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Mechanisms of FAD neurodegeneration may be independent of A β

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Abstract

Alzheimer's disease (AD) is the most common cause of dementia in the aged population. Most cases are sporadic although a small percent are familial (FAD) linked to genetic mutations. AD is caused by severe neurodegeneration in the hippocampus and neocortical regions of the brain but the cause of this neuronal loss is unclear. A widely discussed theory posits that amyloid depositions of A β peptides or their soluble forms are the causative agents of AD. Extensive research in the last twenty years however, failed to produce convincing evidence that brain amyloid is the main cause of AD neurodegeneration. Moreover, a number of observations, including absence of correlations between amyloid deposits and cognition, detection in normal individuals of amyloid loads similar to AD, and animal models with behavioral abnormalities independent of amyloid, are inconsistent with this theory. Other theories propose soluble A β or its oligomers as agents that promote AD. These peptides, however, are normal components of human CSF and serum and there is little evidence of disease-associated increases in soluble A β and oligomers. That mutants of APP and presenilin (PS) promote FAD suggests these proteins play crucial roles in neuronal function and survival. Accordingly, PS regulates production of signaling peptides and cell survival pathways while APP functions in cell death and may promote endosomal abnormalities. Evidence that FAD mutations inhibit the biological functions of PS combined with absence of haploinsufficiency mutants, support the hypothesis that FAD mutant alleles promote autosomal dominant neurodegeneration by interfering with the functions of wild type alleles.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder of the central nervous system (CNS) leading to the most common form of age-associated dementia. Clinical symptoms of AD include loss of recent memory, faulty judgment, personality changes and progressive loss of reasoning power. The neuropathology of AD is characterized by the presence of large numbers of neuritic plaques (NPs) and neurofibrillary tangles (NFTs) in the striatum and neocortex of the CNS (Newell et al., 1999). It is now believed that dementia is caused by extensive neuronal and synapse losses in the affected areas of the brain. NPs are complex extracellular structures containing at their core amyloid depositions of fibrillar A β -

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protein surrounded by reactive astrocytes, microglia, and dystrophic neurites. In contrast to the extracellular localization of the NPs, NFTs accumulate intracellularly and consist mainly of paired helical filaments of hyper-phosphorylated tau protein. AD patients often display high levels of amyloid depositions of A β peptides in brain blood vessels, a condition termed cerebrovascular amyloidosis (CVA) (Glenner and Wong, 1987). Most AD cases occur after the ages of 65 or 70 and are termed sporadic because they lack a clear genetic etiology, but about 5% of all cases are linked to specific genetic mutations and are classified as familial AD (FAD). These usually occur at younger ages and follow a more aggressive clinical course than does sporadic AD. The brain neuropathology however, is similar in sporadic and familial AD, suggesting the involvement of common cellular mechanisms in both forms of AD. Despite intense efforts, there is still no chemical test for AD and a definite diagnosis of the disease is made after clinical symptoms are combined with post-mortem examination of brain tissue for detection of plaques and tangles. AD is a serious health problem as it is estimated that by 2020 30 million people will be afflicted worldwide.

