Molecular aspects of neurodegeneration in Alzheimer’s disease

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Summary

Alzheimer’s disease (AD) is a degenerative disease of the brain, and the most common form of dementia. It is estimated that more than 22 million individuals worldwide will have AD by 2025. The causes of the disease are still unknown and recent hypotheses suggest that an aberrant protein processing initiates the neurodegeneration. Several lines of research are centered on the study of proteins that are genetically associated with this syndrome, such as amyloid precursor protein (APP) and presenilins. This review focuses on recent advances in the processing of APP and on the neuropathological role of its amyloidogenic fragments, which have been shown to be directly involved in neurodegeneration and glial inflammation and which likely influence the development of AD.

KEY WORDS: Alzheimer’s disease, amyloid beta-peptides, amyloid precursor protein.

Introduction

Alzheimer’s disease (AD) is among the most common diseases of the elderly, affecting almost 10% of individuals who survive beyond the age of 65 years, and another 10% for each additional decade thereafter (1,2). The prognosis of the disease is an inexorable decline of mental functions and, to date, there is no effective therapy. It is still unclear which features of AD represent the causes of the disease and which are, instead, consequences of the metabolic processes that lead to neurodegeneration and dementia (1-3). Pathological hallmarks of the disease are the presence in brain parenchyma of extracellular lesions, in the form of aggregated proteins and peptides defined as amyloid plaques, intracellular aggregates of hyperphosphorylated proteins in dystrophic neurons, and neuronal loss followed by secondary inflammation or gliosis that contributes to the neuropathological process (1-6). The parenchymal deposition of heavily aggregated amyloid peptides in the brains of AD patients is thought to be a central event in the pathogenesis of the disease and the so-called “amyloid hypothesis” links plaque formation, neurodegeneration and AD pathology with the abnormally enhanced formation of these amyloid peptides in the brain (1,2). The amyloid present in plaques and meningeal vessels is predominantly composed of a 40/42 amino acid peptide that, defined as amyloid β-peptide (Aβ), is derived from the proteolysis of the amyloid precursor protein (APP) through the action of two enzymes called β and γ-secretase (1,4,7) (Fig. 1). The APP holoprotein may be cleaved to form a number of transmembrane polypeptides, previously referred to as C22 kDa, C18 kDa, C100, C99, or collectively as carboxy-terminal fragments of APP (CTFs) which, depending on their length and sequence, may contain the entire Aβ sequence and are considered amyloidogenic (1,7-9). The putative β-secretase recently identified (10) is responsible for the generation of the C99 polypeptide while α-secretase is responsible for the formation of the C83 fragment, sites of secretase cleavage, Aβ region and the major point mutations that are linked with the familial AD phenotype.

