Lysosome and calcium dysregulation in Alzheimer’s disease: partners in crime

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Abstract

Early-onset FAD (familial Alzheimer’s disease) is caused by mutations of PS1 (presenilin 1), PS2 (presenilin 2) and APP (amyloid precursor protein). Beyond the effects of PS1 mutations on proteolytic functions of 

Introduction

Autosomal dominant mutations of PS1 (presenilin 1), PS2 (presenilin 2) and APP (amyloid precursor protein) cause EOAD (early-onset Alzheimer’s disease). Although EOAD accounts for fewer than 5% of all AD cases, investigation of the three genes responsible has so far provided many of the available clues to suspected pathogenic mechanisms in AD. Mutations of PS1, which are responsible for the vast majority of EOAD cases, can accelerate disease onset to ages as early as the late 20s. In most cases of EOAD, the defining lesions of AD, i.e. NFTs (neurofibrillary tangles) and neuritic plaques, as well as characteristic autophagic–lysosomal pathology, resemble the features of later-onset sAD (sporadic AD), although these abnormalities are usually more severe. Notable clinical and neuropathological heterogeneity, however, is sometimes seen among families carrying one of the >120 known PS1 mutations [1–3].

PS1, a ubiquitous protein with nine transmembrane domains, exists as a 65 kDa holoprotein in the ER (endoplasmic reticulum). The molecule is cleaved by a furin-like ‘presenilinase’ to generate a two-chain form [4,5], which constitutes the catalytic subunit of the γ-secretase enzyme complex composed of four additional subunits.

Delivered from the ER to diverse vesicular destinations in the cell, γ-secretase mediates the intramembranous cleavage of well over 25 different substrates, which are mainly type 1 membrane proteins [6,7], including most notably APP. The γ-secretase generates aneurotoxic Aβ (amyloid β-peptide) from a membrane-bound C-terminal fragment of APP generated by BACE-1 (β-site APP-cleaving enzyme 1) [8]. The pathogenic effects of PS1 mutations in AD are commonly ascribed to a small increase in the generation of a toxic 42-amino-acid peptide (Aβ42) relative to a less toxic 40-amino-acid form (Aβ40) [7,9,10]. Recent findings, however, indicate that AD (Alzheimer’s disease)-causing PS1 mutations often confer loss of function of the secretase, so that, even if the Aβ42/Aβ40 ratio is modestly higher, as is often, although not invariably, the case [11], the absolute levels of these peptides may be markedly lowered [12,13] and not always a higher Aβ42/Aβ40 ratio [11]. Thus additional or alternative explanations for pathogenicity of PS1 mutations have been sought [14–16].

PS1 may contribute to AD pathogenesis through loss of its other functions [17] while serving as a component of γ-secretase, which may include cell adhesion, neurite outgrowth and synaptic plasticity [18,19] or, alternatively, by acting via its secretase-independent roles as a PS1 holoprotein, which include lysosomal acidification essential for autophagic–lysosomal pathology, both of which are considered strong pathogenic factors in AD. Loss of PS1 function compromises assembly and proton-pumping activity of the vacuolar-ATPase on lysosomes, leading to defective lysosomal acidification and marked impairment of autophagy. Additional dysregulation of cellular Ca2+ by mutant PS1 in FAD has been ascribed to altered ion channels in the endoplasmic reticulum; however, rich stores of Ca2+ in lysosomes are also abnormally released in PS1-deficient cells secondary to the lysosomal acidification defect. The resultant rise in cytosolic Ca2+ activates Ca2+-dependent enzymes, contributing substantially to calpain overactivation that is a final common pathway leading to neurofibrillary degeneration in all forms of AD (Alzheimer’s disease). In the present review, we discuss the close inter-relationships among deficits of lysosomal function, autophagy and Ca2+ homeostasis as a pathogenic process in PS1-related FAD and their relevance to sporadic AD.

Key words: Alzheimer’s disease, calcium regulation, calpain, lysosome

Abbreviations used: Aβ, amyloid β-peptide; AD, Alzheimer’s disease; APP, amyloid precursor protein; EOAD, early-onset AD; ER, endoplasmic reticulum; ERK, extracellular-signal-regulated kinase; FAD, familial AD; H6P70.1, heat-shock protein 70.1; kV1, kV1.4, kV1.5, potassium receptor; LRRK2, leucine-rich repeat kinase 2; MEF, mouse embryonic fibroblast; NCL, neuronal ceroid lipofuscinosis; NFT, neurofibrillary tangle; PS1, presenilin 1; PS2, presenilin 2; RyR, ryanodine receptor; sAD, sporadic AD; SERCA, sarcoplasmic/endoplasmic reticulum Ca2+-ATPase; TPC2, two-pore channel 2; v-ATPase, vacuolar ATPase.