Despite intense research efforts in the last twenty five years, the cause of the accelerated neuronal degeneration of AD remains unclear. It is generally accepted however, that the pathogenesis of this disease is complex, driven by both environmental and genetic factors. Presently, age and the apolipoprotein allele E4 (Corder et al., 1993) are the two highest known risk factors for sporadic AD. In contrast to sporadic AD, FAD is driven by specific genetic mutations localized in at least three distinct genetic loci including the genes encoding the amyloid precursor protein (APP) (Chartier-Harlin et al., 1991), presenilin1 (PS1), (Sherrington et al., 1995) and PS2 (Levy-Lahad et al., 1995; Rogaev et al., 1995). APP is important to all forms of AD because in addition to its specific contribution to FAD, it is the precursor of the A β peptides (Robakis et al., 1987a; Goldgaber et al., 1987; Kang et al., 1987; Tanzi et al., 1987) that form the amyloid depositions used to define AD. APP is also important to the neuropathology of Down syndrome (DS) as almost all patients over the age of 40 develop neuropathology similar to AD including depositions of A β amyloid (Wisniewski et al., 1985). Localization of the APP gene on chromosome 21 revealed a direct genetic linkage between these two disorders (Robakis et al., 1987b). It is important to note however, that neither NPs nor NFTs are specific to AD as both pathological structures are also found in normal aged people, usually at lower numbers compared to AD (see also below). NFTs are found in other neurodegenerative disorders in addition to AD, including most forms of frontotemporal dementia (FTD), Down syndrome and often in Parkinson's disease. The presence of NFTs in several neurodegenerative disorders of distinct etiology suggests that tau-based structures may represent a neuronal reaction to stress conditions induced by genetic lesions or environmental factors such as oxidative stress.

The etiology of AD: A β and derivatives

In the last quarter of a century much attention has been focused on A β peptides and their soluble and insoluble derivatives as the main causative agents of AD. A β peptides are a family of small proteins with heterogeneous ends ranging in length from 35 to 43 amino acids with A β 40 and 42 being the predominant species (Miller et al., 1993; Mori et al., 1992). Under conditions that favor aggregation, A β peptide chains form extended β -pleated sheet structures held together in anti-parallel oligomeric arrangements by hydrogen bonds. These A β oligomers may aggregate further to form amyloid fibrils that precipitate in the neuropil as NPs or in blood vessels as CVA. It should be noted that amyloid is a generic term that describes a precipitate of β -pleated sheet fibrillar proteins able to bind Congo red and display optical polarization properties (Glenner, 1980). Thus, the term amyloid should not be used to describe soluble A β peptide or its non-amyloid oligomeric forms. A β peptides are derived from the amyloidogenic processing of APP through the combined action of two distinct enzyme activities termed β (beta) and γ (gamma) secretases (Fig 1). β -secretase

(Vassar et al., 1999) acts on APP to produce peptide C99 that is then processed by the PS/ γ -secretase complex (Wolfe et al., 1999) at γ sites within the membrane to produce A β peptides including the most abundant species A β 1–40 and 1–42 commonly found in amyloid plaques and CVA (Fig 1). In the non-amyloidogenic processing, APP is cleaved by α -secretase close to the extracellular face of the plasma membrane within the A β sequence (blue arrow in Fig. 1) thus inhibiting production of A β peptides (Anderson et al., 1991). α -Secretase fragments are also processed by γ -secretase at the ϵ site (Fig. 1) to produce intracellular peptides containing the carboxyl c-terminal fragment (CTF) of APP. Recent work revealed a number of cell surface proteins and receptors that similar to APP are processed by γ -secretase at the ϵ site to produce intracellular peptides termed CTFs or intracellular domains (ICDs). Importantly, a number of these peptides have been shown to act as signal transduction and gene expression factors (Marambaud and Robakis, 2005; see also below).

