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SPECIAL ISSUE

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Genes and mechanisms involved in β-amyloid generation and Alzheimer's disease

Abstract Alzheimer's disease is characterized by the invariable accumulation of senile plaques that are predominantly composed of amyloid β-peptide (Aβ). Aβ is generated by proteolytic processing of the β-amyloid precursor protein (βAPP) involving the combined action of β- and γ-secretase. Cleavage within the Aβ domain by α-secretase prevents Aβ generation. In some very rare cases of familial AD (FAD), mutations have been identified within the βAPP gene. These mutations are located close to or at the cleavage sites of the secretases and pathologically effect βAPP processing by increasing Aβ production, specifically its highly amyloidogenic 42 amino acid variant (Aβ42). Most of the mutations associated with FAD have been identified in the two presenilin (PS) genes, particularly the PS1 gene. Like the mutations identified within the βAPP gene, mutations in PS1 and PS2 cause the increased generation of Aβ42. PS1 has been shown to be functionally involved in Notch signaling, a key process in cellular differentation, and in βAPP processing. A gene knock out of PS1 in mice leads to an embryonic lethal phenotype similar to that of mice lacking Notch. In addition, absence of PS1 results in reduced γ-secretase cleavage and leads to an accumulation of βAPP C-terminal fragments and decreased amounts of Aβ. Recent work may suggest that PS1 could be the γ-secretase itself, exhibiting the properties of a novel aspartyl protease. Mutagenesis of either of two highly conserved intramembraneous aspartate residues of PS1 leads to reduced Aβ production as observed in the PS1 knockout. A corresponding mutation in PS2 interfered with βAPP processing and Notch signaling suggesting a functional redundancy of both presenilins.

Key words Alzheimer's disease · Amyloid β-peptide · Notch · Presenilin · γ-secretase

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In this issue, some of the recent work on the molecular mechanisms involved in Alzheimer's disease (AD) as well as novel diagnostic approaches and risk factors for AD will be discussed. In the first article, we like to give an overview on mechanisms involved in the proteolytic generation of Amyloid β-peptide (Aβ), the major pathological player of this devastating disease. In the second part of this article recent results will be described, which demonstrate an unexpected biological and pathological function of an AD associated gene.

Proteolytic processing of the β-Amyloid precursor protein by secretases

Alzheimer's disease is by far the most common cause of dementia. This neurodegenerative disorder is characterized by two major pathological hallmarks: accumulation in the brain parenchyma of senile plaques and aggregates called tangles, which originate from neuronal cell bodies.

Aβ is the major constituent of amyloid plaques and is generated through proteolytic processing of the β-amyloid precursor protein (βAPP; Haass and Selkoe 1993) (see Fig. 1). The combined activity of β- and γ-secretase is required to generate Aβ (Fig. 1). Once generated, Aβ is immediately secreted into the media of cultured cells or biological fluids such as plasma and cerebrospinal fluid (Haass and Selkoe 1993). The third secretase, the α-secretase, cleaves in the middle of the β-amyloid domain and thus prevents Aβ generation. Amyloid production and plaque formation are influenced by several genetic risk factors, including mutations in the genes encoding the two homologous presenilins, PS1 and PS2, and βAPP itself (Haass 1997; Selkoe 1998). Specifically the discovery of the PS genes turned out to be very helpful for our understanding of the molecular mechanisms behind Aβ production and AD pathology, since they appear to directly promote γ-secretase activity.