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**Figure 1** | An overview of macroautophagy (autophagy)

Multiple signalling pathways and protein modification assemblies initiate the formation of an isolation membrane which is then elongated around the selected substrate or region of cytoplasm (phagophore). The inner and outer bilayers of the isolation membrane close to form a double-membrane-limited autophagosome. Lysosomes fuse with either an autophagosome or an amphisome created by autophagosome–endosome fusion, yielding autolysosomes. The contents of the autolysosome are degraded by hydrolytic enzymes activated within a highly acidic environment by the v-ATPase. Digestion yields basic metabolites which are released into the cytoplasm to be used for new synthesis or as sources of energy and can also modulate TORC1 (target of rapamycin complex 1) activity and other aspects of autophagy induction [26,102]. MVB, multivesicular body; PAS, pre-autophagosomal structure. Modified with permission from Nixon, R.A. (2013) The role of autophagy in neurodegenerative disease. Nat. Med. 19, 983–997.

**Mutations or deletion of PS1 cause autophagy defects by disrupting v-ATPase assembly and lysosome acidification**

Autophagy is a lysosomal degradative pathway for recycling diverse cellular constituents [25,26], particularly under conditions of metabolic stress (Figure 1). Essential for survival of neurons, autophagy is solely responsible for the cellular turnover of damaged or obsolete organelles and is vital for eliminating misfolded and aggregated proteins, which are poorly degraded by the ubiquitin–proteasome system. Autophagy has been reported to be altered in different neurological disorders, and is considered to be a pathogenic factor in several of these diseases, particularly AD and Parkinson’s disease [27]. In AD, disruption of autophagy results in a particularly profuse accumulation of autophagic vacuoles within grossly swollen dystrophic neurites of affected neurons [28] which stems from defects in the lysosomal clearance of autophagy substrates by lysosomes [29,30]. Growing evidence has linked lysosome system failure during autophagy to multiple pathological outcomes in AD, including accelerated amyloidogenesis, neuritic dystrophy, apoptosis and, possibly, tauopathy [31–34].

Mutations of PS1 considerably exacerbate autophagy pathology in EOAD and mouse models of AD [35], and similar pathology can be induced in neurons and non-neuronal cells by deleting PS1 [20,36,37]. Exploring the basis for these effects, Lee et al. [20] demonstrated that the PS1 holoprotein serves as a chaperone in the ER for the v-ATPase (vacuolar ATPase) V0a1 subunit, a six-pass transmembrane protein constituent of the proton pump responsible for full acidification of lysosomes. Binding to the PS1 holoprotein, as this subunit is being translated in the ER, stabilizes the correct folded state and facilitates its glycosylation, believed to be necessary for transit from the ER [20,38–40]. In cells lacking PS1, the V0a1 subunit of the v-ATPase is poorly glycosylated and unstable, which prevents adequate delivery to lysosomes and assembly of the multisubunit v-ATPase, leading to greatly diminished levels and activity of the proton pump [41] and resulting in failure to fully acidify lysosomes, which is a requirement for lysosomal protease activation and autophagy [20,30]. Similar lysosomal acidification defects have now been
observed in PS1-knockout blastocysts, PS1-knockout and PS1/2-knockout MEFs (mouse embryonic fibroblasts), PS1-FAD (familial Alzheimer’s disease) human fibroblast lines, PS1-knockout neurons and PS1/APP mice [30,41]. The roles of the V0a1 subunit and of PS1 in lysosome acidification have been confirmed in studies of PS1-ablated cells or mutant PS1 mouse models and other systems [30,38–40,42], although Coen et al. [43] has proposed that neither of these components is involved in lysosomal pH regulation. The importance of this PS1 chaperone function in AD pathogenesis is underscored by the ability of pharmacological inhibitors of v-ATPase to induce identical AD-related autophagy dysfunction in normal cells and by the complete reversal of this phenotype in PS1-deficient cells by pharmacologically normalizing lysosomal acidification [41]. The connection of PS1 loss of function to Wnt signalling was demonstrated recently in which the loss of PS1 led to an increase in nuclear β-catenin and phosphorylated LRP6 (low-density lipoprotein receptor-related protein 6) due to increased Wnt activity, all of which was attributed to defective acidification of the multivesicular body [38].

