Therapeutic potential of amyloid β -degrading enzymes in Alzheimer's disease

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'Promoting activities of proteases ... could represent an effective way to induce A β clearance and reverse A β -dependent pathology in AD.'

Alzheimer's disease (AD), the most common dementia in the elderly, is a progressive neurodegenerative disease characterized by significant loss of cognitive functions [1]. Prevalence of the disease is predicted to drastically increase as the population ages if no effective therapeutic strategies are developed [2]. Currently four drugs, including acetylcholinesterase inhibitors and the *N*-methyl-D-aspartate antagonist memantine, are routinely used to treat AD. However, they provide only modest benefits to a subset of patients [3]. Clearly, there is an urgent need to identify new disease-modifying strategies to treat this devastating disease.

One promising therapeutic strategy – the anti-amyloid β (A β) approach – aims at decreasing the levels of A β peptides [4]. A β peptides, which are key components of amyloid plaques in AD brains, are derived from the processing of amyloid precursor protein (APP) by β - and γ -secretases [5,6]. Compelling evidence supports the amyloid cascade hypothesis, namely the accumulation of A β peptides, particularly as small aggregates [7–9], as being critical in causing the neuronal and behavioral deficits of AD [10].

A β levels result from a balance between production and clearance. Increased A β levels in AD could be due to A β overproduction, as in the case of early-onset familial AD cases that are linked with autosomal mutations in the *APP* or *presenilin* genes [11]. These familial mutations either enhance β -secretase cleavage on the *APP* gene or alter the activity of γ -secretase to increase the ratio of amyloidogenic A β 42 to A β 40, which is less prone to aggregation. However, in most late-onset sporadic AD cases there is little evidence of elevated A β production, suggesting that a failure in A β clearance might underlie the accumulation of A β in AD brains [12]. In the past decade, identifying and disrupting the mecha-

nisms of A β production has been the major focus of the anti-A β approach. More recently, significant progress has been made in deciphering the processes of A β clearance, which involve proteolytic degradation of A β , receptor-mediated cellular uptake and transfer of A β from brain tissue to the cerebrospinal fluid and plasma [13]. This editorial will focus on enzymes that degrade A β , especially the recently identified cathepsin B (CatB), and perspectives on promoting A β degradation as an anti-A β therapeutic strategy.

Proteases that degrade A β

Known A β -degrading enzymes include neprilysin (NEP), insulin-degrading enzyme (IDE), matrix metalloproteinases (MMPs), endothelin-converting enzyme (ECE), angiotensin-converting enzyme, the plasmin system and CatB [14–20]. Studies in knockout mice demonstrated that NEP, IDE, ECEs and MMP-2 or -9 are involved in degrading endogenous murine A β [16,21,22]. Their distinct cellular localizations suggest that they play complementary roles. The primary localization of NEP at the presynaptic terminals in neurons suggests that NEP is capable of degrading A β released from the nerve terminals [23]. IDE is mainly cytosolic and peroxisomal [24], but can also degrade A β extracellularly [14]. MMP-2 and -9, which are secreted in a latent form from astroglia, are localized with the amyloid plaques [25]. ECEs, on the other hand, may degrade A β in acidic compartments represented by the trans-Golgi network [26].

In AD-related animal models overexpressing IDE or NEP, the levels of human A β 1–42 and A β 1–x were markedly decreased [27], strongly supporting effective degradation of human A β peptides by IDE and NEP. Unlike murine A β peptides, which mostly remain monomeric, human A β peptides, especially A β 1–42, are prone to aggregation. In fact, the small human A β aggregates, not A β monomers, are believed to be critical in inducing AD-related neuronal and cognitive deficits [9,28]. However, A β aggregates are largely resistant to degradation. IDE and ECEs appear to target monomers exclusively, whereas NEP showed limited ability to degrade dimers *in vitro* [29]. Although lentiviral overexpression of

NEP in the brain of adult human (h)APP transgenic mice modestly decreased the pre-existing A β deposits [30], the ability of NEP to efficiently degrade protofibrillar and fibrillar A β remains unclear. Plasmin, which does not appear to be involved in degrading endogenous murine A β , can degrade A β aggregates *in vitro* and block A β toxicity in primary neuronal cultures [31]. The proteolytic efficiency with which plasmin degrades fibrillar A β , however, is approximately 1% of that for freshly dissolved (i.e., largely monomeric) A β [17]. A recent study demonstrated that MMP-2 and -9 degrade A β fibrils *in vitro* [19]. However, the ability of plasmin or MMPs to degrade A β aggregates in AD-related animal models remains to be established.

Recently, we identified CatB as an enzyme that efficiently degrades human A β aggregates *in vivo* [20]. CatB has been implicated in AD owing to its prominent association with neuritic plaques and enlarged endosomes in AD brains [32–36]. However, it had been postulated that CatB might contribute to A β generation through proteolysis of APP.

By directly testing this hypothesis, we discovered that CatB ablation had no effect on levels of full-length APP or C-terminal fragments of APP [20]. Surprisingly, CatB inhibition significantly increased plaque deposition and the relative abundance of A β 1–42 in hAPP mice. In primary neurons overexpressing hAPP, CatB inhibition increased, while CatB overexpression reduced, A β levels. These findings suggest that CatB reduces A β levels, possibly by proteolytic degradation. Indeed, under cell-free conditions, CatB acted as a dipeptidyl carboxypeptidase that sequentially converts A β 1–42 into A β 1–40 and A β 1–38, and an endopeptidase that generates A β 1–33. The generation of distinct C-terminally truncated A β fragments by CatB offers a unique catabolic mechanism to modulate the levels of A β , especially A β 1–42. We also found that CatB cleaves both fibrillar and nonfibrillar A β assemblies as efficiently as nonaggregates. Incubation of CatB with A β aggregates led to a decrease in A β oligomers and fibrils *in vitro*. In old hAPP mice with well-developed plaque pathology, lentiviral-mediated overexpression of CatB significantly reduced A β deposits [20]. Remarkably, CatB gene transfer also induced a marked reduction in neuritic plaques containing compact A β fibrils, whereas overexpression of NEP did not affect these neuritic plaques despite its strong effects on overall plaque load. These results suggest that CatB is more effective than NEP in

degrading aggregated A β within plaques. However, since many aspects of AD-related neuronal and functional deficits have been attributed to nonfibrillar, nondeposited A β , it would be important to determine if CatB also reduces levels of toxic A β oligomers *in vivo*.

