Mechanisms of Amyloid-β Peptide Clearance: Potential Therapeutic Targets for Alzheimer's Disease

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Abstract

Amyloid-β peptide (Aβ) is still best known as a molecule to cause Alzheimer's disease (AD) through accumulation and deposition within the frontal cortex and hippocampus in the brain. Thus, strategies on developing AD drugs have been focused on the reduction of Aβ in the brain. Since accumulation of Aβ depends on the rate of its synthesis and clearance, the metabolic pathway of Aβ in the brain and the whole body should be carefully explored for AD research. Although the synthetic pathway of Aβ is equally important, we summarize primarily the clearance pathway in this paper because the former has been extensively reviewed in previous studies. The clearance of Aβ from the brain is accomplished by several mechanisms which include non-enzymatic and enzymatic pathways. Nonenzymatic pathway includes interstitial fluid drainage, uptake by microglial phagocytosis, and transport across the blood vessel walls into the circulation. Multiple Aβ-degrading enzymes (ADE) implicated in the clearance process have been identified, which include neprilysin, insulin-degrading enzyme, matrix metalloproteinase-9, glutamate carboxypeptidase II and others. A series of studies on Aβ clearance mechanism provide new insight into the pathogenesis of AD at the molecular level and suggest a new target for the development of novel therapeutics.

Key Words: Amyloid-β peptide, Amyloid-β peptide degrading enzyme, Alzheimer's disease, Clearance, Proteases

INTRODUCTION

Alzheimer's disease (AD) is a devasting neurodegenerative disease and the most common form of dementia. The prevalence of AD is approximately 5.7% in people over 65 years in South Korea (Kim *et al.*, 2011a). Data from the US shows a rather higher prevalence of 10%. Many countries, including South Korea, are experiencing a fast rate of aging in their population and the prevalence of AD has been continuously increasing along with the rise in life expectancy. Unfortunately, aging is the top risk factor for AD and the total number of patient is expected to double every 20 years in South Korea. Thus, a better understanding of causes of this disease has been urgent and the development of disease-modifying therapy is the biggest issue in the 21st century.

AD is characterized by the accumulation and deposition of amyloid-β peptide peptides (Aβ) within the brain, leading to neuronal cell loss and perturbation of synaptic function (Tanzi and Bertram, 2005). Studies from the last decade revealed that disturbance in Aβ metabolism in the brain is thought to be central to the pathogenesis of the disease. The role of amyloid precursor protein (APP) processing and resulting Aβ production in disease development was established from the

genetic analysis of familial forms of AD. However, the blocking of Aβ synthesis does not appear to be effective for reducing the brain Aβ levels as we expected. Recently, the importance of Aβ clearance in AD pathogenesis, especially in late-onset sporadic AD (LOAD) has been raised, and the understanding of Aβ clearance mechanism have provided new therapeutic targets.

REVISIT TO THE AMYLOID HYPOTHESIS

The Aβ cascade hypothesis of AD was originally proposed (Selkoe, 1991; Hardy and Higgins, 1992) by the theory that accumulation of Aβ, in particular $A\beta_{1-42}$, is the initial trigger for neurodegeneration. However, the chemical nature and the precise biological roles of Aβ in AD pathogenesis have been elusive (Castellani and Smith, 2011). Furthermore, the failure of developing clinically effective disease-modifying drugs has underestimated Aβ-based therapeutic approaches but the genetic studies still strongly place Aβ as a favorable target. Early-onset type of AD (EOAD) occurs as a result of gene mutation involving APP, and presenilin genes (PSEN1, PSEN2). Mutation of these genes showed a common phenomenon of

an increase of $A\beta_{1-42}$ or of the ratio of $A\beta_{1-42}$ to $A\beta_{1-40}$; $A\beta_{1-42}$ is more hydrophobic and more prone to aggregate than $A\beta_{1-40}$ (Jarrett *et al.*, 1993). Furthermore, we also found in Asian population including Korean that beta-site APP cleaving enzyme (BACE)-1 polymorphism in exon 5 influences a risk for LOAD in those carrying the ApoE ε4 allele (Jo *et al.*, 2008) although Caucasian may not be the case. These observations strongly suggest a cause-and-effect relationship between $A\beta$ accumulation and AD pathogenesis.

Previously, it has been generally accepted that amyloid plaques, fibrils, or much complicated forms synthesized from $A\beta$ are the major pathological species causing impaired synaptic and neuronal dysfunctions. However, $A\beta$ oligomers are highly toxic and cause synaptic dysfunction (Hardy and Selkos, 2002), while amyloid plaques or fibrils induce proliferation and activation of glial cells which secret cytotoxic factors and indirectly induce neuronal damage. It was recently proposed that certain receptors were necessary for binding with $A\beta$ oligomers to produce neurotoxicity. For example, $A\beta$ binds to prion proteins (Laurén *et al.*, 2009) or A7 nicotinic acetylcholine receptor (Wang *et al.*, 2000), which causes neuropathy. We found from *in silico* assay that several neurotransmitters including acetylcholine can bind $A\beta$ more favorably than their corresponding receptors (Hong *et al.*, unpublished data). This result suggests that the binding of neurotransmitters with $A\beta$ might play an important role in AD pathogenesis through the disturbance of the normal signaling of neurotransmitters.

