Symposium

Amyloid-Independent Mechanisms in Alzheimer's Disease Pathogenesis

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Despite the progress of the past two decades, the cause of Alzheimer's disease (AD) and effective treatments against it remain elusive. The hypothesis that amyloid-β (Aβ) peptides are the primary causative agents of AD retains significant support among researchers. Nonetheless, a growing body of evidence shows that Aβ peptides are unlikely to be the sole factor in AD etiology. Evidence that Aβ/amyloid-independent factors, including the actions of AD-related genes, also contribute significantly to AD pathogenesis was presented in a symposium at the 2010 Annual Meeting of the Society for Neuroscience. Here we summarize the studies showing how amyloid-independent mechanisms cause defective endo-lysosomal trafficking, altered intracellular signaling cascades, or impaired neurotransmitter release and contribute to synaptic dysfunction and/or neurodegeneration, leading to dementia in AD. A view of AD pathogenesis that encompasses both the amyloid-dependent and -independent mechanisms will help fill the gaps in our knowledge and reconcile the findings that cannot be explained solely by the amyloid hypothesis.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that begins as mild short-term memory deficits and culminates in total loss of cognition and executive functions. Currently, the precise cause of the disease is not known and there is no cure. Genetic studies (Price et al., 1998) have identified mutations in amyloid precursor protein (APP) and presenilin 1 and 2 (PS1, PS2) that cause rare, dominantly inherited familial AD (FAD). Proteolytic processing of APP by BACE (β-site APP cleaving enzyme) followed by PS-containing γ-secretase complex generates amyloid-β (Aβ) peptides that deposit in amyloid plaques. Genetic and cell biological studies show increased production of more amyloidogenic Aβ peptides associated with FAD-linked mutations, providing strong support for the amyloid hypothesis (Hardy and Selkoe, 2002), which posits that Aβ peptides play a pivotal role in AD pathogenesis. However, Aβ peptides are also generated as a part of normal metabolism and there is no consensus regarding the identity of the disease-causing, pathological form of Aβ.

Despite the genetic and cell biological evidence that supports the amyloid hypothesis, it is becoming clear that AD etiology is complex and that Aβ alone is unable to account for all aspects of AD (Pimplikar, 2009). For example, recent neuroimaging studies confirm the previous autopsy findings that amyloid deposits are present in cognitively normal individuals, whereas some AD patients show no amyloid deposits in PET (positron emission tomography) scans (Edison et al., 2007; Li et al., 2008). Similarly, it is possible that all of the amyloid-focused clinical trials failed because they were started too late in the disease progression, but the negative outcome is also consistent with the notion that AD can be caused by Aβ/amyloid-independent factors. The fact that vast overproduction of Aβ peptides in the mouse brain failed to cause neurodegeneration raises further questions as to whether accumulation of Aβ peptides is indeed the culprit for neurodegeneration in AD. Also, a large number of preclinical studies support roles for calcium dysregulation, proteolysis failure, altered cell signaling, oxidative stress and inflammation in neuronal dysfunction, and neurodegeneration similar to those observed in AD. This article highlights the findings that were presented in a symposium and is not meant to be a comprehensive review of AD pathogenesis. Here we discuss studies showing that mutations in APP and presenilins can contribute to AD pathology by amyloid-independent mechanisms.

FAD mutations in APP and PS1 lead to defective endo-lysosomal trafficking and proteolysis

The lysosomal network, comprising the endocytic and autophagic pathways, mediates the processing, sorting, and turnover of proteins and other cellular constituents. Endocytosis is especially

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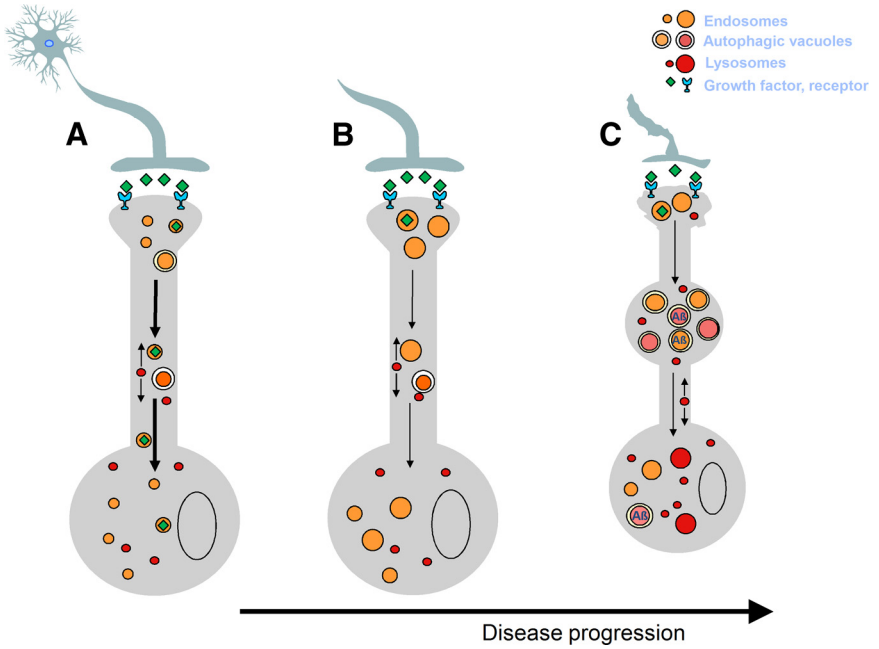