Fig. 1 - Schematic representation of amyloid precursor protein, sites of secretase cleavage, Aβ region and the major point mutations that are linked with the familial AD phenotype.
which is cleaved at residue 17 of Aβ and is therefore considered non-amyloidogenic. γ-secretase is responsible for the cleavage at the C-terminus of CTFs and for the formation of Aβx-40 or Aβx-42, the latter being more amyloidogenic, more toxic, and whose increased presence is correlated with familial AD and Down’s syndrome (DS) (1,2,11-16). The amyloid hypothesis proposes that plaque amyloid deposition, or partially aggregated soluble Aβ, trigger a neurotoxic cascade, thereby causing neurodegeneration and AD (1,2). The first evidence that linked APP and Aβ to AD was the early finding that the APP gene is located on chromosome 21, since virtually all individuals carrying an extra copy of this chromosome (trisomy 21; Down’s syndrome) show AD-like neurodegeneration as early as the middle of their third decade (2,16). Further evidence suggesting that APP is pivotal in AD pathogenesis is provided by specific point mutations present in the APP gene, which cause some forms of familial AD (1,2,17,18). The amyloid theory is also based on in vitro studies, which demonstrate that Aβ is fibrillogenic, toxic for neuronal cells and that its formation is enhanced in cells carrying familial AD (FAD) mutant genes (1,12,13,17). In addition to APP, other proteins are in fact involved in FAD and several mutations in genes that encode for presenilins – presenilin 1 (PS-1), and presenilin 2 (PS-2) – have been identified (19,20). Ever since the discovery of PS-1 and its homolog PS-2, researchers have attempted to correlate the role of PS-1 and PS-2 with the neuropathological features present in FAD patients and therefore with the proteolytic processing of APP and the formation of Aβ (1,2,21). Several lines of evidence suggest that mutations on presenilins cause an increased production of the Aβx-42 peptide, corroborating strongly the idea that abnormal processing of APP is pivotal in the pathogenesis of the disease (1,21). Other investigators have demonstrated that presenilin mutations associated with FAD may increase neuronal apoptosis by altering the stability of the beta-catenin and cadherin/catenin cell-cell adhesion system, and that mutations in PS-1 may increase the cells’ susceptibility to apoptosis and neuronal cell death (1,22,23). Extensive studies have been conducted in recent years to decipher the influence of PS-1 and PS-2 on the interaction with APP and on the secretase cleavage of APP (1,12,13,21). The results are not unambiguous: some researchers propose that PS-1 may only “influence” β- and γ-secretase, while others feel that PS-1 may be “the” γ-secretase that generates Aβ. Also, presenilin mediates the γ-secretase-like cleavage of Notch receptors to enable signaling by their cytoplasmic domains (23). Therefore, APP and Notch might be substrates for this unique intramembranous aspartyl protease. In any case, it is widely accepted that presenilins may regulate APP cleavage and Aβ formation. In addition to amyloid plaques, neurofibrillary degeneration constitutes a key histopathological brain lesion of AD that is shared with related neurodegenerative disorders commonly referred to as taoopathies (2,5). The microtubule-associated protein tau is a family of six isoforms that become abnormally hyperphosphorylated and accumulate in neurons undergoing neurodegeneration in the brains of patients with AD and taoopathies (1,2,5). Hyperphosphorylated tau is the major protein subunit of paired helical filaments (PHFs), which accumulate in the soma (as neurofibrillary tangles, or NFTs) and dystrophic neurites (as neuropil threads and as dystrophic neurites surrounding the beta-amyloid core in neuritic plaques in AD) of the affected neurons (2). Unlike normal tau, which stimulates assembly and stabilizes microtubules, the abnormally hyperphosphorylated tau inhibits assembly and disrupts microtubules therefore leading to neurodegeneration (2,5). A Manichean separation between “tautists” and “Baptists” (24) divides those who believe that tau is the cause of neurodegeneration and dementia from those who consider Aβ to be the culprit. But recent data suggest that Aβ and tau might be pathophysically related in AD. Transgenic animal models expressing mutant tau, in which fibrillar Aβ was injected, and double mutant tau/APP, which overexpress Aβi, both showed accelerated and enhanced NFT formation (25,26). Therefore a pathological interaction between Aβ and tau has been reproduced in these mice, suggesting a pivotal action of Aβ over tau in inducing the neurodegeneration. Most studies on APP therefore focus on Aβ and on its formation as the leading cause of AD. In this review we look at recent advances in the understanding of the pathophysiological role that APP, CTFs and Aβ heterogeneity may play in AD.