In 1987 it was suggested that CVA depositions promote AD by compromising the blood-brain barrier, causing micro-hemorrhages and allowing neurotoxic serum products into the neuropil, thus initiating neurodegenerative cascades that promote formation of NFTs, the second hallmark of AD (Glenner and Wong 1987). This model raised for the first time the possibility that AD is caused by CVA depositions of A β . Subsequent work however showed that a number of patients had little cerebrovascular damage indicating that AD neurodegeneration can develop in the absence of significant CVA. The amyloid cascade hypothesis, a variant of the CVA theory, proposed that depositions of A β amyloid in NPs trigger neurotoxic cascades causing neurodegeneration, NFTs and AD (Hardy and Higgins, 1992; Hardy and Selkoe, 2002). Despite extensive efforts in the last two decades however, there is no agreement on a specific mechanism that explains the proposed neurotoxicity of NPs and many workers doubt these structures are the main causative agents of AD (Neve and Robakis, 1998; Smith et al., 2000; Robinson and Bishop, 2002). Although amyloid depositions may contribute secondarily to neuronal dysfunction, it now seems unlikely that these are the main cause of the AD neurodegeneration as studies have failed to show significant correlations between brain NPs and degree of dementia or neuronal loss (Arriagada et al., 1992; Bouras et al., 2006; Crystal et al., 1988; Davis et al., 1999). Importantly, amyloid depositions at levels similar to those seen in AD are often detected in normal aged individuals (Crystal et al., 1988; Davis et al., 1999), and transgenic (Tg) animal models with high levels of brain amyloid deposits that show no significant neurodegeneration have been reported (Hsia et al., 1999). Interestingly, some Tg mouse models overexpressing APP show synaptic and electrophysiological abnormalities independent of amyloid depositions (Mucke et al., 2000), probably as a result of the abnormally high levels of exogenous APP expressed by these models (see also below). Furthermore, recent studies in humans showed that clearance of amyloid depositions resulted neither in cognitive improvement nor in a decreased rate of mental deterioration (Holmes et al., 2008) suggesting that NPs may not be the driving force of the neurodegeneration and cognitive decline of AD. It seems therefore unlikely that clearance of brain amyloid depositions will result in significant improvements of the cognitive deterioration of AD patients.

AD neurodegeneration may be independent of A β and oligomers

A more recent A β peptide-based theory posits that soluble oligomers of extracellular or intracellular A β 42 may represent the neurotoxic forms of A β . This theory is based on reports suggesting that soluble oligomers of A β are toxic and interfere with synaptic plasticity *in vitro* or with memory function in experimental animals (Klein et al., 2001; Walsh et al., 2005; Cleary et al., 2005). These models however are based on Tg animals or cell lines overexpressing exogenous APP, an artificial condition that may not apply to AD

where there is no evidence of APP overexpression (Robakis et al., 1987a). In addition, behavioral abnormalities in animal models overexpressing APP cannot unambiguously be assigned to A β because APP is metabolized to a large number of derivatives some of which are neurotoxic (Nalbantoglu et al., 1997). It should be noted that over-expression of protein in animal brain often results in neurotoxicity due to, among other factors, trafficking abnormalities driven by the over-expressed protein. It is thus unclear whether the behavioral abnormalities detected in Tg APP models are due to specific A β species, to toxicities derived from other toxic metabolites of APP or to interference with cellular pathways due to the high levels of exogenous APP. Importantly, soluble A β peptides are normal components of human serum and cerebrovascular fluid (CSF) and recent reports from several groups suggest they may have useful biological functions (Paris et al., 2004; Plant et al., 2003; Chen and Dong 2009; Giuffrida et al., 2009). Presently there is little evidence of AD-associated increases in the levels of soluble A β or its oligomers. Absence of data supporting disease-associated increases in soluble toxic A β oligomers is a serious weakness of the theory that such oligomers are the causative agents of the neurodegeneration of AD.

Absence of disease-associated increases in soluble A β makes it unclear what drives aggregation and precipitation of A β peptides as amyloids in AD. Although increased expression of APP and production of A β may lead to amyloid formation, neither condition is necessary for A β amyloid precipitation in the brain. This suggestion is supported by sporadic AD cases in which amyloid depositions form in the absence of any significant increases in either APP expression or A β production. A plausible explanation for the increased amyloid formation in AD is that neurodegeneration may affect the ability of the brain to keep A β peptides in a soluble state. For example, healthy neurons may produce a factor that inhibits aggregation of A β peptides thus keeping them soluble. Neurons compromised by the disease may produce lower levels of this factor, a condition that would allow aggregation and precipitation of soluble A β thus decreasing its concentration. This hypothesis is in agreement with a relatively small but consistent decrease in soluble A β commonly found in the CSF of AD patients compared to normal ones (Hulstaert et al., 1999; Andreasen et al., 1999; Mattsson et al. 2009). In contrast, amyloidosis in experimental animal models is likely driven by the high levels of A β resulting from the overexpressed exogenous APP. Recently, it was reported that an extracellular metabolite of APP activates death receptor 6 (DR6) triggering neuronal degeneration. Based on this observation, it was proposed that the APP-DR6 system promotes the neuronal cell death seen in AD (Nikolaev et al., 2009). Interestingly, this report may explain the increased neuronal cell loss detected in experimental models of AD that are based on APP overexpression (Hsia et al., 1999; Mucke et al., 2000; Cleary et al., 2005), as increased production of the DR6-binding secreted APP fragment would be expected to stimulate neuronal cell death.