Figure 1. Dysfunction of autophagic and endocytic pathways to lysosomes driven by relevant genes and other risk factors in Alzheimer's disease. ***A–C***, A normal neuron is depicted in ***A***. At the earliest stages of AD (***B***), an abnormal acceleration of endocytosis, mediated partly by rab5, is known to be caused by *App* gene duplication (via β-CTF) in early onset FAD and Down syndrome and promoted by ApoE4 and elevated cholesterol in late-onset AD. Adverse consequences include endosome enlargement and defective endosome retrograde transport and neurotrophin signaling functions, which promote apoptotic pathway activation and neurodegeneration, particularly of cholinergic neuronal populations. Subsequently (***C***), failure of autophagy, prominently involving impaired lysosomal proteolysis, leads to massive selective accumulation of autophagic vacuoles (autophagosomes, autolysosomes etc,) containing partially digested autophagic and endocytic substrates within swollen "dystrophic" neurites. The diminished clearance by autophagy of toxic organelles and proteins, including Aβ, ubiquitinated proteins, activated caspases, and possibly tau, leads to neurodegeneration via multiple pathways. Failure of lysosomal proteolysis and autophagy in AD is driven directly by PS1 mutations in early-onset FAD, and is also promoted by normal aging, oxidative stress, Apo E4, intracellular Aβ, and other AD-related genetic and environmental risk factors.

critical in neurons, as it supports such specialized functions as synaptic transmission and retrograde trophic signaling (Nixon et al., 2008). Autophagy, the principal degradative pathway for organelles and long-lived proteins, involves the sequestration of cytoplasmic constituents within autophagosomes followed by digestion of these substrates within autolysosomes that are formed by fusion of autophagosomes with lysosomes. Autophagy is essential for neuronal survival in part by clearing damaged, aggregated, or obsolete proteins in disease states and cellular aging (Wong and Cuervo, 2010). Notably, longevity and cellular aging mechanisms are closely linked to the efficacy of autophagy (Madeo et al., 2010), and during aging, a *sine qua non* for the development of Alzheimer's disease, autophagy efficiency declines (Cuervo, 2008). The neuron's unique reliance on the lysosomal system is well documented in many primary lysosomal disorders where the defect in a ubiquitous lysosomal protein produces severe neurodegenerative phenotypes (Bellettato and Scarpa, 2010), including pathologies characteristic of AD (Ohm et al., 2003; Ohmi et al., 2009). The close connection between neurodegeneration and lysosomal system dysfunction is further highlighted by the growing numbers of lysosomal system proteins identified as pathogenic in familial late-onset neurodegenerative disorders, including forms of Parkinson's disease (McCray and Taylor, 2008; Nixon et al., 2008; Cherra et al., 2010).

Recent evidence shows that mutations of PS1 and APP (or *APP* gene duplication), independently of Aβ, directly disrupt autophagy or alter endocytosis, which impairs neuronal function and reduces neuron survival (Fig. 1). PS1 has recently been found to be essential for lysosomal proteolysis and autophagy by enabling the acidification of lysosomes required for protease activation (Lee et al., 2010). In PS1-lacking neurons, vacuolar ATPase (vATPase), the proton pump that acidifies lysosomes, is not delivered to lysosomes. Failed lysosome acidification blocks substrate proteolysis during autophagy, causing incompletely degraded proteins to accumulate in autolysosomes. The underlying mechanism involves a novel role for PS1, independent of γ-secretase, in which PS1 holoprotein, before being cleaved and assembled into γ-secretase, facilitates *N*-glycosylation of the V01A subunit of vATPase in the endoplasmic reticulum, which is required for its efficient delivery to lysosomes and for assembly of the proton pump. In familial early-onset AD, PS1 mutations lead to a similar loss of lysosome function by the same mechanism (Lee et al., 2010), most likely resulting from a dominant-negative effect of mutant PS1. Deficient lysosomal proteolysis leads to the extensive "neuritic dystrophy" of AD (Suzuki and Terry, 1967; Masliah et al., 1993) characterized by grossly swollen neurites packed with autophagic vacuoles containing Aβ and other incompletely degraded substrates (Nixon et al., 2005; Yu et al., 2005) that are potentially neurotoxic (Yang et al., 2008). This massive "storage" of waste proteins, reminiscent of lysosomal storage diseases, can be experimentally reproduced in neurons by inhibiting lysosomal cathepsin proteolysis (Boland et al., 2008). Interestingly, impaired autophagy in the AD brain results in Aβ accumulation in autolysosomes, and this reservoir of intracellular Aβ may exert further toxicity to the lysosome system (Glabe, 2001). Experimental measures may stimulate autophagy restore lysosomal proteolysis to more normal levels have yielded promising therapeutic effects on neuronal function and cognitive performance in mouse models of AD (Sun et al., 2008; Spilman et al., 2010) and in certain other neurodegenerative diseases (García-Arencibia et al., 2010).