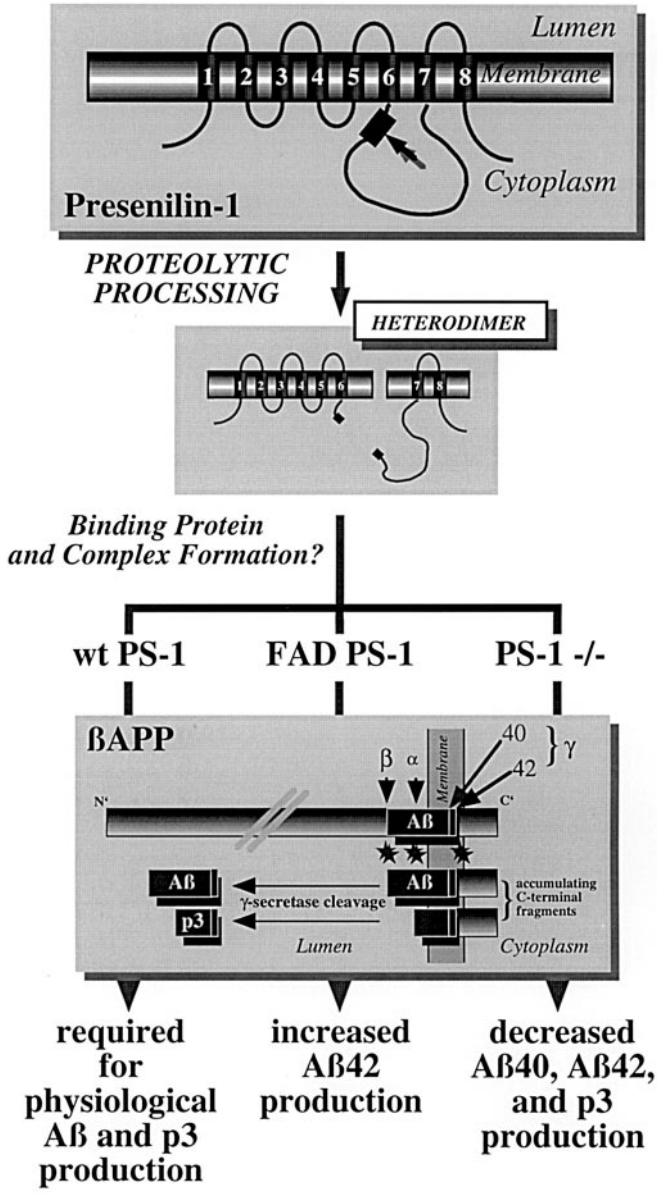

Fig. 1 Proteolytic processing of βAPP and presenilins. PS is cleaved within its cytoplasmic loop (Thinakaran et al. 1999) to generate the N-terminal fragment (NTF) and the C-terminal fragment (CTF). These fragments bind to each other and form the biologically active PS complex. This complex is required for physiological and pathological Aβ generation. The knock out of the PS1 gene prevents the γ-secretase cleavage and results in the accumulation of C-terminal fragments of βAPP. Alzheimer's disease associated mutations of βAPP are indicated by stars

Mutations in Alzheimer's disease associated genes cause the pathological production of Aβ

In about 10–15% of the cases, AD is caused by autosomal dominant mutations within three genes (Selkoe 1998). A very limited set of families have been identified as carrying mutations in the βAPP gene (Selkoe 1998). These mutations occur at, or close to the cleavage sites of the βAPP processing enzymes (Fig. 1), the secretases (Haass and Selkoe 1993; Selkoe 1998). βAPP mutations located close to the β- and γ-secretase sites cause an enhanced production of Aβ, specifically the highly pathogenic 42 amino acid variant, Aβ42 (Fig. 1; Selkoe 1998). The longer form of Aβ plays a central role in AD. Aβ42 is a major component of amyloid plaques (Selkoe 1998), which are the pathological hallmark of the disease (Selkoe 1998). Furthermore, Aβ42 exhibits an enhanced neurotoxicity, which might be due to its increased ability to form insoluble fibers (Jarret and Lansburry 1993). Therefore, these findings suggest a central role of Aβ for the pathogenesis of AD. This was strongly supported by the recent findings that numerous mutations in the PS genes cause exactly the same type of aberrant Aβ42 production (Haass 1997; Selkoe 1998; Price and Sisodia 1998). Therefore, mutations in three different genes appear to affect very similar pathological mechanisms, which at the end accelerate Aβ aggregation. Accelerated aggregation of Aβ results in early amyloid plaque formation and enhanced neurotoxicity.