Aβ origin and heterogeneity

Aβ is the major constituent of extracellular plaques and perivascular amyloid deposits, the pathognomonic neuropathological lesions of AD. The Aβ42 form is abundantly present in plaques, is highly fibrillogenic and is present in the early stage of the deposition process (4,14,16). An increased presence of Aβ42 is a common pathogenetic marker present in APP familial AD, in PS-1 familial AD and in DS, and a plethora of studies, in vitro and in vivo, shows the Aβ42 form to have a significantly higher propensity to aggregate and a higher toxicity than the Aβ ending at 40 (1,2,14,15). Soluble and oligomeric aggregated Aβ may induce cell death either through physical damage of membranes (and therefore initiating a cascade of events such as oxidative damage, lipoperoxidation, apoptosis, etc.) or even through a sort of receptor-mediated mechanism that involves APP itself (14,15,27). In PS-1 mutant models both in vitro and in vivo, a selective increase in the production of Aβ42 is correlated with that familial phenotype, linking pathogenic PS-1 function to an abnormally enhanced γ-secretase cleavage at residue 42 and strengthening the hypothesis that PS-1 is the γ-secretase (1,12,13,21). Aβ may exist in vitro and in vivo as soluble or highly insoluble derivatives, as a monomer or as dimers or oligomers, and all forms have been associated with highly toxic properties (14,15,28). The Aβ water-soluble derivatives have been described in different reports and are considered early markers of amyloid formation because they are detectable only in brains of subjects with AD or at risk of AD, such as young or even fetal individuals with DS who still lack plaques and amyloid deposits (28,29). Notably, soluble-Aβ is undetectable in normal tissues, possibly because in non-AD subjects it is complexed with apolipoprotein E (apoE) (30). In AD individuals, the soluble complex apoE-Aβ is unstable and most apoE-Aβ exists as an insoluble aggregate (30). This is an important observation since inheritance of the apoE ε4 allele is a prevailing risk factor for sporadic and familial AD and it has been suggested that.
the effects of traumatic head injury and apoE ε4 are, in synergy, risk factors for the development of a late dementia (30-32). Furthermore, the low-density lipoprotein receptor-related protein gene, which is involved in cholesterol transport, is often mentioned as a candidate gene for AD because of its role as a receptor for apoE and because of its interaction with APP (33). Heterogeneity of Aβ arises at cellular level too; newly-formed Aβ has, in fact, been detected both in intracellular compartments as well as at the cell surface (9,34). The latter finding made issues of the multiple sites of origin of Aβ and of the multiple sites of cellular action of the enzymes involved in APP cleavage. It is only recently that attention has focused on the heterogeneity of Aβ peptides, and we and others have detected in human brain, in addition to the Aβ peptides starting with L-Asp at the N-terminus (Aβ1-42/40), a great heterogeneity of N-terminally truncated forms, which occur in senile plaques as aggregates and also as soluble derivatives (35,36). The fact that Aβ exists in the AD brain as a heterogeneous family of differently cleaved peptides is shown in figure 2, in which, by Western blotting and MALDI-TOF mass spectrometry, different isoforms are electrophoretically separated and sequenced. Aβ peptides may be further modified, and isomerization and racemization of the aspartate position at 1 and 7 and cyclization of glutamate at residues 3 and 11 have been described (35-37). Recent data indicate that the pyroglutamate-containing isoform at position 3 (Aβ3(pE)-40/42) represents the prominent form (25-50% of the total Aβ amount) among the N-truncated species present in senile plaques (35-37). Also, we reported that N-truncated peptides are overproduced in early-onset subjects with mutated PS-1 and proposed a direct correlation between disease severity and the content of Aβ N-truncated species (38,39). As a consequence we hypothesized an influence of PS-1 mutations on the activity of β-secretase, which is responsible for the cleavage at the N-terminus of CTFs and of Aβ as well (38). The putative β-secretases recently identified (BACE1 and 2) seem to be responsible only for the cleavages at residues 1 and 11 of the Aβ sequence leaving a question mark over the formation of other Aβ sequences previously identified with different N-termini such as the pyroglutamate at residue 3 (10). Also, pyroglutamate-modified Aβ are known to appear early and to increase with age in DS brains (16,36). Finally, while the toxicity and the aggregation propensity of Aβ1-40/42 have been extensively analyzed, little and contrasting information is, at the moment, available on the fibrillogenic capabilities and toxic properties of AβN3(pE)-40/42 peptides (40). Moreover, even their formation is matter of discussion: do they arise from the 1-42 peptide after proteolytic amino-terminal degradation or do they originate directly at β-secretase level as pyroglutamate-modified CTFs, which are then cleaved by γ-secretase to give rise to Aβ peptides?

**CTFs and Aβ formation**

As mentioned above, the β-secretase cleavage of APP forms different transmembrane polypeptides or CTFs which contain the entire Aβ sequence, are considered amyloidogenic and neurotoxic, and whose expression in vivo can induce neurodegeneration (1,8,9). It seems widely accepted that the β-secretases BACE1 and 2 generate the free N-terminus of Aβ at Asp1 and likely at Glu11 before the γ-secretase cleavage (10). We have recently analyzed the electrophoretic profile of CTFs detected in brain extracts from AD, control and DS subjects. CTFs, which migrate as five bands between 8 and 14 kDa, are present in brain extracts from human brain samples (41). Their expression is increased in DS fetal cases in comparison to sporadic AD and non-mentally control samples. The CTF content does not differ significantly between AD and control cases, but we must consider that in AD patients these species have been heavily cleaved by γ-secretase to form Aβ. CTFs as amyloidogenic fragments constitute, in young plaque-free DS subjects, a pathogenic event that might be directly correlated with the early occurrence of amyloidotic deposits in these patients (16). Indeed, increased β-secretase activity, due to the increased presence of both enzyme (BACE2 is encoded in the 'Down critical region' in 21q22.3 and substrate (APP in the same region), is probably responsible for the increased formation of CTFs and their toxic species in DS (20-22). We also provided evidence (41) that pyroglutamate-modified CTFs are detected among the CTF bands and that the pyroglutamate 3-99 polypeptide is likely to be the substrate for γ-secretase, like the well-known C99, which starts at Asp1 of Aβ (Fig. 3). Therefore, aminoterminally truncated AβN3(pE)-40/42 are not generated in the human brain by the progressive proteolysis of Aβ 1-40/42, as previously hypothesized, but their formation is, rather, an event that precedes the γ-secretase cleavage (41). Study of PS-1 familial cases has suggested that the formation of N-truncated Aβ may be related to the severity of the disease and to the amyloidogenic process. Therefore these might constitute an independent amyloidogenic entity whose origin may be unrelat-