Autophagy deficits in AD are part of a continuum of lysosomal system deficits, including endocytic abnormalities that may be manifested as the first specific signs of AD (Cataldo et al., 2000; Jiang et al., 2010; Rothenberg et al., 2010) (Fig. 1). Abnormal acceleration of neuronal endocytosis is evident before amyloid is deposited in the neocortex (Cataldo et al., 1997, 2000). Genes related to endocytosis, such as *Rab5*, *Rab7*, and *Rab4*, are among the first group to be upregulated in AD (Ginsberg et al., 2010) and are abnormally recruited to endosomes, which progressively enlarge. This pattern is specific for AD among studied aging-related neurodegenerative diseases and is accelerated by inheritance of the ε4 allele of APOE (apolipoprotein E), the major genetic risk factor for late-onset AD (Cataldo et al., 2000). In a form of AD caused by *App* gene duplication and in Down syndrome, where a chromosome 21 segment containing *App* is trisomic, endosome dysfunction can be attributed to the extra copy of *App* (Cataldo et al., 2003; Salehi et al., 2006; Jiang et al., 2010) and has been linked

to altered trophic signaling and cholinergic neurodegeneration (Salehi et al., 2006), and activation of apoptotic pathway (Neve et al., 1996). Recently, these effects of increased *App* dosage were shown to be mediated specifically by the β-cleaved C-terminal fragment of APP (Jiang et al., 2010), previously known to have neurotoxic properties relevant to AD (Oster-Granite et al., 1996; Kim et al., 2000; Choi et al., 2001; Mathews et al., 2002; Arbel et al., 2005; Lee et al., 2006).

FAD mutations in APP and PS1 can exert deleterious effects independent of Aβ

It has been proposed that PS FAD mutations promote neurodegeneration by increasing neurotoxic peptide Aβ42. More recent work, however, shows that many FAD mutants increase neither production of Aβ42 nor the Aβ42/40 ratio that has been thought to initiate AD pathology (Bentahir et al., 2006; Shioi et al., 2007; Batelli et al., 2008). Also, the Swedish mutation of APP increases production of both Aβ42 and Aβ40 but does not change the ratio (Duering et al., 2005). Thus, although some FAD mutations increase Aβ42 and/or the Aβ42/40 ratio, not all mutations show this phenomenon. Furthermore, Aβ peptides are normal components of human serum and CSF, and there is little evidence that Aβ is neurotoxic at *in vivo* concentrations, which are severalfold lower than the concentrations Aβ is used in *in vitro* neurotoxicity assays (for review, see Robakis, 2010).

Currently more than 30 FAD mutations have been mapped on the APP gene. Some of these mutations do not change the primary sequence of Aβ while others fall within the Aβ region. A mutation within this region, Glu693Gln, does not increase Aβ production but increases its tendency to form amyloid. Carriers of this mutation develop the fatal syndrome of hereditary cerebral hemorrhage with amyloidosis of Dutch type (HCHWA-D), characterized by recurrent cerebral hemorrhages due to accumulation of amyloid depositions in cerebral blood vessels. These patients are not classified as AD as they are usually not demented. Other APP mutations on residues 692 and 694, however, are associated with FAD, but these increase neither Aβ production nor the 42/40 ratio. Interestingly, APP mutations of the London type, which cause relatively small increases in Aβ, induce AD at earlier ages than the Swedish mutation, which causes much higher increases in Aβ than the London mutations. It should be noted that in APP-based mouse models of AD, all products of APP metabolism are increased together with Aβ, and there is evidence that some of these non-Aβ products are neurotoxic (Nalbantoglu et al., 1997; Ghosal et al., 2009; Nikolaev et al., 2009). Thus, behavioral abnormalities of animal models overexpressing APP need to be interpreted with caution, as in addition to Aβ, other APP metabolites may influence the final outcome and contribute to the mechanism(s) of neurodegeneration (Robakis, 2010).

