Are A β and Its Derivatives Causative Agents or Innocent Bystanders in AD?

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Key Words

Alzheimer's disease · A β peptides · Amyloid precursor protein · Neuronal loss

Abstract

Alzheimer's disease (AD) is characterized by neurodegeneration in neocortical regions of the brain. Currently, A β -based theories, including amyloid depositions and soluble A β , form the basis of most therapeutic approaches to AD. It remains unclear, however, whether A β and its derivatives are the primary causative agents of neuronal loss in AD. Reported studies show no significant correlations between brain amyloid depositions and either degree of dementia or loss of neurons, and brain amyloid loads similar to AD are often found in normal individuals. Furthermore, behavioral abnormalities in animal models overexpressing amyloid precursor protein seem independent of amyloid depositions. Soluble A β theories propose toxic A β 42 or its oligomers as the agents that promote cell death in AD. A β peptides, however, are normal components of human serum and CSF, and it is unclear under what conditions these peptides become toxic. Presently, there is little evidence of disease-associated abnormalities in soluble A β and no toxic oligomers specific to AD have been found. That familial AD mutations of amyloid precursor protein, PS1 and PS2 promote neurodegeneration suggests the biological functions of these proteins play critical roles in neuronal survival. Evidence shows that the PS/ γ -

secretase system promotes production of peptides involved in cell surface-to-nucleus signaling and gene expression, providing support for the hypothesis that familial AD mutations may contribute to neurodegeneration by inhibiting PS-dependent signaling pathways.

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Introduction

Alzheimer's disease (AD) is defined by large numbers of neuritic plaques (NPs) and neurofibrillary tangles (NFTs) in neocortical regions of the brain, but it is now accepted that dementia is caused by extensive neuronal and synapse losses in the hippocampus and neocortex. NPs are complex extracellular structures containing a core of amyloid depositions of fibrillar A β -proteins surrounded by reactive astrocytes, microglia, and dystrophic neurites. NFTs accumulate intracellularly and consist mainly of paired helical filaments of overphosphorylated tau protein [1]. Most AD cases occur after the age of

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Fig. 1. Amyloidogenic and signaling cleavages of APP are distinct but closely related. Both depend on the PS1/ γ -secretase system. β - and γ -secretase cleavages promote production of A β peptides. APP and other cell surface receptors are cleaved extracellularly by metalloproteinases (MPs) and then by γ -secretase at the ϵ -site to promote production of biologically active CTFs (ICDs). In the nonamyloidogenic processing, APP is cleaved by α -secretase close to the extracellular face of the plasma membrane (blue arrow, see [37]). This cleavage inhibits production of A β peptides.

65 or 70 and are termed sporadic because they lack a clear genetic etiology, but a small percent of all cases display clear genetic linkages and are classified as familial AD (FAD). Three genetic loci have been linked to FAD including the genes encoding the amyloid precursor protein (APP), the precursor of the A β peptides, PS1 and PS2. Despite intense research efforts in the last 25 years, it is still unclear what causes the accelerated neuronal cell death of sporadic AD although aging and apoE alleles are risk factors. In contrast, FAD is driven by specific genetic mutations although the biological mechanisms by which these mutations promote neurodegeneration remain unclear.

Processing of APP and Production of A β

In the last quarter of a century, much attention has been focused on A β peptides and their derivatives including soluble A β oligomers and fibrillar amyloid depositions as the main causative agents of AD. A β peptides are a family of small proteins ranging in length from 39 to 43 amino acids. Under conditions that favor aggregation, soluble A β peptide chains form extended β -pleated

sheet structures held together in oligomeric arrangements by hydrogen bonds [2]. These A β oligomers aggregate further to precipitate as amyloid fibrils in NPs and blood vessels (cerebrovascular amyloid, CVA). A β peptides are derived from the amyloidogenic processing of APP through the sequential cleavages by β - and γ -secretases. β -Secretase cleaves first at the extracellular N-terminus of the A β sequence of APP to produce a membrane-bound fragment termed C99 that contains the A β sequence plus the transmembrane and cytoplasmic domains of APP (fig. 1). C99 peptide is then processed by the PS/ γ -secretase complex at the γ -sites located in the middle of the transmembrane sequence producing A β peptides with distinct C-terminal ends (fig. 1).

Recent evidence shows that in addition to the amyloidogenic γ -cleavages of APP, the PS/ γ -secretase system promotes the ϵ -cleavage of a large number of cell surface type I transmembrane proteins, including APP, Notch1 receptor, E-cadherin, N-cadherin and CD44 [reviewed in 3]. This alternative cleavage takes place downstream from the γ -cleavages at the end of the transmembrane sequence of the substrate (fig. 1). Cleavage at the ϵ -site follows a metalloproteinase cleavage at the extracellular domain of the substrate and results in the release of soluble

cytosolic peptides containing the intracellular domains (ICDs or C-terminal fragments, CTFs) of cleaved substrate. Similar to γ -cleavages, the ε -cleavage is also sensitive to γ -secretase inhibitors. To date, at least 20 cell surface proteins have been shown to be cleaved at the ε -sites by γ -secretase producing soluble peptides containing the CTFs of the cleaved substrates. Research in the last several years showed that these peptides may migrate to the nucleus where they act as regulators of gene expression or they may remain in the cytoplasm where they regulate the metabolism of transcription factors [3].