Presenilin 1 promotes γ-secretase activity

A very provocative hypothesis was recently published by Selkoe and his colleagues (Wolfe et al. 1999). The work is

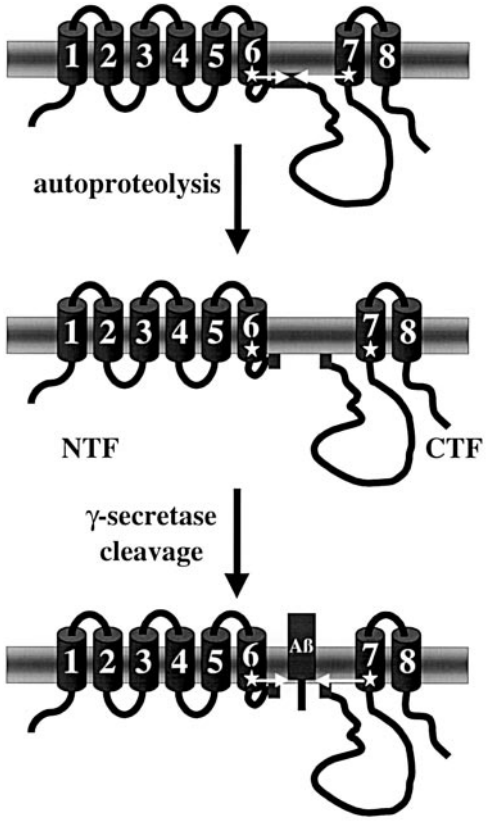

Fig. 2 A model demonstrating the hypothesis that PS1 exhibits a γ-secretase activity. Two critical asparate residues, which are required in the catalytic center of aspartyl proteases, are indicated by stars within transmembrane domains 6 and 7. PS1 is produced as a holo-protein, which is activated by autoproteolysis. The resulting PS1 complex then acts as the γ-secretase

based on the functional requirement of two unusual intramembraneous aspartate residues (see Fig. 2), which are highly conserved in all known presenilin genes (see below). Selkoe and colleagues mutagenized these aspartate residues in PS1 and analyzed the consequences for PS1 endoproteolysis and Aβ generation. Strikingly, all the mutations completely blocked endoproteolysis of PS1. This was accompanied by a pronounced decrease in both major Aβ species, Aβ40 and Aβ42. Moreover, C-terminal fragments of βAPP produced either by α- or β-secretase accumulated to high levels. This indicates a major defect in the γ-secretase activity that would normally process these fragments to Aβ and p3. Therefore, the aspartate mutants have a dominant negative effect on γ-secretase activity. These findings are strikingly similar to the effects of a PS1 knockout on Aβ production (De Strooper et al. 1998). Neurons derived from mice lacking the PS1 gene produced substantially reduced levels of Aβ and also accumulated C-terminal fragments of βAPP (De Strooper et al. 1998; Fig. 1). Based on their findings, Selkoe and colleagues proposed a very exciting model. In this model, PS1 is an unusual aspartyl protease and is in fact the long-sought γ-secretase (Fig. 2). Aspartyl proteases require two aspartate residues within their enzymatically active domain. Mutagenizing either one of them results in a complete loss of proteolytic activity. Therefore, Selkoe and coworkers proposed a simple model in which presenilins autocatalytically activate themselves, and then cleave βAPP within the membrane to generate Aβ and p3 (Fig. 2). This model would be supported by the findings that the two PS fragments are bound to each other in vivo (Capell et al. 1998; Yu et al. 1998; Thinakaran et al. 1998). Moreover, fragment formation is tightly regulated and both fragments are required for PS1 activity (Baumeister et al. 1997; Saura et al. 1999, Steiner et al. 1998). Interestingly, the recombinant NTF by itself is not sufficient to promote PS function (Baumeister et al. 1997; Steiner et al. 1998; Saura et al. 1999). Based on Selkoe's hypothesis this is due to the lack of one of the critical aspartate residues.