Indeed, as seen above in the case of vacuolar ATPase, there is ample evidence to indicate that PS1 performs γ-secretase-independent functions in addition to its role as a catalytic subunit

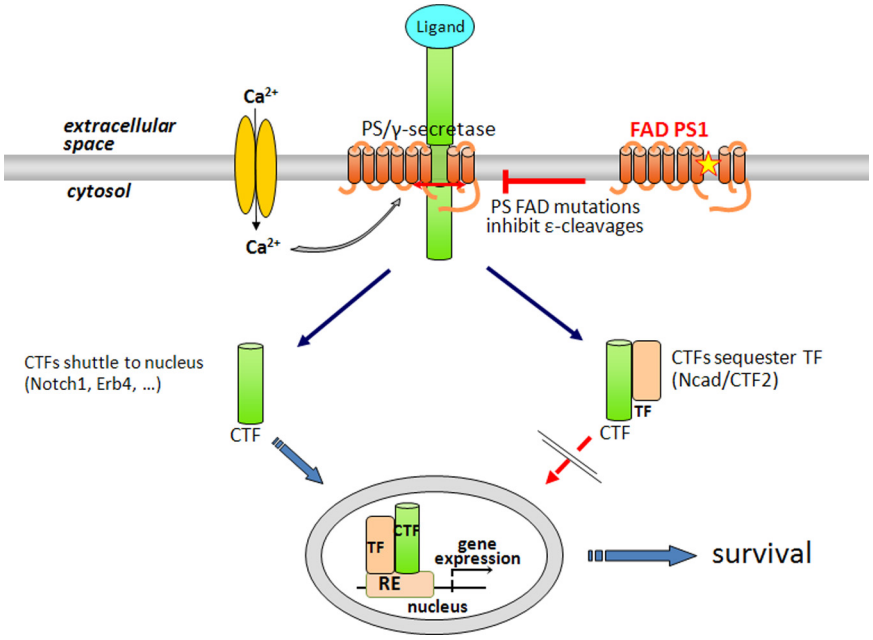

Figure 2. The ε-cleavage of receptors is mediated by γ-secretase and inhibited by PS FAD mutations. This cleavage produces biologically active peptides containing the CTFs of substrates. ε-cleavages can be stimulated by ligand binding or calcium influx (Litterst et al., 2007). CTFs can travel to nucleus where they can regulate gene expression or sequester transcription factors (TF) in the cytoplasm. Many PS1 FAD mutants inhibit the ε-cleavage indicating FAD mutations cause a loss of γ-secretase function (Marambaud et al., 2003). PM, Plasma membrane; RE, response element.

of the γ-secretase complex. In addition to its role in regulating calcium homeostasis, wild-type PS1 also stimulates the phosphoinositide 3-kinase (PI3K)/Akt and MEK/ERK (MAP kinase kinase/extracellular signal-regulated protein kinase) signaling pathways and promotes cell survival and growth. By contrast, a number of PS1 FAD mutations fail to stimulate the cell survival pathways and interfere with γ-secretase-independent functions. These observations reveal the presence of additional mechanisms by which FAD mutations in PS1 may promote neurodegeneration and tau hyperphosphorylation (Baki et al., 2004, 2008; Kang et al., 2005; Tu et al., 2006; Dreses-Werringloer et al., 2008).

Recent data show that in addition to the γ-cleavage of APP, the PS1–secretase complex promotes the ε-cleavage of other transmembrane proteins, including Notch1 receptor, cadherins, APP, and EphB receptors. This cleavage takes place downstream from the γ-cleavages site resulting in the release of soluble cytosolic peptides containing the intracellular C-terminal fragments (CTFs). Several of these peptides, including APP intracellular domain (AICD), migrate to the nucleus and regulate gene expression (Cao and Sudhof, 2001; Gao and Pimplikar, 2001), while others remain in the cytoplasm where they regulate stability of transcription factors (Kopan and Ilagan, 2004; Marambaud and Robakis, 2005). Interestingly, γ-cleaved AICD fragment has been shown to exert deleterious effects and recapitulate multiple AD pathological features in a mouse model (Ryan and Pimplikar, 2005; Ghosal et al., 2009, 2010; Vogt et al., 2009), and peptide EphB2/CTF2 generated by the ε-cleavage of EphB2 receptor stimulates phosphorylation of NMDA receptor subunit NR2B (Xu et al., 2009). Recent data show that in contrast to the proposed gain of function, PS1 FAD mutations may inhibit the cleavage of proteins including APP, cadherins, ephrinB, Notch1, and EphB receptors, leading to reduced production of the corresponding CTF peptides (Song et al., 1999; Marambaud et al., 2003; Wiley et al., 2005; Georgakopoulos et al., 2006; Litterst et al.,