FAD, Presenilins and A β

Mutations in three distinct genes, presenilin-1 (PS-1), PS2 and APP, have been implicated as causative agents of FAD, with mutations in the PS1 gene responsible for most cases. PSs are important components of γ -secretase (Wolfe et al., 1999) and to date more than 150 FAD mutations have been linked to PS1 gene. Support for a causative role of A β in AD is derived from reports that FAD mutations of PS invariably increase production of neurotoxic A β 42 by causing a gain of γ -secretase function, the activity involved in the production of this peptide (Klein et al., 2001; Borchelt et al., 1996; Scheuner et al., 1996; Citron et al., 1997). More recent work (Shioi et al., 2007; Bentahir et al., 2006; Walker et al., 2005) however, showed that many PS1 FAD mutants fail to increase production of A β 42, suggesting that not all FAD mutations increase the amyloidogenic processing of APP. Such a specific gain of function is also unexpected for a large set of mutations distributed throughout the PS1 polypeptide. Similarly, reports that FAD mutations promote neurotoxicity by increasing the

A β 42/40 ratio may need further examination because many FAD mutants fail to increase this ratio (Shioi et al., 2007) and although the APP Swedish FAD mutation induces a robust increase in both A β 42 and A β 40 it does not significantly alter the A β 42/40 ratio (Duering et al., 2005). Additional reports indicate that affected individuals carrying PS1 mutants associated with FAD show no abnormalities in either the *in vivo* levels of soluble A β peptides or in the A β 42/40 ratio (Batelli et al., 2008). Regarding the *in vitro* neurotoxicity of A β 42, it is important to note that this toxicity is detectable at A β 42 concentrations that are at least ten thousand times higher than the peptide concentrations found *in vivo* which is usually at the picomolar range (Hulstaert et al., 1999; Mattsson et al., 2009). Attempts in our laboratory to show neurotoxicity for either the monomeric or aggregated forms of A β 42 at concentrations below 1 μ M have been unsuccessful (Famer and Robakis unpublished observations). In contrast, emerging evidence shows that A β 42 promotes neuronal survival, growth and differentiation (Plant et al., 2003; Chen and Dong, 2009; Giuffrida et al., 2009)

That many FAD mutations have no significant effect on the production of A β 42 supports the idea that the effects of these mutations on the neurodegeneration of AD may be independent of their effects on A β (Shioi et al., 2007). The neurodegeneration caused by FAD mutants suggests that the wild type proteins play critical roles in neuronal survival. FAD mutations may interfere with these neuronal survival activities, thus promoting neuronal cell death. The precise mechanism of this interference however, remains unclear. Although the autosomal dominant mode of FAD transmission may be consistent with the hypothesis that FAD mutants cause gain of a toxic function, such a specific gain of function is unexpected for a large number of FAD mutations distributed throughout the PS1 polypeptide chain. Recent evidence from the field of FTD show that progranulin (PGRN) mutations cause autosomal dominant transmission of neurodegeneration by reducing the levels of functional protein (haploinsufficiency) (Goedert and Spillantini, 2006). Unlike the PGRN FTD mutations however, no FAD mutations have been shown to reduce protein levels. A solution to this conundrum is the suggestion that in addition to causing inactivation of the function of the mutant allele, FAD mutations may also cause a loss of function of the wild type allele. The protein product of the mutant allele could for example physically interact with the wild type allele thus interfering with its activity. This mechanism of “allelic interference in FAD” is supported by recent evidence that FAD mutations inhibit the biological functions of PS (see below) and that PSs, as well as APP, form dimers (Schroeter et al., 2003; Hébert et al., 2003; Scheuermann et al., 2001). This mechanism is also consistent with both the autosomal dominant transmission of FAD neurodegeneration and the absence of haploinsufficiency mutations in FAD.

