,69] By contrast, when AV clearance is impeded experimentally[33,69] AVs resembling those in AD dystrophic neurites rapidly accumulate.

Lysosomal System Function is Disrupted by Factors Causing or Increasing Risk for Alzheimer Disease

Genetic factors that cause or increase risk for AD have significant effects on autophagic-lysosomal function, which secondarily contribute to β-amyloidogenesis and possibly lysosome-mediated neuronal cell death (Table 2). Aβ is generated in the endocytic and autophagic pathways.[7,67] Among its various pathophysiological effects, Aβ exerts neurotoxicity in part through effects on lysosomal functions.[70-72]

Presenilin-1 (PS1) mutations are the most common cause of early-onset familial AD (FAD).[73] PS1, a ubiquitous transmembrane protein, has diverse biological roles in cell adhesion, apoptosis, neurite outgrowth, calcium homeostasis and synaptic plasticity.[74] Many, but not all, of these functions involve PS1 as the catalytic subunit of an enzyme complex, termed γ-secretase, which mediates the intramembranous cleavage of various type 1 membrane proteins, including amyloid precursor protein (APP) and Notch.[75] Although the pathogenic effects of PS1 mutations in AD are commonly ascribed to increased generation of the neurotoxic Aβ peptide from APP, loss of PS1 function is increasingly suspected of playing a key role.[76] One of the vital functions of PS1 is its essential involvement in the autophagic turnover of proteins. Macroautophagy is completely eliminated in blastocysts from mice lacking PS1 and PS2 genes but is rescued by reintroducing wild-type PS1 (Lee, Yu, Nixon, unpublished data), accounting for the previously reported retarded autophagic-lysosomal turnover of specific proteins.[77,78] Moreover, in fibroblasts from patients with FAD, mutant PS1 substantially slows AV clearance and rates of autophagic turnover of long-lived proteins while having minimal effects on mTOR signaling and autophagosome formation. In the brains of patients with PS-FAD or mice modeling the disease, PS1 mutations potentiate lysosomal system pathology, amyloidogenesis and neurodegeneration.[79,80]

App duplication causes early-onset AD in some families and in individuals with Down Syndrome (DS).[81] Among the earliest responses of neurons in AD and in DS and its mouse model (Ts65Dn) is marked enlargement of Rab5-positive endosomes[82] and late endosomes[83] which reflects upregulated endocytosis and substrate delivery to MVB/late endosomes, amphisomes and lysosomes. The presence of three copies of *App* is responsible for the endocytic pathway dysfunction, which is associated with defective neurotrophic signaling and neurodegeneration in the Ts65Dn mouse.[84-86] Upregulated endocytosis at early stages of AD[83] increases delivery of substrates, including APP-rich membranes, to the autophagic pathway thereby further burdening an already stressed lysosomal system.