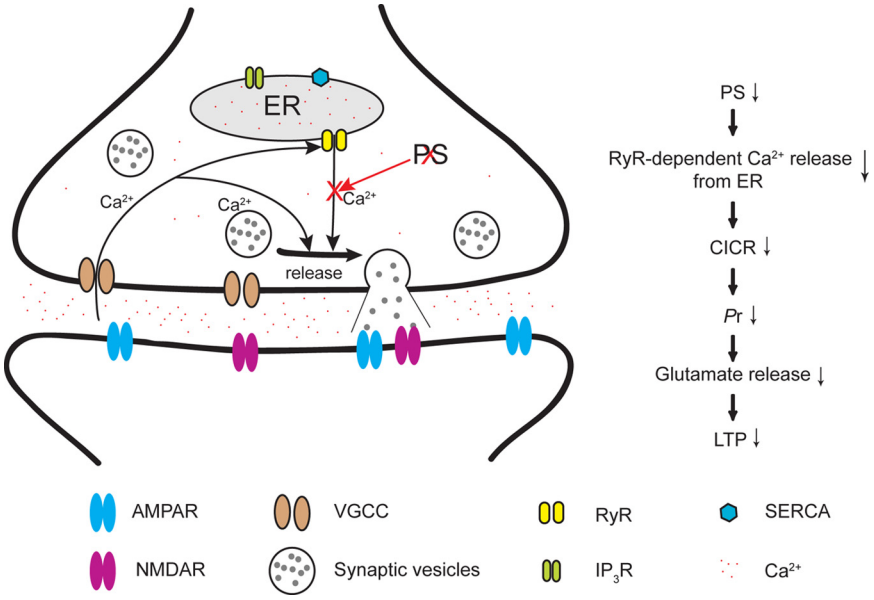

Figure 3. A model depicting the role of presenilins in the regulation of neurotransmitter release. Upon stimulation, calcium concentration at the presynaptic terminal is drastically elevated due to calcium influx through VGCCs and calcium-induced calcium release (CICR) from intracellular stores, which is mediated through both ryanodine receptors and IP_3 receptors. Loss of PS function in the presynaptic terminal specifically disrupts ryanodine receptor-mediated Ca^{2+} release from the ER store, thus reducing CICR and resulting in reduced increases of calcium-induced by action potentials in the presynaptic terminal. This reduction in calcium increases impairs the probability of neurotransmitter release, and the decreased glutamate release causes LTP impairment in *PS*-deficient presynaptic terminals. (Figure taken from supplementary information in Nature 460:632–636, 2009. Reprinted with permission.)

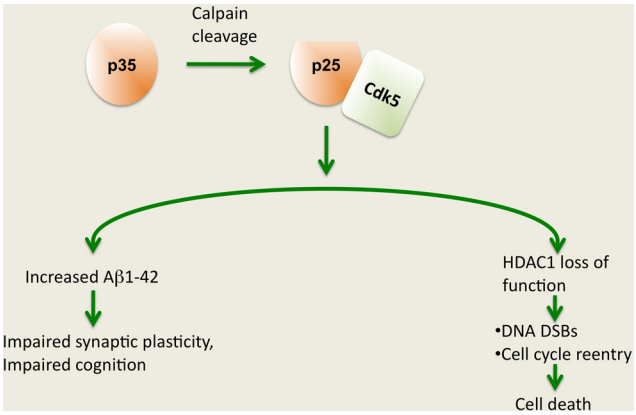

Figure 4. p25/Cdk5 in the pathogenesis of Alzheimer's disease. The p25/Cdk5 kinase exerts two parallel processes in the course of neurodegeneration. First, it increases β-amyloid production which contributes to synaptic impairment and memory loss. Second, p25/Cdk5 in the nucleus reduces HDAC1 activity, which leads to increased expression of cell cycle genes and DNA double-strand breaks. These pathologies eventually lead to neuronal loss and neurodegeneration. DSBs, double-strand breaks.

2007). These data raise the possibility (Fig. 2) that FAD mutations promote neurodegeneration by altering the production of peptides with important transcriptional and signal transduction properties (Robakis, 2003).

The autosomal-dominant mode of FAD transmission is consistent with the notion that these mutations cause gain of a toxic function. However, there is a possibility that some FAD mutations in PS1 may function through allelic interference. Evidence that PS1 FAD mutants inhibit the γ-secretase-catalyzed ε-cleavages of many substrates (loss of function, see above), combined with absence of FAD haploinsufficiency mutants, raises the possibility of a model of allelic interference in which products of inactive FAD mutant alleles of PS promote autosomal-dominant neurodegeneration by also inhibiting the functions of the wild-type protein (Robakis, 2010).