Recent data from several laboratories show that the PS/ γ -secretase system promotes not only the amyloidogenic γ -cleavages of APP but also the ϵ -cleavage of a number of type I transmembrane proteins, including APP, Notch1 receptor, cadherins, EphB receptors and CD44 (Marambaud and Robakis, 2005; Kopan and Ilagan, 2004). The ϵ -cleavage takes place downstream from the amyloidogenic γ -cleavages (Fig. 1) and results in the release of soluble cytosolic peptides containing the intracellular carboxyl-terminal fragments (CTFs or ICDs) of cleaved substrates. To date more than 20 cell surface transmembrane proteins and receptors have been shown to be processed at the ϵ -site by the PS/ γ -secretase system, producing soluble peptides. Additional work showed that a number of these peptides migrate to the nucleus where they act as regulators of gene expression while others remain in the cytoplasm where they regulate metabolism of transcription factors (Marambaud and Robakis, 2005; Kopan and Ilagan, 2004). Together, these data indicate that in addition to producing A β , the γ -secretase system plays central roles in diverse signaling pathways leading to regulation of gene expression.

Importantly, recent reports show that in contrast to the proposed gain of γ -secretase function, PS1 FAD mutations inhibit the γ -secretase-catalyzed ϵ cleavage of a number of cell surface proteins including APP, cadherins, ephrinB, Notch1 and EphB receptors thus reducing production of the corresponding ICD peptides (Marambaud et al., 2003; Wiley et al., 2005; Georgakopoulos et al., 2006; Litterst et al., 2007; Song et al., 1999). These data provide support for the hypothesis that FAD mutations may promote neurodegeneration by inhibiting production of peptides with important transcriptional and signal transduction properties (Fortini, 2003; Robakis, 2003). On the other hand, in addition to PSs, the functional γ -secretase complex comprises at least three other protein components including nicastrin, Aph-1 and Pen-2 (Kopan and Ilagan, 2004). The lack of FAD mutations linked to the genes of the other γ -secretase components raises the possibility that the neurodegenerative functions of the FAD mutants of PS may be independent of γ -secretase activity. Indeed, recent evidence from several laboratories suggests that in addition to their role in γ secretase proteolysis, PSs have γ -secretase-independent functions including stimulation of the PI3K/Akt and MEK/ERK cell survival signaling (Kang et al., 2005; Baki et al., 2004), regulation of the glycogen synthase kinase-3 (Baki et al., 2004; Pigino et al., 2003) and calcium homeostasis (Tu et al., 2006; Dreses-Werringloer et al., 2008; Zhang et al., 2009). Importantly, a number of PS1 FAD mutations have been reported to interfere with the γ -secretase-independent functions of PS1, revealing additional mechanisms by which these mutations may promote neurodegeneration and tau overphosphorylation (Kang et al., 2005; Pigino et al., 2003; Baki et al., 2008). In summary, research in the last decade revealed specific biological functions of PS1 and provided evidence that these functions are inhibited by FAD mutations. It would be interesting to examine whether “allelic interference” (see above) is involved in the mechanism(s) by which FAD mutants inhibit the biological functions of PS.