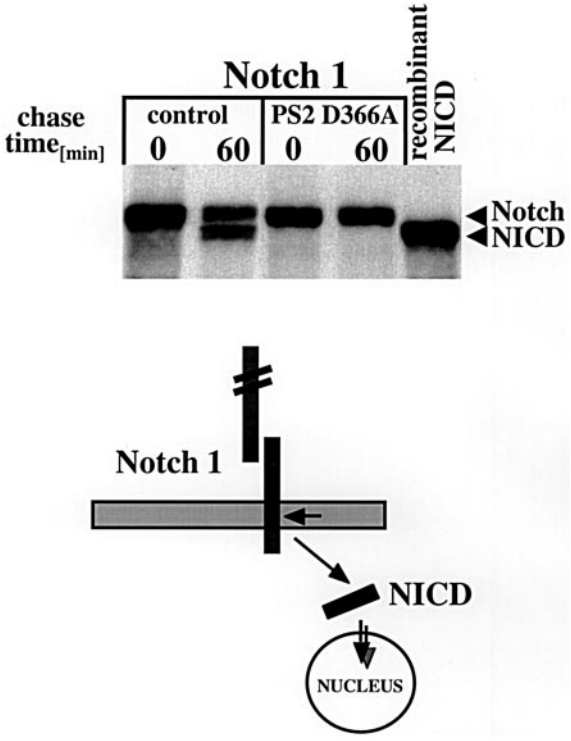

Fig. 3 A loss of function mutation of PS2 (Asp366A1a) affects NICD formation. Cells expressing PS2 wt or PS2 D366A were transfected with a Notch cDNA construct, pulse labeled with ^{35}S-methionine and chased for the indicated time points. Proteolytic formation of NICD occurs within 60 min in cells expressing wt PS2. In contrast, cells expressing the mutant PS2 variants lost their ability to proteolytically generate NICD (Steiner et al. 1999)

Presenilin 1 facilitates Notch signaling by promoting proteolytic processing of Notch 1

Mice lacking PS1 exhibit an embryonic lethal phenotype, which resembles at least partially that caused by the deletion of the Notch 1 gene (Wong et al. 1997; Shen et al. 1997). Moreover, work carried out in the nematode Caenorhabditis elegans demonstrated that PS proteins play a fundamental role in Notch signaling and cellular differentiation. A defective PS homologue of the worm was found to cause major problems in signal transduction pathways mediated by Notch (Levitan and Greewald 1995). This defect could be completely rescued by transgenic expression of human PS genes in the mutant worm (Levitan et al. 1996; Baumeister et al. 1997). A direct involvement of PS1 in Notch signaling has now been demonstrated by the finding that cells lacking PS1 show reduced levels of the proteolytically generated cytoplasmic domain of Notch 1 (NICD; De Strooper et al. 1999) as well as by genetic evidence derived from multiple model systems including Caenorhabditis elegans, Drosophila melanogaster, and mice (De Strooper et al. 1999; Struhl and Greenwald 1999; Ye et al. 1999). Proteolytic generation of NICD is known to be critically required for Notch signaling (Schroeter et al. 1998; Struhl and Adachi 1998). Notch is a type I integral membrane protein, which is proteolytically processed in its extracellular domain by furin and/or the metalloproteinase kuzbanian (Fig. 3). Notch signaling is initiated by binding to a member of the DSL (Delta, Serrate, Lag-2) ligand family at the cell surface. Upon binding, the cytoplasmic domain of Notch is cleaved and translocates to the nucleus (Schroeter et al. 1998; Struhl and Adachi 1998). NICD by itself has no DNA binding properties, but acts as a co-activator by binding to a member of the CSL (CBF1, Su (H), Lag-1) transcription factor family. Strikingly, NICD generation is blocked in neurons derived from mice lacking the PS1 gene (De Strooper et al. 1999). Since the proteolytic cleavage of Notch appears to occur at or within the transmembrane domain the similarities to the γ-secretase cleavage of βAPP are obvious. This again supports the idea that PS1 is either identical with the γ-secretase or activates the activity of this protease. The lack of γ-secretase activity then results in reduced Aβ production as well as problems in Notch signaling due to reduced proteolytic

cleavage of the corresponding precursor proteins. The defect in Notch signaling in cells expressing dominant negative mutations of PS1 as well as in mice lacking the PS1 gene is therefore explained by the dramatically reduced production of NICD.