Synaptic dysfunction and neuronal degeneration caused by loss of PS

The *presenilin* genes harbor most of the FAD-linked mutations and are highly expressed in pyramidal neurons of the adult cerebral cortex, where AD pathogenesis manifests. Analysis of viable *presenilin* conditional knock-out mice, in which presenilin expression is selectively inactivated in excitatory pyramidal neurons of the postnatal forebrain, revealed important PS functions relevant to AD pathogenesis (Yu et al., 2001; Saura et al., 2004). Specifically, loss of presenilins affects both short- and long-term plasticity, in the absence of neurodegeneration. Furthermore, NMDA receptor-mediated responses are impaired and synaptic levels of NMDA receptor subunits are reduced in the absence of PS. Interestingly, loss of PS reduces levels of cAMP response element-binding protein (CREB)-binding protein (CBP) and transcription of CREB/CBP target genes (Saura et al., 2004; Beglopoulos and Shen, 2006), even though it was subsequently observed that CREB-mediated transcription is regulated indirectly by PS (Watanabe et al., 2009). Strikingly, *PS* cDKO mice (conditional double knock-out mice lacking both presenilins in the postnatal forebrain) develop in an age-dependent manner synaptic, dendritic, and neuronal degeneration with accompanying astrogliosis and hyperphosphorylation of tau, demonstrating an essential role for PS in neuronal survival (Beglopoulos et al., 2004; Saura et al., 2004; Wines-Samuelson et al., 2010). Specifically, while presenilins are inactivated at 4 weeks of age postnatally in *PS* cDKO mice, significant increases (~8-fold, compared with control mice) of apoptotic cell death are first detected at 2 months of age. However, this represents only ~0.1% of cortical neurons that are undergoing apoptosis; thus, the total cortical neuron number and volume are not significantly altered at this age. By 4 months of age, ~9% of cortical neurons are lost in *PS* cDKO mice, followed by 18% and 24% neuronal loss at 6 and 9 months of age, respectively. Furthermore, presenilins appear to promote memory and neuronal survival in a γ-secretase-dependent manner, as conditional inactivation of nicastrin, another component of the γ-secretase complex, in the adult cerebral cortex similarly resulted in progressive memory impairment and neurodegeneration (Tabuchi et al., 2009). These *in vivo* findings and a large number of reports on the effects of FAD-linked mutations in culture and *in vitro* systems as well as in *Caenorhabditis elegans* raised the possibility that PS mutations may cause dementia and neurodegeneration in AD via a partial loss-of-function and dominant-negative mechanism (Shen and Kelleher, 2007). Indeed, a recent report showed that pathogenic mutations in PS1, such as L435F, could result in complete loss of γ-secretase activity (Heilig et al., 2010). The later onset of the disease in AD patients carrying presenilin mutations, compared with *PS* cDKO mouse models, can be explained by the fact that while FAD mutations

confer partial (or complete) loss of presenilin activity, only one of the *PS* alleles is affected in FAD patients.

The fact that synaptic impairments precede progressive neurodegeneration suggests that synaptic dysfunction caused by loss of PS function promotes subsequent neuronal degeneration. The role of presenilins in the synapse was elucidated by systematic genetic analysis through the restriction of presenilin inactivation to hippocampal CA1 or CA3 neurons (Zhang et al., 2009). This strategy permitted analysis of the effects of presenilin inactivation in either presynaptic or postsynaptic neurons of the Schaeffer collateral pathway. It was found that long-term potentiation (LTP) induced by theta burst stimulation is decreased after presynaptic but not postsynaptic deletion of presenilins. Moreover, presynaptic but not postsynaptic inactivation of presenilins impairs short-term plasticity and synaptic facilitation, and the defects in synaptic facilitation are dependent upon the frequency used for stimulation and the external calcium concentration. The probability of evoked glutamate release, measured by the decay curve of the open-channel NMDA receptor antagonist MK-801 [5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (dizocilpine maleate)], is reduced by presynaptic inactivation of presenilins. To explore further the involvement of calcium in the presynaptic defects caused by loss of presenilins, both calcium influx and efflux were evaluated, and it was found that the current–voltage relationship of voltage-gated calcium current is normal in the absence of PS. Strikingly, depletion of calcium internal stores by thapsigargin, a noncompetitive SERCA (sarcoplasmic/endoplasmic reticulum calcium ATPase) calcium pump, mimics and occludes the effects of presynaptic presenilin inactivation, suggesting a defect in calcium influx underlying the presynaptic impairment. Blockade of calcium release from ryanodine receptors using two independent inhibitors had effects similar to those of thapsigargin, whereas blockers of IP_3 receptors had no effect on presynaptic frequency facilitation. These findings were further corroborated using dissociated *PS*-null hippocampal neuronal cultures from postnatal pups, in which the requirement of presenilins in neural development is circumvented. These cultured *PS*-null hippocampal neurons show normal neuronal morphology and synaptic density and recapitulate the presynaptic defects seen in acute hippocampal slices of CA3-*PS* cDKO mice, suggesting that they are relevant experimental systems to assess calcium homeostasis directly. Indeed, depolarization-induced calcium increases in the cytosol, which are composed of both calcium influx and calcium-induced calcium release from intracellular stores, are reduced in hippocampal neurons lacking both presenilins. Furthermore, blockade of ryanodine receptors but not IP_3 receptors mimics and occludes the effects of presenilin inactivation.