Regulation of A β -degrading enzymes

Aging is the predominant risk factor for AD. Levels of both NEP and IDE appear to be regulated with the progression of aging in a region-specific manner [37,38]. During aging, NEP and IDE levels are selectively reduced in vulnerable regions of the brain, such as the hippocampus, where the A β appears to be readily deposited first, but not in the cerebellum and striatum, where no significant A β accumulation occurs [38,39]. The aging-induced reduction of NEP levels has been attributed, at least partially, to age-dependent depletion of somatostatin, which stimulates NEP expression [40]. In AD brains, NEP levels were significantly lower in regions with high levels of plaque, such as the hippocampus and temporal gyrus [41], further supporting the notion that the deficiency in NEP or IDE might contribute to region-specific A β accumulation in sporadic AD.

‘...dysregulation of A β -degrading enzymes in the aging brain may contribute to pathogenic A β accumulation in late-onset sporadic AD cases.’

In an interesting paradox, high levels of A β appear to stimulate the activities of other A β -degrading enzymes, including plasmin, MMPs and CatB. Levels of tissue plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA), which catalyze the transformation of inactive plasminogen into plasmin, are elevated by aggregated A β and in the brains of hAPP mice [17,42]. Astroglial expression of MMP-2 and -9 is enhanced surrounding amyloid plaques in aged AD animal models [25]. We found that CatB levels were markedly increased by A β 1–42 in both neuronal and microglial cells [20,43]. Moreover, CatB levels in the hippocampus were higher in hAPP mice at both 1–3 months and 7–8 months of age, suggesting that the upregulation of CatB by A β in young and middle-aged mice may represent a protective mechanism [20]. However, at 16–20 months, hAPP/A β failed to stimulate CatB, suggesting aging-dependent failure of this protective mechanism [20]. Similarly,

despite stimulation of tPA/uPA by A β , levels of plasminogen and plasmin in the neocortex and hippocampus of AD brains are actually low [44], supporting the idea that upregulation of the plasmin system by A β might have failed in the course of the disease. These findings reinforced the notion that dysregulation of A β -degrading enzymes in the aging brain may contribute to pathogenic A β accumulation in late-onset sporadic AD cases. Thus, promoting A β degradation mechanisms would be of therapeutic value.

Strategies to promote A β -degradation in the brain

Several strategies have been proposed to enhance the activities of the A β -degrading enzymes, a more difficult task than inhibiting protease activities. One strategy is to deliver recombinant proteases. However, this approach could be problematic since these proteases are involved in regulating levels of many peptides of important physiological functions. For example, IDE regulates the levels of insulin, which controls the glucose levels in the blood, whereas NEP regulates the levels of atrial natriuretic peptide, which regulates blood pressure. Thus, a localized and regulated delivery to the vulnerable regions of the brain would be needed. Viral vectors overexpressing NEP and CatB have been used in experimental animal models to reduce A β pathology in the aged mouse brain [20,23,30]. However, extensive preclinical and clinical studies will be required to test the safety of gene therapy in humans.

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In addition, a better understanding of the regulatory pathways of A β -degrading enzymes could lead to reagents that promote A β -degrading enzymes. For example, somatostatin significantly elevated neuronal NEP activity, probably by affecting protein turnover and cellular

localization [40]. Agonists for somatostatin receptors might be used to augment the A β -degrading activities of NEPs [40].

Another potential strategy would be to down-regulate the endogenous inhibitors of proteases. We hypothesize that the balance between A β -degrading proteases and their endogenous inhibitors may be altered in sporadic AD, leading to a deficiency in A β degradation and clearance. Thus, reducing the levels/activities of the inhibitors would tip the balance in favor of A β degradation. For example, CatB activity might be suppressed by its endogenous inhibitor cystatin (Cys)C [45], whose levels are higher in the cerebrospinal fluid and susceptible neurons of AD patients [46,47]. Interestingly, several genetic studies have linked a polymorphism in the *CysC* gene (CST3) with an increased risk of AD [48]. Ongoing studies are testing whether downregulation of CysC will promote A β degradation and reduce A β levels in AD-related animal models.

Conclusion & future perspectives

Significant progress has been made in identifying proteases that degrade A β in physiological and pathophysiological conditions. The specific localizations and proteolytic properties of these enzymes suggest that they may play complementary roles in regulating A β of different assembly states in distinct cellular compartments. A better understanding of the pathways regulating A β -degrading enzymes could lead to reagents that reduce A β levels through enhancing the expression and activation of A β -degrading enzymes. Promoting activities of proteases, especially those capable of degrading A β aggregates and oligomers, such as CatB and NEP, could represent an effective way to induce A β clearance and reverse A β -dependent pathology in AD. However, great caution needs to be taken in evaluating the potential side effects for such strategies owing to the important regulatory peptides that are also substrates of these proteases. It is likely that a combination of approaches that leads to both decreased production and increased clearance would be the most effective anti-A β therapy.

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