What about the function of presenilin 2?

While our understanding of PS1 function is now quite detailed (see above), very little is known about the physiological and pathological role of PS2. FAD associated mutations of the PS2 gene enhance Aβ42 generation like PS1 mutations (see above); therefore a similar amyloidogenic function would be expected. However, in contrast to the PS1 knock out an ablation of the PS2 gene does not result in a Notch-like phenotype and does not appear to affect βAPP processing (Steiner et al. 1999; Herreman et al. 1999). Does that mean that PS2 plays no role in Notch signaling and Aβ production? If that would be true, how would we explain the effect of the PS2 mutations on aberrant Aβ42 generation?

In order to identify the function of PS2, we have therefore recently mutagenized one of the conserved aspartate residues of PS2. The goal was to generate loss of function mutations and to express these mutations in human cells co-expressing βAPP. Similar to the corresponding PS1 mutations (Wolfe et al. 1999), mutagenesis of the asparate located in transmembrane domain 7 of PS2 blocked its endoproteolysis and resulted in the accumulation of large amounts of the uncleaved holoprotein (Steiner et al. 1999). Moreover, the mutant PS2 variant severely interfered with Aβ generation (Aβ40 and Aβ42), thus, indicating that PS2 has a similar function in βAPP processing like PS1. The lack of an obvious phenotype of the PS2 ablation in mice might be explained by the finding that the PS1 gene is expressed about 5–10 fold higher in the brain (Lee et al. 1997). We also looked for a potential function of PS2 in Notch signaling. Since the knock out did not produce a Notch phenotype, we expressed the wt PS2 gene as well as the above described aspartate mutation in C. elegans with a defective PS homologue to analyze the functional properties of PS2 directly. Surprisingly, we found that PS2 fully restored Notch signaling, whereas transgenic expression of the asparate mutation did not allow survival of the worms (Steiner et al. 1999). Therefore, the aspartate mutations of PS2 directly interfered with Notch signaling under in vivo conditions, which strongly indicates a critical function of PS2 in Notch signaling. Finally, we also searched for the effect of that mutation on NICD generation in human cells. Similar to the PS1 deletion, the PS2 aspartate mutation prevented NICD production. These data strongly indicate that the functions of PS2 and PS1 are at least partially redundant. This is supported by the recent finding of Herreman et al. 1999 that a double-knock out of PS1 and PS2 severely enhances the fatal phenotype of the single PS1 deletion and almost fully reproduces the phenotype produced by the ablation of the Notch 1 gene. Moreover, the critical function of the aspartate residues is conserved within both PS genes. The functional importance of these residues is not only conserved within the human PS genes but also in lower vertebrates. We recently cloned the PS1 homologue of zebrafish (Danio rerio) and found subsequently that the critical aspartate residues in transmembrane domains 6 and 7 were again functionally conserved. Mutagenesis of a critical aspartate severely inhibited Aβ production even in a heterologous system such as human cells (Leimer et al. 1999).

Taken together, the new findings on the functions of PS1 and PS2 demonstrate the critical requirement of two intramembraneous aspartate residues for the proteolytic generation of Aβ and NICD. If indeed both PS proteins exhibit a γ-secretase activity or indirectly activate γ-secretase remains to be demonstrated.

Conclusions

The work of many groups all over the world has now clearly established a pivotal role of Aβ42 for AD pathology. We are now starting to understand the detailed molecular mechanisms of Aβ generation specifically due to the intensive work on the biological and pathological function of presenilins. The two homologous presenilins now turned out to be novel and highly unexpected targets for amyloid lowering drugs. However, one should not be too overexcited about the consequences of the new data for therapeutic treatment of AD, since inhibition of presenilin function interferes not only with Aβ production, but also with their essential activity in Notch signaling. However, very minor amounts of NICD are sufficient to allow successful Notch mediated signal transduction, therefore partial inhibition of PS1/PS2 function may indeed lead to a hopeful approach to lower Aβ generation and the subsequent inhibition of amyloid plaque formation.

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