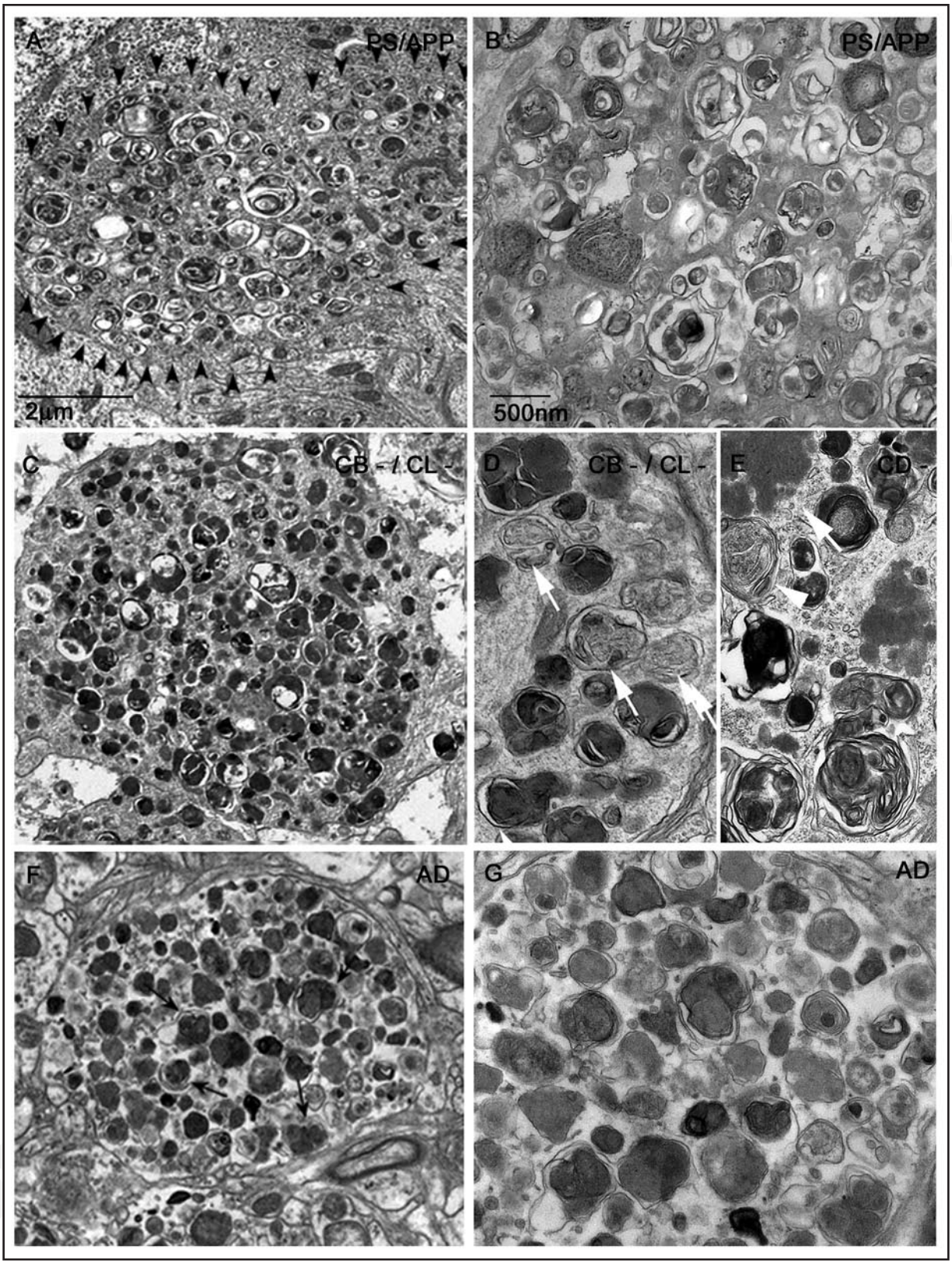

Figure 2. Autophagy pathology in Alzheimer's Disease (AD) brain and models of AD and neuronal ceroid lipofuscinosis (NCL) reveals striking similarities in the appearance of the axonal dystrophy and the nearly complete replacement of the cytoplasm with autophagic vacuoles ranging from autophagosomes to autolysosomes. Depicted here is the ultrastructure of a dystrophic neurite (A, outlined by arrowheads) in the brain of a PS1/APP mouse. The higher magnification image (B) reveals many immature autophagic vacuoles delimited by double membranes. Dystrophic axons in a mouse model of neuronal ceroid lipofuscinosis,the cathepsin B-/cathepsin L-null mouse (C and D) and the cathepsin D-null mouse (E) exhibit similar profiles. These patterns strongly resemble that in human AD brain (F and G). Panel A reproduced with permission from Yu et al., JCB, 2005. (C–E) reproduced with permission from Koike et al., AJP, 2005.

Table 2 **Genetic factors in Alzheimer's disease**

Gene	Coding protein	Cellular location of coding protein	Endo/lysosomal phenotype	Ref
APP	Amyloid precursor protein	Broad-distribution includes endosomes, autophagosomes	Upregulates endocytosis leading to abnormal endosomes and endosomal storage Aβ alters MVB trafficking	84, 86, 114
PSEN	Presenilin	Broad distribution includes endosomes, lysosomes enriched in AVs	Slows autophagic protein turnover of long-lived proteins Massive AV accumulation in neurites	77
APOE	Apolipoprotein E, Epsilson allele	plasma membrane, endosome	Exacerbates endocytosis upregulation and potentiates Aβ1-42 induced lysosome destabilization	88–90, 121
CatD	Cathepsin D polymorphisms	Endosome, lysosome	Effects of polymorphisms unknown, loss of function causes CLN with impaired autophagic degradation and massive AV accumulation	30
SORL 1	Sortilin-related receptor	Plasma membrane, endosome	SORL1 releases APP into the endocytic increasing Aβ generation	122
CST3	Cystatin C polymorphisms,	Cytoplasm, endosome, lysosome	Increased autophagy associated with *lower* AD risk	93

Inheritance of the ε4 allele of the apolipoprotein E gene (APOE), the strongest genetic risk factor for late onset AD,[87] accelerates and magnifies the abnormal endocytosis upregulation seen in early AD.[88,89] Lysosomes proliferate and accumulate intraneuronal Aβ42 in hippocampal neurons of transgenic mice overexpressing APP and ApoE.[90,91]