Collectively, these genetic and electrophysiological studies demonstrated that loss of presenilin function impairs LTP induction and glutamatergic neurotransmitter release by a presynaptic mechanism (Fig. 3). These findings raised the possibility that presynaptic mechanisms may play a primary role in AD pathophysiology (Shen, 2010). In support of this hypothesis, presenilins are localized to presynaptic terminals (Zhang et al., 2009) and APP C-terminal fragments, precursors of Aβ, accumulate in presynaptic terminals of *PS1* cKO mice (Saura et al., 2005). A time course study to identify temporal development of presynaptic and postsynaptic defects in forebrain-*PS* cDKO mice further revealed that presynaptic defects, such as synaptic facilitation, occur at 5 weeks of age, followed by postsynaptic defects, such as NMDA receptor-mediated responses, at 6 weeks of age, providing additional support for the importance of presynaptic roles played by presenilins (Zhang et al., 2010).

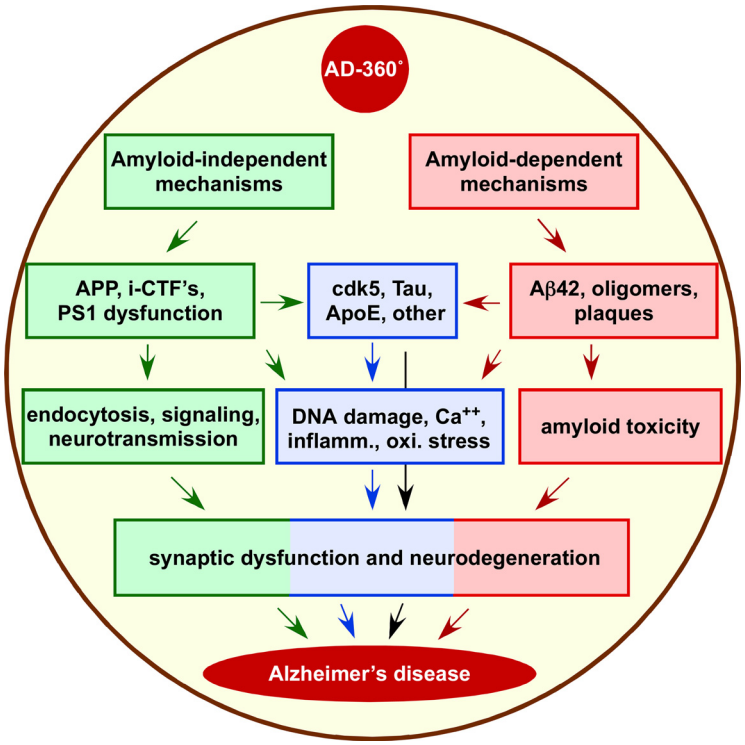

Figure 5. AD-360°. This global view of AD pathogenesis includes both the amyloid-independent (green pathway) and amyloid-dependent (red pathway) mechanisms. The Aβ/amyloid-independent mechanisms are mediated via APP, intracellular fragments (i-CTFs) and PS1 via the cellular processes discussed here (green arrows) while the amyloid mechanisms are mediated via Aβ42 or Aβ oligomers or plaques (red arrows). Cdk5 (blue box) may be influenced by or interacts with both pathways and its activation triggers DNA damage, cell cycle activation and neurodegeneration (blue arrows). Non-APP/PS factors such as Tau and ApoE also contribute to AD pathology (blue and black arrows) and there is strong evidence to suggest that cellular processes such as inflammation, oxidative stress and Ca^{2+} dysregulation implicated in AD pathogenesis can be triggered by both amyloid-dependent and amyloid-independent mechanisms. All of these pathways can lead to synaptic dysfunction and neurodegeneration and AD most likely results from the cumulative effects of multiple pathway.

DNA damage by aberrantly activated cyclin-dependent kinase 5

Cyclin-dependent kinase 5 (Cdk5) is a proline-directed serine/threonine kinase that has important roles in various neuronal functions including brain development, synaptogenesis, synaptic plasticity, and memory formation (Dhavan and Tsai, 2001). Cdk5 is activated when bound to one of its two activators, p35 or p39, which have related primary sequences. Aberrant activation of Cdk5 can occur through the proteolytic cleavage of p35 to p25 via calpain, a calcium-dependent protease (Kusakawa et al., 2000; Lee et al., 2000; Nath et al., 2000). Evidence for a role of Cdk5 in

the pathogenesis of neurodegenerative conditions has been accumulating over the last several years (Patrick et al., 1999; Wang et al., 2003; Qu et al., 2007).

Cdk5 activity is increased in postmortem AD brains; this is likely due to increased p25 levels found in AD brain tissues compared with age-matched control brain tissues (Patrick et al., 1999). Levels of p25 are also increased in AD mouse models, including the PS1-deficient and 5XFAD mice (Oakley et al., 2006). *In vitro*, Aβ1-42 causes p25 production in primary dissociated neurons (Lee et al., 2000). A forebrain-specific inducible p25 (CK-p25) transgenic mouse model exhibits elevated Aβ1-42 peptide, neurodegeneration characterized by massive neuronal and synaptic loss, tau-associated pathology, and learning and memory impairments (Cruz et al., 2003; Fischer et al., 2005; Kim et al., 2008). Inhibition of Cdk5 activity in transgenic p25 mice reduces Aβ1-42 production suggesting that Aβ1-42 processing is regulated by the Cdk5/p25 complex (Wen et al., 2008). Conversely, inhibition of Aβ generation by BACE1 loss of function in the CK-p25 mice rescued the impairments in synaptic plasticity and memory formation (L.H.T., unpublished observation). These results indicate that Aβ plays a crucial role in neurodegeneration exhibited by the CK-p25 mice. Furthermore, they hint at the possibility that p25 and Aβ collaborate to cause neuronal death.