**Defective lysosome acidification: an emerging factor in pathogenesis of degenerative diseases**

Interestingly, disruptions of normal v-ATPase activity appear to be important for the progression of other diseases. Mutations within the a3 subunit of the v-ATPase cause misfolding and ER retention of this subunit, leading to osteopetrosis, a bone disease that may be accompanied by neurodegeneration [44]. Mice lacking the a3 subunit also have impaired acidification of lysosomes in macrophages [45]. In a rare familial form of Parkinson’s disease, the loss of function of ATPase13A2 (Park9), believed to be involved in lysosome acidification, leads to impaired autophagy and neurodegeneration [46]. Mutations in LRRK2 (leucine-rich repeat kinase 2) increase the activity of the protein and lead to late-onset familial Parkinson’s disease. Among other pathological effects that have been attributed to this protein kinase, increase in LRRK2 activity leads to pathologically increased autophagy induction and defective lysosomal acidification [47]. Reduced v-ATPase activity and abnormally elevated lysosomal pH due to a deficiency of VMA21, an integral membrane protein required for the assembly of the v-ATPase V0 complex in the ER, leads to X-linked myopathy, a form of muscle fibre degeneration [48].

Although lysosomal pH is predominantly maintained through the activity of the v-ATPase, other ion channels localized to the lysosomal membrane are suspected to be important in maintaining or regulating pH shifts during lysosomal proteolysis and may be particularly important when the intraluminal lysosomal environment is disturbed in disease states. The Cl− channel CLC7 has been implicated in fine-tuning lysosomal pH in conjunction with v-ATPase [49], and other evidence has suggested an involvement of Ca2+ channels, TRPML1 (transient receptor potential mucolipin 1) and TPC2 (two-pore channel 2), in lysosomal pH regulation [50–52]. Lysosomal storage disorders exhibit elevated lysosomal pH as a part of the progression of the disease. Elevated lysosomal pH has been detected in several forms of NCL (neuronal ceroid lipofuscinosis), including juvenile NCL (Batten disease) and infantile NCL, leading to altered lysosomal enzyme activity [53].

**Ca2+ dysregulation in EOAD: the important contribution of defective lysosomal acidification**

PS1 holoprotein has also been implicated in mediating the γ-secretase-independent effects of AD-causing PS1 mutations (or PS1 deletion) in dysregulating Ca2+ homeostasis at several different subcellular levels, including the ER [54–57], the MAMs (mitochondria-associated membranes) [58] and lysosomes [43,59] (Figure 2). Ca2+ homeostasis in the ER is normally maintained by regulating Ca2+ efflux through RyRs (ryanodine receptors) and IP3Rs [Ins(1,4,5)P3 receptors], and by regulating the import of Ca2+ through the SERCA (sarcoplasmic/endoplasmic reticulum Ca2+-ATPase) channel [60]. Although the mechanism(s) is unresolved, loss of function of PS1 leads to ER Ca2+ dysregulation. Exaggerated Ca2+ release by IP3Rs in response to Ins(1,4,5)P3 was initially reported in 1994 [61] and confirmed by other groups in different cell types [57,62,63], and more recent studies into the underlying mechanism of the IP3R-mediated Ca2+ release linked the exaggerated release to a gain-of-function mechanism in which mutations in PS1 stimulate IP3-R gating [64]. The expression of RyRs is also up-regulated in the brains of transgenic mice harbouring PS1 mutations [65] as well as in PC12 cells expressing PS1 mutations and in primary cells from PS1-knockin mice [63]. This increase in RyR channels mediates Ca2+ release [63,65]. Additionally, PS1 may act as a passive Ca2+ leak channel within the ER: mutations within the PS1 holoprotein are proposed to disrupt the leak function, leading ER stores to overfill, thereby overactivating IP3Rs and increasing Ca2+ release [22,66]. Another group of investigators, however, was unable to observe changes in ER Ca2+ fill or leak rates in cells lacking presenilin or expressing FAD-linked presenilin mutations [24], and the basis for the observed differences is not yet resolved. The removal of Ca2+ from the cytosol back to the ER is controlled by the SERCA pump, but, in cells lacking PS1, PS2 or PS1/2, SERCA activity is diminished, leading to increased cytosolic Ca2+. A similar effect has been seen in cells expressing either PS2 or FAD-linked PS2 mutant [67], although others have observed increased SERCA function leading to the overfilling of ER Ca2+ stores in cells overexpressing PS2 or AD-linked mutant PS1 [68]. Despite these uncertainties about the precise mechanisms, there is consensus in the field that ER Ca2+ is dysregulated in response to loss of PS1 function [56,60].

