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Importance of the Caspase Cleavage Site in Amyloid- β Protein Precursor

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Abstract

Reports from multiple laboratories have now been published analyzing the critical nature of the caspase cleavage site of amyloid- β protein precursor (A β PP) for cell death induction, synaptic loss, hippocampal atrophy, long-term potentiation, memory loss, neophobia, and other aspects of the Alzheimer's phenotype. Here we review the results and implications of these studies for the understanding of Alzheimer's disease pathophysiology and the potential development of therapeutics that target this site in A β PP.

Keywords

Alzheimer's disease; amyloid- β peptide caspases; transgenic mouse

INTRODUCTION

Cells depend on critical trophic support for survival, and the withdrawal of such support may lead to cell death, process retraction, cellular atrophy, or other cellular reactions, depending on the developmental status of the cell, as well as other factors. The cellular response to trophic withdrawal is mediated by specific receptors, called dependence receptors and first described in 1993 [1,2]. These receptors mediate the activation of cysteine aspartyl-specific proteases (caspases) following trophic factor withdrawal, and this effect requires caspase cleavage of the receptors themselves [3] as part of an amplification process. Amyloid- β protein precursor (A β PP), as well as related family members APLP1 and APLP2, exhibit characteristics of dependence receptors, including a critical caspase site [4–6], mediation of cell death [5,7], and binding to a trophic factor [8].

The cleavage of neurodegeneration-associated proteins by caspases was first described by Goldberg et al. [9] for huntingtin (Htt). Although only a small minority of cellular proteins serves as caspase substrates [10], many of the neurodegeneration-associated proteins have been found to be caspase substrates, such as A β PP, TDP-43, presenilins, tau, ataxin-3, atrophin-1, and others [4–6,11–15]. The finding that such proteins appear to be over-

represented as caspase substrates suggests that neurodegeneration-associated proteins may play critical roles in cell suicide programs, such as those mediating neuronal development.

Specific caspase cleavage products were shown to be cytotoxic, such as A β PP-C31 [5]. These studies raised the question of whether the caspase cleavage sites in these neurodegeneration-associated gene products play a key role in the neurodegenerative process. Graham et al. [16] tested this possibility in the YAC (yeast artificial chromosome) model of Huntington's disease (HD). Mutation of the caspase-6 site (Asp586) did indeed result in an almost complete suppression of the Huntington's phenotype, whereas mutation of a caspase-3 site did not. Further studies by Pouladi et al. [17] showed that the Asp586 mutation prevented the depressive phenotype associated with HD, and those of Milnerwood et al. [18] showed that this mutation prevented the extrasynaptic NMDA receptor activation associated with HD. Similarly, mutation of the caspase site in A β PP, Asp664, in an Alzheimer model transgenic mouse prevented the Alzheimer's disease (AD) phenotype, including synaptic loss, dentate atrophy, electrophysiological abnormalities, behavioral deficits, convulsive predisposition, and stem cell response [19–23].

Thus the findings from neurodegeneration-associated proteins such as Htt and A β PP suggest a fundamental relationship between the neurodegenerative process and the developmental process – more specifically, dependence receptor-mediated developmental neurite retraction and cell death following trophic factor withdrawal [24]. This relationship has been strengthened further by the demonstration that trophic factor withdrawal in multiple primary neuronal culture systems leads to cleavage of A β PP to produce N-A β PP, which in turn binds to death receptor 6 (DR6) and induces caspase-6 activation [25]. The specificity for caspase-6 is especially noteworthy in light of the requirement for caspase-6 cleavage of Htt in pathogenesis [26] and the finding that the caspase cleavage site of A β PP – VEVD'A – is susceptible to cleavage by caspase-6 [5, 27,28], that caspase-6 is activated in aging and mild cognitive impairment [29], and that activated caspase-6 and caspase-6-cleaved tau are present in neuropil threads, neuritic plaques, and neurofibrillary tangles of AD patients [30,31].

CASPASE CLEAVAGE OF A β PP

The caspase cleavage of A β PP was first demonstrated by Gervais et al. [4], and subsequently it was shown that one of the resulting products – A β PP-C31 – induces apoptosis [5]. Following those reports, the peptide product of the cleavage of A β PP by the γ -secretase complex and caspase, Jcasp, was also shown to be pro-apoptotic [32]. Finally, N-A β PP, the aminoterminal peptide derived from sA β PP, was shown to bind to death receptor 6 (DR6) and induce caspase-6 activation [25]. Thus all four of the peptides derived from A β PP by BACE cleavage, γ -secretase cleavage, and caspase cleavage – i.e., sA β PP, A β , Jcasp, and C31 – induce caspase activation and programmed cell death [33]. Interestingly, the pro-apoptotic effects attributed to both C100 (/C99) and AICD (A β PP intracytoplasmic domain) also require the caspase cleavage site, suggesting that C31 and Jcasp may be the mediators of cytotoxicity in these cases [34–36].