To decipher the cellular mechanism(s) that leads to neurodegeneration, microarray gene expression profiling experiments were performed in CK-p25 mice before the detection of astrogliosis and neuronal loss (Kim et al., 2008). Surprisingly, two main classes of genes were profoundly upregulated in the brain of CK-p25 mice compared with control mice. The first class is cellular proteins known to participate in mitotic cell division including cyclins, mitotic Cdks, proliferating cell nuclear antigen, and E2F. The second class is components of the DNA damage response pathway, especially DNA double-stranded break, including RAD51, ATM, and DNA polymerase ε. Indeed, reactivation of cell cycle genes and DNA double-stranded break lesions were demonstrated in the hippocampal neurons of CK-p25 mice before the onset of neurodegeneration indicating that these events may cause neuronal loss. Reactivation of cell cycle genes has been reported in postmortem AD patients. Preliminary data indicate that the numbers of neurons with DNA double-stranded break are significantly increased in AD brains (L.H.T., unpublished observations).

It was postulated that a common mechanism may underlie both cell cycle reactivation and DNA double-stranded breaks, as the two events were largely observed in the same neurons in CK-p25 mice. Histone deacetylase 1 (HDAC1) has been shown to repress the expression of numerous cell cycle proteins by binding to their upstream regulatory elements (Brehm et al., 1998; Stiegler et al., 1998; Lagger et al., 2002; Rayman et al., 2002). In CK-p25 mice, HDAC1 activity was downregulated, and its association with the genes encoding cell cycle proteins as well as with chromatin was reduced. Furthermore, HDAC1 loss of function caused DNA double-stranded breaks, reactivation of cell cycle genes, and neuronal death. Conversely, HDAC1 gain of function ameliorated p25-induced DNA damage and cell death. Thus, reduced HDAC1 activity resulting from p25 accumulation may contribute to several early events leading to neurodegeneration.

In conclusion, we think that p25/Cdk5 concurrently exerts two parallel processes within the neuron (Fig. 4). One aspect involves deficits in synaptic plasticity, and learning and memory in which p25/Cdk5 leads to increased β-amyloid, which in turn contributes to synaptic impairments and memory loss. Meanwhile, p25/Cdk5 also exerts nuclear activity, resulting in dysregulation of HDAC1. HDAC1 loss of function causes the upregulation of cell cycle genes and DNA double-strand breaks. These pathologies eventually lead to neuronal loss and neurodegeneration. Our results suggest that p25 generation plays a very upstream and critical role in the signaling cascade leading to neurodegeneration, contributing to both cognitive symptoms and cellular demise. Thus, targeting Cdk5 or p25 generation may provide new promising avenues for therapeutic intervention of Alzheimer's disease.

Concluding remarks

Today, research in the field of AD is on the precipice of a crucial and somewhat paradoxical juncture. On the one hand, progress made in multiple fields of investigation (genetics, biochemistry, cell biology, epidemiology, neuroimaging, etc.) has yielded tremendous insights into the possible cause of AD, and we are closer to finding a disease-modifying treatment. On the other hand, it has become increasingly clear that disproportionate reliance on Aβ/amyloid-based mechanisms to explain AD etiology and nearly exclusive emphasis on amyloid as a therapeutic target have not yielded the desired results. Genetic studies of FAD, which accounts for ~3–5% of AD cases, have been considered the strongest evidence supporting the amyloid hypothesis; however, as discussed above, there is increasing evidence that even the FAD mutations in APP and presenilins can act via amyloid-independent mechanisms. Indeed, it is clear from the data discussed above that the amyloid-dependent and amyloid-independent mechanisms are not mutually exclusive and both can contribute to AD pathology.

An important challenge for future studies will be to determine the extent to which amyloid-independent mechanisms contribute to AD. The implicit assumption underlying the current drug trials is that the prime causative agent of AD is amyloid/Aβ-peptides, and therefore that blocking amyloid accumulation will prevent AD. However, if amyloid-independent mechanisms also make significant contributions to AD, then the current drug trials will yield only moderately positive results, as the case has been. Another challenge facing future investigations will be to produce a satisfactory explanation as to why AD takes so long to manifest itself and why certain parts of the brain (limbic system) are more susceptible in AD. As future studies seek answers to these questions, it is becoming clear that both amyloid-dependent and amyloid-independent mechanisms (Fig. 5) are involved in contributing to AD pathology and that an effective disease-modifying treatment will arise only from a strategy that addresses both of these mechanisms.

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