Amyloid Depositions Cannot Explain the Neurodegeneration Responsible for Dementia

In 1987, it was suggested that CVA depositions promote AD by compromising the blood-brain barrier allowing neurotoxic serum products into the neuropil [4]. This serum-born toxicity promotes neurodegeneration and formation of NFTs. This was an important concept as it raised for the first time the possibility that amyloid depositions of A β may be causally related to AD. Subsequent work however, indicated that many AD patients had little cerebrovascular damage, suggesting that AD neurodegeneration can develop in the absence of significant CVA depositions. The amyloid cascade hypothesis of AD, a variant of the CVA theory, suggested that depositions of A β amyloid fibrils in NPs trigger a neurotoxic cascade causing neurodegeneration and dementia [1, 5]. In the last two decades, many workers concentrated on the specific mechanism(s) by which NPs may promote neuronal cell death, but presently there is not a clear mechanism that could explain the proposed neurotoxicity of amyloid depositions, and many workers doubt these depositions are the main causative agents of AD [6, 7]. Although amyloid pathology may contribute secondarily to the neuronal dysfunction of AD, it seems unlikely that amyloid plaques constitute the main pathological agent as a number of studies failed to show significant correlations between concentration or brain distribution of NPs and degree of dementia, loss of neurons, distribution of dystrophic neurites, or cytoskeletal abnormalities [8, 9]. Furthermore, amyloid depositions at levels similar to those seen in AD are often detected in normal individuals [10, 11], and transgenic (Tg) mouse models constructed to develop high levels of brain amyloid deposits failed to show significant neurodegeneration. Interestingly, several of these models showed synaptic and electrophysiological abnormalities before detection of amyloid de-

posits, probably as a result of the abnormally high levels of exogenous APP [12, 13] expressed by these animals. Finally, recent studies in humans showed that clearance of amyloid depositions resulted neither in cognitive improvement nor in decreased rate of mental deterioration [14], suggesting that NPs are probably not 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 functions of AD patients.

AD Neurodegeneration May Be Independent of A β and Oligomers

The inability of the amyloid plaque theories to explain the neurodegeneration of AD prompted the development of the soluble oligomer A β theories which posit that soluble oligomers of extracellular or intracellular A β 42 represent the neurotoxic forms of A β . Indeed, recent reports suggest that soluble oligomeric A β may interfere with synaptic plasticity of cell cultures or memory function in experimental animal models [15, 16]. Most of these models however, are based on Tg animals or cell lines constructed to overexpress exogenous APP, an artificial condition that does not apply to AD where there is no evidence of APP overexpression [17]. More important, behavioral abnormalities in animal models overexpressing APP cannot be unambiguously assigned to soluble A β oligomers because in addition to A β , APP is metabolized to a large number of derivatives, some of which, including CTFs, have been reported to be neurotoxic. It is important to note that overexpression of proteins in animal brain often results in neurotoxicity due to, among other factors, trafficking abnormalities driven by the overexpressed protein. It is thus unclear whether the behavioral abnormalities detected in Tg APP mice are due to specific A β species or to additional toxicities of the overexpressed exogenous APP.

Soluble A β peptides are produced by most cells and are found in all people. Apparently, these peptides are normal components of human serum and CSF, and it has been proposed that they have useful biological functions [18]. Efforts to show AD-associated abnormalities in soluble A β have failed, and it is thus unclear under what conditions these normal peptides become toxic, especially in sporadic AD where no significant disease-associated abnormalities have been detected in their production or degradation. Similarly, there is no evidence of a soluble toxic A β oligomeric species specific to AD (detection of

such a species would also be important to the diagnosis of this disease). Without such evidence, it is hard to accept the concept that A β oligomers identified in animal models overexpressing APP are relevant to AD, a disease that displays no significant overexpression of APP. Regarding the in vitro neurotoxicity of A β 42, it is important to note that this neurotoxicity becomes detectable when A β is used at concentrations which are at least ten thousand times higher than the peptide concentrations found in vivo (usually less than 500 pM). Repeated 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, unpubl. obs.].

Reports that FAD mutants of PS1 invariably increase production of neurotoxic A β 42 by causing a gain of γ -secretase function seemed to support a causative role of A β in AD. Based on these reports, it was proposed that FAD mutations promote dementia by increasing production of A β 42 (gain of function) and, by extrapolation, this mechanism may also be central to other forms of AD [19, 20]. Additional work, however, showed that many FAD mutations of PS1 inhibit the γ -secretase cleavage at the ϵ -site of several substrates including cadherins, ephrin B and APP, suggesting that PS1 FAD mutants may cause a loss, rather than a gain, of γ -secretase activity [21–23]. These findings seemed inconsistent with the suggestion that all PS1 FAD mutations cause a gain of γ -secretase cleavage activity. Furthermore, such a specific gain of function is rather unexpected for a large number of mutations distributed throughout the PS1 polypeptide. More recent work from our [24] and other [25–27] laboratories showed that many PS1 FAD mutants fail to increase production of A β 42, indicating that not all FAD mutations increase the amyloidogenic processing of APP.