The caspase site in A β PP is at Asp664 (A β PP₆₉₅) in the intracytoplasmic domain, aminoterminal to the YENPTY domain that interacts with multiple phosphotyrosine-binding proteins. As noted above, the P4-P1' residues are VEVD'A, suggesting that this is a caspase-6 site [37]; however, other caspases such as caspase-3 and caspase-8 have also been shown to be capable of cleaving A β PP [5,34]. This site is highly conserved, both in orthologs and in family members APLP1 and APLP2, both of which have been shown to be cleavable by caspases, as well [6].

DOES CASPASE CLEAVAGE OF A β PP OCCUR IN ALZHEIMER'S DISEASE?

The activation of caspases in AD was initially shown indirectly through the resulting cleavage of actin [38] at its previously described caspase cleavage site [39]. Subsequently the activation of caspase-3 and caspase-9 were shown to occur in AD [5,40]. A β PP was shown to be a caspase substrate, both *in vitro* and *in vivo*, and both in AD patients and transgenic mouse models of AD [4, 5,19,27,41]. Furthermore, the activation of caspase-6 was shown to precede the clinical and pathological diagnosis of AD [29].

Comparison of A β PP caspase cleavage in AD brains versus age-matched non-AD control brains showed a four-fold increase in caspase cleavage of A β PP in the AD brains, occurring predominantly in the hippocampus and cortex [41]. Interestingly, however, control brains showed an inverse relationship between age and degree of caspase-cleaved A β PP, such that the younger control brains showed the greatest degree of caspase-cleaved A β PP. While this level of A β PP cleavage exceeded the AD-associated caspase cleavage in older patients, the pattern was clearly distinct, since the non-AD controls displayed cleavage along fiber tracts, whereas the AD-associated cleavage was observed in cell bodies.

IS CASPASE CLEAVAGE OF A β PP A MEDIATOR OF ALZHEIMER'S DISEASE PATHOGENESIS?

As noted above, multiple studies have indeed demonstrated that the caspase cleavage of A β PP is a critical mediator of the pathogenesis of AD in transgenic mouse models and in cell culture models. Lu et al. [5] showed that the caspase cleavage of A β PP is critical for its induction of programmed cell death in cultured neuroblastoma cells. McPhie et al. [34], showed the pro-apoptotic effect of A β PP-C31 in primary cultures of neurons. Furthermore, the toxicity of C100 and AICD were also shown to require the caspase cleavage site [5,36]. The finding that A β itself was capable of inducing the caspase cleavage of A β PP, and that this cleavage mediated part but not all of the A β toxicity [35], is compatible with the notion that A β is upstream of the caspase cleavage in AD brain (although it does not exclude the possibility that it may also be downstream as part of an amplification loop). This possibility was supported by the subsequent finding that systemic caspase inhibition by Q-VD-OPh (quinolyl-valyl-O-methylaspartyl-[-2, 6-difluorophenoxy]-methyl ketone) reduced caspase-related pathology but did not reduce A β [42]. Park et al. found that the A β PP-C31 toxicity is associated with its interaction with A β PP itself [36].

The *in vivo* effects of preventing this A β PP caspase cleavage were evaluated by several groups (summarized in Table 1): Galvan et al. [19] assessed hippocampal synaptic density (as measured by synaptophysin staining), dentate gyrus volume, Morris water maze performance, and neophobia. Four different lines of transgenic mice were compared, all on a C57Bl background: two with human A β PP with Swedish and Indiana mutations – a lower expressor, J9, and a higher expressor, J20 – and two with Swedish and Indiana mutations and an additional caspase cleavage site mutation, D664A – B21, which expresses A β PP at a higher level than J9 but lower than J20, and B254, which expresses A β PP at a higher level than J20 and J9. Thus the B21 experimental line was bracketed by the two positive controls, J9 and J20, and the J20 positive control was bracketed by the two experimental lines, B21 and B254. Although in all four lines the concentrations of A β peptides 1-40 and 1-42 were found to be commensurate with the A β PP expression, and therefore unlikely to have been affected by the D664A mutation, the phenotypes of the B21 and B254 were otherwise not significantly different than the non-transgenic controls, indicating that the D664A mutation played a critical role in preventing the AD phenotype. Whether this effect was due to the lack of generation of the A β PP-C31 and Jcasp peptides from A β PP, or whether it was due to other effects such as structural effects on the intracytoplasmic domain of A β PP, or to some

combination of these various possible effects, could not be determined from the study. However, whatever the underlying mechanism(s), the effect on the AD phenotype in this transgenic model was marked. These studies were followed up by late behavioral studies carried out at 13 months of age, in order to determine whether the mutation simply delayed but did not ultimately prevent the behavioral abnormalities of the AD mouse model [21]. These latter studies showed that, although there was still no significant difference between the D664A mice and the non-transgenic control littermates, there was a trend toward poorer performance, suggesting that the D664A mutation delayed but may not have completely prevented the AD phenotype. Furthermore, qualitative differences in rescue were subsequently reported: the reversal of learning deficits in the D664A mice was found to require early experience, suggesting that the caspase site mutant rescued retention more than learning itself [23].