Among the many possible genetic modifiers of AD risk investigated, only a few have been confirmed in meta-analyses of multiple studies. These include two lysosomal system proteins, cathepsin D and cystatin C (Alzforum: Alzgene website http://www.alzforum.org/res/com/gen/alzgene/). In most but not all populations studied AD risk is increased in carriers of Cat D T-224C polymorphism that alters pro-Cat D trafficking.[92] Specific polymorphisms of cystatin C, an inhibitor of cysteine proteases that is neuroprotective in injury settings, is associated with reduced AD risk.[93]

Aging, a sine qua non for AD development, is accompanied by slowed degradation of long-lived proteins[94] reflecting declines in macroautophagic proteolysis and chaperone-mediated autophagy.[95] Lipofuscin accumulation, a hallmark phenomenon of cellular aging that was previously thought to be innocuous, interferes with the fusion of lysosomes with autophagosomes.[96]

Pathological Consequences of Autophagic-Lysosomal Failure in Alzheimer's Disease

Defining the cell death process in AD and other chronic late-onset neurodegenerative disorders is confounded by the heterogeneity of neurons and non-neuronal cells in brain, each of which is potentially capable of different primary or secondary responses to a toxic stimulus. Cat D deficiency, for example, causes two distinct types of cell death in the outer and the inner nuclear layer in the retina.[97] Loss of one neuronal population from a primary insult may deprive another neuronal population of trophic support and trigger a second pattern of cell death. Caspases, cathepsins and calpains which govern different cell death cascades cross-talk extensively and, in a chronic disorder, such as AD, all of these proteases may become activated in a slowly dying neuron.[98-100] Despite the difficulties in pinpointing the triggering events in this degenerative process, a relatively strong case can be made that autophagy failure, through several different routes, contributes significantly to neuronal cell death in AD and, by extension, to some of the other diseases previously discussed.

Neurons are dependent on autophagy for survival: they accumulate ubiquitinated proteins and degenerate within weeks after macroautophagy is inactivated genetically in mice.[101,102] Death of these neurons involves, at least in part, the toxicity of proteins accumulating in the cytoplasm.[103] Autophagy failure reduces the cytoprotection afforded by the elimination of potentially toxic mutant and oxidized proteins, protein aggregates and damaged organelles. For example, activated caspase-3 generated constitutively in hippocampal neurons is degraded by autophagy normally but it accumulates in AVs in the dystrophic neurites of PS/APP mice (Yang et al., unpublished). Efficient autophagy also delays or prevents apoptosis by turning over non-essential cell constituents to provide substrates for energy during states of nutritional deprivation or trophic factor withdrawal.[104]

More specific to the mechanism of degeneration in Alzheimer's disease, lysosomal degradative failure enables Aβ and possibly tau to accumulate and further disrupt proteolysis[105] or destabilize AVs/lysosomes. Aβ peptide is generated during the macroautophagic turnover of APP[79] before being degraded in lysosomes[106] and accumulates when degradation in AVs is impaired within dystrophic neurites of AD brain. The accumulation of Aβ1-42, which is more slowly degraded than Aβ1-40, has been reported to cause leakage of lysosomes enzymes into the cytosol prior to other morphological signs of cellular toxicity.[107,108] ApoE4, a major AD risk factor, potentiates Aβ1-42-induced lysosome proliferation and promotes leakage of acid hydrolases and apoptosis in cultured neuronal cells.[109] The low pH of lysosomes accentuates the conversion of ApoE4 to a molten globule, inducing reactive intermediates that bind avidly to phospholipid vesicles and destabilize cellular membranes.[110] Calpains, which are activated in AD brain,[111] are also known to labilize lysosomal membranes. Cataclysmic disruption of lysosomal membranes induces rapid necrosis during which released hydrolases act as both the trigger and executioner in the death process:[112,113] slow release of cathepsins more likely operates through signaling pathways to trigger apoptosis.[113]

Conclusions

Endocytic and autophagic activity is high in synapses and along the long neuritic processes of neurons. Efficiently clearing these cargo-laden vesicular compartments represents a unique challenge. It is, therefore, not surprising that neurons seem to be particularly vulnerable to lysosomal system disruption. This vulnerability is amply evidenced by the recent identification of primary genetic defects affecting protein sorting and degradation within the endocytic and lysosomal pathways in a growing number of inherited neurodegenerative disorders across the entire age spectrum. Despite the universal cellular role of the lysosomal system, relatively selective neurodegenerative phenotypes might be favored in the CNS when impairments of lysosomal dysfunction are mild enough to be tolerated by cells in peripheral tissues but not by neurons as they are confronted with additional aging-related lysosomal degradative impairments. Autophagic-endocytic-lysosomal pathology in Alzheimer's disease is particularly prominent and new studies are now revealing that the genetic factors that lead to increased Aβ production and deposition also substantially affect lysosome system function and stability, which independently contributes to neuronal dysfunction, amyloidogenesis, and the complex cell death process in this disease.

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