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**Fig. 2.** MALDI-TOF mass spectrometry and Western blotting (box) of soluble Aβ peptides extracted from AD brain samples. Aβ peptides, immunoprecipitated from an AD cortical sample, are electrophoretically separated by SDS-PAGE and are identified by Western blotting with the 4G8 antibody. Aβ peptides migrate in three distinct bands; the upper migrating band co-migrates with a synthetic peptide Aβ1-42 as a reference. MALDI-TOF analysis of similarly immunoprecipitated Aβ peptides shows the sequences of differently cleaved Aβ, which are present in the bands identified by Western blotting. Besides the full-length 1-40 and 1-42 sequences, other peptides N-truncated at residues 2, 3, 4 etc., are also present.
secretase activity was further complicated by a so-called “spatial paradox” caused by the fact that γ-secretase cleavage of APP-C99 might occur in specialized subcellular compartments where little or no PS-1 is present, calling into question the equation PS-1 equals γ-secretase (43). The neuronal subcellular localization of presenilins, their integration in multiprotein complexes, possibly together with APP or CTFs, and the recent identification of additional modulators of γ-secretase, like nicastrin (44), suggest that several proteins may be involved in the as yet unknown function of APP and PS-1, as well as in the formation of Aβ. The cytoplasmic portion of APP contains a NPTY motif, which is present in the cytodomains of several tyrosine-kinase receptors (TKRs) and in non-receptor tyrosine kinase (TK). In TKRs, the tyrosine residue of this motif is phosphorylated upon TK activation and the NPXXpY motif functions as a docking site for the phosphotyrosine-binding domain (PTB) present in several adaptor proteins interacting with TRKs and non-receptor-TK. In APP and in APP-related proteins APLP1 and APLP2, the NPTY motif interacts with adaptor proteins Fe65, X11 and mDab1, and this interaction has been demonstrated to be independent of tyrosine phosphorylation (45,46) (Fig. 4). We have recently described (41) the presence in human brains (of both AD and non-AD control subjects) of a subset of tyrosine phosphorylated CTFs. Moreover, we have evidence that in vitro the APP cytodomain too may be tyrosine-phosphorylated by the non-receptor tyrosine kinase Abl, which however phosphorylates a tyrosine residue upstream (Y682, numbering for APP695 isoform) the NPTY motif (47). This phosphorylation generates a pYENP motif which is recognized by the SH2 domain of Abl itself. Tyrosine-phosphorylated APP fragments might therefore constitute a docking site for PTB or SH2-containing adaptors, linking APP metabolism to signal transduction. Our data (41) show that only Aβ-bearing CTFs are tyrosine-phosphorylated, while the α-secretase derived C-83 remains unphosphorylated. This implies either that unphosphorylated APP is an unsuitable substrate for β-secretase cleavage and a good substrate for α-cleavage, or that C83 is quickly dephosphorylated. Some questions also arise about Aβ formation: are tyrosine-phosphorylated CTFs available substrates for γ-secretase? Which are the tyrosine residues and binding motifs involved? Which kinase(s) are responsible? What is the pathophysiological significance of these modifications? Other phosphorylation sites in serine and threonine residues have been described on APP and these modifications seem to be responsible for an effect on neuronal differentiation and on the stability of Fe65/APP complexes (1,33,48-50). The answers to these questions might help to define the physiological role of APP and provide insight into the APP-dependent pathogenesis of AD. Since the cytoplasmic domain of APP is anchored to a complex protein network that might function in neuronal cell migration, axonal elongation and dendritic arborization, the proteolysis of APP might be critically involved in intracellular signaling events (46-50). And if, on the one hand, the interaction between APP and Fe65 has been implicated in the regulation of cell movement, on the other, X11alpha binds to the YENPTY sequence in the cytoplasmic carboxyl terminus of APP prolonging APP’s half-life and inhibiting Aβ secretion (51). Aβ generation and APP function are therefore strictly linked and disruption of the normal function of APP is at least one cause of the neurodegeneration and consequent dementia in AD. The treatment of AD remains a major challenge and many pieces of the puzzle still need to

Fig. 3 - Densitometric quantitation of C99 polypeptide purified from brain samples of sporadic AD subjects (AD ••), Down’s syndrome fetal subjects (DSf-•) and control subjects (CO-•), identified by western blotting (box). In DSf cases a significantly higher amount of C99 is present in comparison to AD and control cases.
be put together in order to get the complete picture of the pathogenetic events that trigger the neurodegeneration. Besides APP, new proteins have been seen to be involved and a deeper comprehension of the complex network of interaction in which APP seems entangled might be the key to disclose the molecular mechanisms that lead to neuronal death and memory loss in AD.

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