APP in FAD

In addition to its role as the precursor of the plaque and cerebrovascular amyloids that define AD, APP, like the PSs, is involved in the development of FAD. Currently close to 20 pathogenic mutations have been mapped on the APP gene locus. Some of these mutations are located at one or the other end of the A β sequence of APP and these do not change the primary sequence of A β . Other mutations are found within A β on APP residues 692–694. The later mutations change the sequence of A β and may be associated with disorders other than AD. The first known mutation of this type, Glu693Gln, increases the tendency of the A β peptides to aggregate to form amyloid but it causes no increases in A β production. Carriers of this mutation develop a fatal syndrome known as hereditary cerebral hemorrhage with amyloidosis of the Dutch type (HCHWA-D) characterized by recurrent cerebral hemorrhages due to accumulation of amyloid depositions in cerebral blood vessels (Wisniewski et al., 1991). These patients are not classified as having AD as they are usually not demented and are mostly free of NPs and NFTs. Other pathogenic mutations located on residues 692 and 694 however are associated with AD and its neuropathology but they do not generally increase production of A β peptides and cause no significant changes in the 42 to 40 ratio (Nilsberth et al., 2001; Brooks et al., 2004). A common explanation for the dementia-causing APP mutations that are located outside the A β sequence is that they increase production of neurotoxic A β . This explanation however seems inconsistent with observations that several APP mutations of the London type (APP770 codon 717) which cause relatively small increases in A β production are more toxic (cause AD at earlier ages of onset) than the Swedish mutation of APP which causes much higher increases in A β than the London mutations (Citron et al., 1992; Suzuki et al., 1994). Together, these discrepancies raise the possibility that additional factors, probably related to the biological functions of APP including its role as a modulator of a death receptor (Nikolaev et al., 2009), may contribute significantly to the mechanism(s) by which the APP FAD mutations

promote neurodegeneration. The important role of APP in neuronal cell death is illustrated by recent reports that certain early onset FAD families contain a duplication in the genomic locus that encodes APP suggesting that overexpression of this protein even at a 50% level may cause neurotoxicity (Rovelet-Lecrux et al., 2006). The precise mechanism of this toxicity remains to be determined although that it may be related to the cell-death function of extracellular APP derivatives (Nikolaev et al., 2009) and to recent evidence that the β -CTF fragments of APP are involved in the endocytic dysfunctions of DS and AD (Jiang et al., 2010).

In summary, the main mechanisms responsible for the neurodegeneration of AD are still poorly understood. It is unclear for example why certain neuronal populations such as cholinergic neurons are more vulnerable to AD than other neurons and how factors like the apoE4 allele, the process of aging, and genetic FAD mutations promote specific degeneration of these neuronal populations. There are additional indications that environmental factors such as oxidative stress and inflammatory processes may also contribute to the neuronal cell death of AD (Pappolla et al., 1992; Weggen et al., 2007), although neither antioxidants nor anti-inflammatory agents seem to have a significant effect on the course of dementia. This may be due to the fact that by the time AD is detected a significant number of neurons have been eliminated and these are difficult to replace. It is reasonable to assume however that in the majority of AD cases such as sporadic AD, the final outcome is determined by both genetic and environmental factors. Presently the FAD mutations are the only identifiable causative agents of AD and these mutations may offer the best available models for the study of the cellular and molecular mechanisms involved in the development of the more common sporadic disease. Since the clinical manifestations and neuropathology seems similar in both sporadic and familial AD, lessons learned from studying the mechanisms of FAD should also be applicable to sporadic AD.

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Figure 1. The amyloidogenic and signaling processing of APP and other type I transmembrane proteins

The α- and ε-site cleavages of APP and other type I transmembrane (TM) proteins (blue arrows) mediated by metalloproteinases (usually ADAMs) and γ-secretase respectively, promote production of biologically active signalling peptides (termed CTFs or ICDs). The β- and γ-site cleavages of APP catalyzed by β and γ secretase (red arrows) respectively promote production of Aβ peptides. The second step of both processing pathways (γ and ε cleavages), is catalyzed by the PS/γ-secretase system. N and C, protein N- and C-terminus respectively.