Although the ER contains the largest stores of Ca2+, with concentrations in the low millimolar range [69],
In ERR, PS1-FAD induces an up-regulation of RyR expression, induces a gain of function in IP₃R, enhances SERCA function and inhibits passive leak function of PS1. At the lysosomes, PS1-FAD elevates lysosomal pH by blocking v-ATPase maturation in the ERR and delivery to lysosomes, leading to increased Ca²⁺ efflux though TPC2 and/or MCLN (mucolipin 1). Consequently, cytosolic Ca²⁺ levels are increased, leading to calpain activation. All of which contributes to impairments of cellular function and neurodegeneration (depicted by skull and crossbones). Black asterisks are symbolic of PS1-FAD. Arrows depict movement of Ca²⁺.

**Figure 2 | Ca²⁺ dysregulation in wild-type PS1 (PS1wt; left) compared with mutant PS1-linked FAD (PS1-FAD; right) conditions**

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(cyclin-dependent kinase 5) (through the cleavage of p35 to p25) [84], ERK1/2 (extracellular-signal-regulated kinase 1/2) [85], and GSK3β (glycogen synthase kinase 3β) [86] and consequent hyper phosphorylation of tau leading to NFT formation. Calpains can also cleave IP3Rs making them constitutively active which may further disrupt ER Ca2+ homeostasis in PS1-related EOAD [87]. The pathogenic role of calpains in AD is indicated further by the marked depletion in AD patients’ brains of calpastatin [88], the specific endogenous inhibitor of calpains [89,90]. Moreover, overexpressing calpastatin reduced calpain activity, tau phosphorylation, ERK activation and amyloidoid plaque load in either of two different mouse models of AD [91,92]. A synthetic calpain inhibitor, BDA-410, improved spatial memory, fear conditioning and synaptic plasticity associated with increased CREB (cAMP-response-element-binding protein) phosphorylation in APP/PS1 transgenic mice [93]. Finally, we have recently observed that calpastatin overexpression in the JNPL3 tauopathy mouse model leads to decreased tau phosphorylation, increased lifespan and loss of motor neurons (MK. McBrayer, unpublished work). Because ER and lysosomal Ca2+ dysregulation is altered in mutant PS1-linked FAD and PS1-knockout cell lines [43,59], it is not surprising that we have also found calpains to be robustly activated in PS1-knockout blastocysts and mutant PS1-linked FAD fibroblasts as reflected in marked increase in calpain-cleaved spectrin and significantly decreased calpastatin levels (MK. McBrayer, unpublished work).

Conclusions: lysosomal and Ca2+ dysfunction in sAD

Although Ca2+ dysregulation may be initially driven by presenilin mutations in FAD, additional cellular Ca2+ derangements are also seen in the more prevalent late-onset forms of AD [94] and probably have a multifactorial origin, including contributions from excitotoxicity, ischaemia and β-amyloid neurotoxicity [95,96]. It is tempting to speculate that the notable disruption of lysosomal function in AD [97,98] may give rise to additional lysosome-related Ca2+ dysregulation. Interestingly, a further connection has been suggested by Yamashima [95] between calpain activation and lysosomal dysfunction leading to lysosomal membrane rupture, which involves increased calpain-mediated cleavage of carbonylated HSP70.1 (heat-shock protein 70.1). HSP70.1 has roles as both a protein chaperone and stabilizer of lysosomal membrane integrity. Calpain-mediated loss of HSP70.1, coupled with other factors impairing autophagic clearance [98], are proposed to lead to lysosomal membrane disruption and are likely to disrupt downstream pathological events [95]. Calpain activation is observed in patients with sAD, and research indicates that Ca2+ homoeostasis is also disrupted in sAD [99–101]. EOAD and sAD probably have different initiation points leading to dysregulated lysosome dysfunction and Ca2+ dysregulation, but ultimately share pathological outcomes, including calpain overactivation, that should prove to be useful drug targets for AD therapy.

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