Saganich et al. [20] evaluated long-term potentiation (LTP) as well as field excitatory post-synaptic potentials (fEPSPs), following Schaffer collateral stimulation and recording at CA1. Saganich et al. independently confirmed the rank order of A β PP expression and A β production as B254 > J20 > B21 > J9, and noted that the A β ₁₋₄₂ concentration was markedly higher (approximately five-fold higher) in B254 than in all other groups. Despite this, the fEPSPs from B254 were highly significantly improved over those of the J20 ($p < 0.001$), and those from B21 were indistinguishable from those of the non-transgenics. Saganich et al. also showed that the D664A mutation prevented LTP deficits and Morris water maze abnormalities.

Nguyen et al. [43] evaluated the effects of the D664A mutation on cell signaling in PDA/ β PP mice. Zhao et al. [44] had shown previously that p21-activated kinase (PAK) signaling is reduced in AD and in the late stages of transgenic mouse model AD. This was of special interest since PAK had been shown to interact with the cytoplasmic domain of A β PP [45], and since PAK is a key contributor to dendritic spine morphology [44]. Nguyen et al. quantitated hippocampal phospho-PAK at three months of age and at 13 months of age in PD A β PP mice (J20) and in PD A β PP-D664A mice (B21 and B254). At three months of age, a marked increase in hippocampal phospho-PAK of three-four fold was observed in the J20 mice, and this effect was completely prevented by the D664A mutation. At 13 months of age, and in keeping with the previous observations of Zhao et al., a reduction in phospho-PAK was observed in the J20 mice. Once again, the D664A mutation completely prevented this effect.

Vogt et al. [22] evaluated the electroencephalography and seizure propensity of the B254 mice vs. the R1.40 model of AD. The D664A mutation normalized the EEG (although some modest slowing remained, the spiking abnormality was completely prevented) and the seizure propensity of the mice, and reduced the mortality following kainic acid administration from 90% (in the R1.40) to zero, the same value observed for the non-transgenic controls.

In contrast to the marked *in vivo* effects of the D664A mutation reported by the groups noted above, a single report described only modest effects [46]. However, in this report, the B254 line, with six times as many A β plaques as the J20 line, a two-fold higher concentration of total A β , and a 2.5-fold increase in A β ₁₋₄₂, was compared to the J20 line. Thus there was no appropriate control for comparison to the B254, and unlike in the studies by Galvan et al. [19] and Saganich et al. [20], multiple lines bracketing the experimental and control groups were not included in the study. Therefore, it was not clear whether the modest improvement of the B254 over the J20 in these studies (much more modest than reported by the other groups) was due to a lack of effect of the D664A mutation or, alternatively, to a marked effect of the D664A mutation in preventing the B254, with its much higher concentration of

$A\beta$ than the J20, from being more severely affected than the J20. Given that the J20 is much more severely affected than the J9, it would be expected that the B254 would indeed be more severely affected than the J20, so the latter interpretation may be more likely to be the correct one. Specifically, the J9 PDA β PP mice display $A\beta$ levels approximately half those of the J20 [47] and their phenotype is much less severe than that of the J20; the J20 mice, in turn, display $A\beta$ levels approximately half those of the B254, so the assumption that the phenotypes of the J20 and the B254 should be similarly severe is invalid.

Thus with this single exception, studies to date have all concluded that the D664A mutation does have a major impact on the AD phenotype in both transgenic mouse models and cell culture models. However, as noted above, the effect was not complete, and this is not surprising given that the mutation prevented the production of two of the four neurotoxic peptides derived from $A\beta$ PP – i.e., Jcasp and C31 – but not the other two – i.e., $A\beta$ and s $A\beta$ PP (from which N- $A\beta$ PP is derived).