It has been suggested that although a number of PS FAD mutations are unable to increase production of neurotoxic A β 42, these mutants cause an increase in the ratio of A β 42/40 and this increase may somehow cause neurodegeneration and the AD phenotype [27, 28]. Our data [24], however, show that although some PS1 FAD mutations may cause an increase in this ratio, others do not. Five FAD mutants tested in two different cell systems failed to show a significant increase in A β 42/40 ratio. Our data are similar to reports that several PS2 FAD mutants [25] have no significant effects on the production of A β 42 or on the ratio of A β 42/40. Additional studies have failed to show a significant correlation between age of FAD onset induced by PS mutations and the increase in

either A β 42 or the A β 42/40 ratio induced by these mutants. Importantly, brain A β 42 levels do not correlate with the age of disease onset [27], and although the APP Swedish FAD mutation induces a robust increase in both A β 42 and A β 40, it does not significantly change the A β 40/42 ratio [28]. Finally, there is no known mechanism to drive the postulated change in the 40/42 ratio in sporadic AD.

The lack of disease-associated increases in soluble A β peptides or their oligomeric forms makes it unclear what drives aggregation and precipitation of A β amyloid in AD. Since there is no evidence of A β overexpression in AD, a plausible explanation is that neurodegeneration affects the ability of the brain to keep the A β peptides soluble. For example, healthy neurons may produce a factor that inhibits aggregation of A β . Neurons compromised by the disease may produce lower levels of this hypothetical factor thus promoting aggregation and precipitation of the soluble A β . This explanation is in agreement with a relatively small but consistent decrease in soluble A β found in AD brains. In contrast, amyloidosis in experimental animal models is driven by high levels of A β produced by the overexpressed exogenous APP.

Effects of PS FAD Mutants on Neurodegeneration May Be Independent of Their Effects on A β 42 or the A β Ratio

That many FAD mutations have no significant effect on the production of A β 42 supports the suggestion that the effects of these mutations on neurodegeneration and AD may be distinct from their effects on A β [24]. This suggestion is supported by reports that PS mutations can cause neurodegeneration in the absence of either amyloid depositions or increased A β and that loss of PS1 may result in neurodegeneration and cognitive decline through amyloid-independent mechanisms [29]. A specific APP mutation rescued neurodegeneration and behavioral abnormalities in Tg animals without affecting A β production or amyloid depositions [30], and patients of an Italian family carrying a PS1 mutant associated with FAD showed no abnormalities in either the in vivo levels of soluble A β peptides or in the A β 42/40 ratio [31]. In summary, a large number of experimental observations seem inconsistent with the theory that FAD mutations promote neurodegeneration by increasing production of neurotoxic A β 42. Similarly, there is no evidence of any A β oligomers specific to AD, neither are there any AD-specific changes in the concentration of these oligomers.

Such evidence is necessary to support the hypothesis that oligomers are causally involved in AD and would also be valuable findings for the diagnosis of the disease.

Although for the last quarter of a century A β -based theories have been the leading explanation for the dementia of AD, it is still unclear that these peptides are the primary causative agents of AD. In contrast, a number of data suggest that the ability of FAD mutations to promote neurodegeneration may be independent of their effects on the production of A β or the ratio of A β species. The FAD mutations of APP, PS1 and PS2 suggest that the biological function of these proteins play critical roles in neuronal survival and function. A recent report indicates that APP interacts with cell death receptor 6 (DR6) and triggers neuronal degeneration. The data suggest that a secreted extracellular fragment of APP binds to and activates DR6 stimulating cell death. Based on these observations, it was hypothesized that the APP-DR6 system may contribute to the neuronal cell death of AD [32]. Interestingly, this report may explain how overexpression of APP stimulates neurodegeneration in animal models as increased production of the DR6-binding secreted APP fragment will stimulate neurodegeneration. Evidence from other groups support the hypothesis that the functions of PS1 in cell survival [33, 34] may be involved in the effects of the FAD mutations on neurodegeneration.

Additional evidence indicates that the PS FAD mutants affect γ -secretase-dependent transcriptional pathways independently of A β and that these effects may contribute to neurodegeneration and AD [35, 36]. It is now clear for example, that the ϵ -cleavage of many cell surface receptors including Notch1, cadherins, EphB receptors and even APP, catalyzed by the γ -secretase proteolytic system, promotes release of ICD peptides (fig. 1), which may act as transcriptional factors, suggesting that this system plays central roles in diverse signaling pathways leading to transcriptional regulation [3]. Importantly, recent reports show that FAD mutations inhibit the ϵ -cleavage of cell surface receptors, and this inhibition results in a dramatic decrease in the production of ICDs involved in signal transduction and gene expression [21, 22]. Together, these data provide support for the hypothesis that transcriptional dysregulation may be another mechanism by which FAD mutations contribute to the neurodegeneration and memory defects characteristic of AD [35].

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