THERAPEUTIC PROSPECTS

Many of the current therapeutic strategies now in clinical trials involve some aspect of modifying $A\beta$ production or clearance [48]. Despite these advances, recent clinical trials targeting $A\beta$ have been disappointing [49,50]. It is important, therefore, to identify new potential drug targets for the treatment of AD. The cleavage of $A\beta$ PP to produce C31 clearly represents such a target for AD therapy, and developing molecules that can inhibit this cleavage and affect neurotoxicity is important and may potentially lead to a new therapeutic approach for AD. Certainly such inhibitors may prove to be an important part of the cocktail that would ultimately be used for optimal therapy for AD. Furthermore, caspase cleavage of tau represents a related therapeutic target, especially in light of the recent demonstration that this event precedes neurofibrillary tangle formation [51]. Thus caspase-6 inhibition may prove to be valuable both for AD and HD. While the development and administration of systemic caspase inhibitors, even for specific caspases such as caspase-6, may be complicated by extraneural effects, the identification of substrate-specific inhibitors, targeting the $A\beta$ PP and tau sites, would theoretically provide needed specificity.

With the development of neo-epitope antibodies that detect the caspase cleavage of $A\beta$ PP [4,19], screening for small molecule inhibitors of this event in a high-throughput format is now possible. Support for this approach has been provided by Rohn et al. [42], who found that the caspase inhibitor Q-VD-OPh reduced the AD-associated pathology in a transgenic model of AD, without affecting the accumulation of $A\beta$. Of course the caspase inhibitor was not substrate-specific for $A\beta$ PP, and clearly affected other substrates, such as tau. However, given the emerging evidence that $A\beta$ PP may be cleaved to produce multiple peptides that mediate synaptic inhibition, neurite retraction, and ultimately programmed cell death, it may be that the development of a therapeutic cocktail that targets not just the $A\beta$ peptides but rather all of these $A\beta$ PP-derived peptides will be optimal.

CONCLUSION

The pathogenetic aspects of AD are still being defined. Although the vast majority of the studies to date have focused on the chemical and physical effects of the $A\beta$ peptide, such as metal binding, peptide radical formation, and detergent-like effects on membranes, multiple recent reports have provided complementary mechanisms implicating $A\beta$ PP-mediated signaling effects and depicting $A\beta$ peptide as a physiological neuromodulatory peptide. In this emerging model, $A\beta$ PP may be processed to produce four peptides that mediate neurite retraction and/or programmed cell death: s $A\beta$ PP (from which N- $A\beta$ PP is derived), $A\beta$, Jcasp, and C31. Two of these – Jcasp and C31 – require caspase cleavage of $A\beta$ PP at

Asp664, and reports from several different laboratories have now shown that this site is required for multiple characteristics of the AD phenotype in transgenic mice, including synapse loss, hippocampal atrophy, electrophysiological abnormalities (LTP, EPSPs, EEG, and seizure propensity), behavioral abnormalities (Morris water maze, Y-maze, and neophobia), and neural precursor abnormalities. Similar phenotypic effects have also been obtained by mutating the caspase-6 site in huntingtin, suggesting parallels in the pathogenesis of HD and AD. These studies point to the caspase cleavage of A β PP and Htt as targets for therapeutic development.

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Fig. 1.

Cleavage of A β PP at Asp664 occurs in the PDAPP model of Alzheimer's disease, both in the hippocampus and in the cortex, as demonstrated by a neo-epitope antibody directed against the eight residues (P8-P1) preceding the caspase cleavage site of human A β PP. Note that this cleavage is prevented in the PDAPP-D664A mice. The minimal signal in the NTg controls may be due to cross-reaction with cleaved endogenous mouse A β PP.

Table 1

Comparison of the effects of the D664A mutation on PDAPP transgenic mice modeling Alzheimer's disease

Study	Age(s) at evaluation	Tests	Improvement (relative to control NTg)	Comments
Galvan et al., 2006 [19]	3–12 mo.	Behavioral, anatomic, IHC	Significant	D664A were indistinguishable from NTg controls
Saganich et al., 2006 [20]	3–6 mo.	Behavioral, EP	85% (B254), 100% (B21)	
Galvan et al., 2008 [21]	13 mo.	Behavioral	Significant	Trend toward poorer water maze than NTg
Nguyen et al., 2008 [43]	3 mo., 13 mo.	IHC	100%	
Vogt et al., 2009 [22]	3–4 mo.	Behavioral, EEG	100% (for seizure propensity)	Seizures prevented, but modest EEG slowing present
Zhang et al., 2010 [23]	7 mo.	Behavioral	Significant	Pre-training required for effect
Harris et al., 2010 [46]	2–7 mo.	Behavioral, IHC, EP	N.S. *	

* Not significant. Trends toward improved retention in the Morris water maze were observed in the B254 mice as compared to the J20 mice, as well as delays in the development of abnormalities in the elevated plus maze and open field tests.

Abbreviations: IHC, immunohistochemical; EP, electrophysiological; EEG, electroencephalographic; NTg, non-transgenic; D664A, aspartate to alanine mutation at residue 664 (APP695 numbering) of amyloid- β protein precursor.