Aβ CLEARANCE

$A\beta$ is generated from APP by sequential cleavages by BACE-1 and the γ-secretase complex (Fig. 1). Scientists have focused on this pathway and these enzymes for a long time in order to develop an AD drug with an idea that blocking the activity of these enzymes might reduce the generation of $A\beta$ and thus $A\beta$-mediated cellular toxicity. Recently, however, a new concept of $A\beta$ accumulation has been emerged; $A\beta$ clearance or degradation rather than its synthesis have been found to be more critical in accumulation of $A\beta$. Furthermore, another mechanism responsible for controlling the brain $A\beta$ levels shows the influx or re-entry into the brain mainly through the receptor for advanced glycation end products. Since the steady state levels of brain $A\beta$ represent a dynamic equilib-
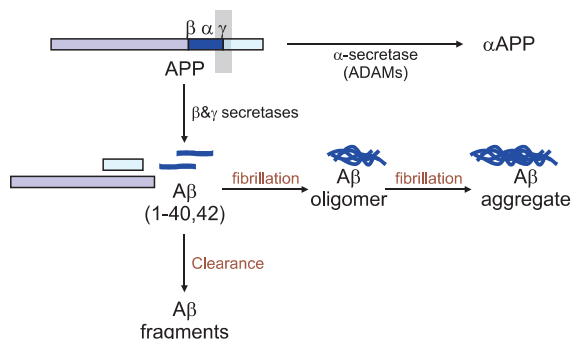
rium between synthesis, re-uptake and clearance, any factors that result in the reduced rate of $A\beta$ removal is likely to cause $A\beta$ accumulation. Thus, $A\beta$ clearance pathways including protease-mediated $A\beta$ degradation have been emerged as a new therapeutic target for AD treatment, which are mostly handled in this review.

$A\beta$ synthesis and clearance rates in ordinary adults are measured in the cerebrospinal fluid (CSF) and estimated to be 7.6% and 8.3%, respectively (Bateman *et al.*, 2006) Thus, $A\beta$ is unlikely to accumulate in the normal brain. However, small defects in $A\beta$ clearance could be sufficient to cause $A\beta$ accumulation leading to cell toxicity. Many data clearly suggest that in the central nervous system (CNS), decreased $A\beta$ clearance is more responsible for the development of AD rather than increased $A\beta$ synthesis (Weller *et al.*, 2000). In particular, defects in $A\beta$ clearance process is also likely to be relevant for the accumulation of $A\beta$ in the blood vessel walls in addition to within the brain, resulting in cerebral amyloid angiopathy (CAA) which is present in approximately 90% of AD patients (Love, 2004) and the most common cause of lobar intracerebral hemorrhage in the elderly (Viswanathan and Greenberg, 2011).

Clearance of $A\beta$ from the brain can be accomplished by several mechanisms including non-enzymatic and enzymatic pathways. The non-enzymatic pathway includes 1) the bulk flow of the interstitial fluid (ISF) into the CSF followed by ISF drainage pathway through perivascular basement membranes, 2) the uptake by microglial or astrocytic phagocytosis, and 3) the transport across the blood vessel walls into the blood vessel which is mediated by a series of clearance receptors such as low-density lipoprotein receptor-related protein 1 (LRP1), very low-density lipoprotein receptor (VLDLR) and P-glycoprotein localized predominantly on the abluminal side of the cerebral endothelium (Shibata *et al.*, 2000; Deane *et al.*, 2004). The enzymatic clearance involves several proteases, including neprilysin (NEP), insulin-degrading enzyme (IDE), matrix metalloproteinase (MMP)-9 and glutamate carboxypeptidase II (GCPII).

INTERSTITIAL FLUID DRAINAGE PATHWAY

In addition to accumulation within senile plaques, accumulation of $A\beta$ in AD brain is also found in the walls of capillaries and arteries as characterized in CAA. Several lines of evidence suggested that $A\beta$ deposit in the wall reflects a failure of the elimination of $A\beta$ along the perivascular ISF drainage pathways of the brain (Weller *et al.*, 2000). As shown in Fig. 2, increased production of $A\beta$ or blockage of $A\beta$ drainage through bulk flow of ISF followed by drainage pathways into the blood across the perivascular Virchow-Robin arterial spaces in the brain (Fig. 2). As an example, in NEP gene knock–out in a human APP (hAPP) mouse model, NEP reduction in cortical blood vessels contributes to the accumulation of $A\beta$ in the vessel walls enough to lead to the development of CAA (Farris *et al.*, 2007). Furthermore this response was observed in gene dosage-dependent manner.

Fig. 1. Anabolic and catabolic pathways of amyloid-β peptide (Aβ). Aβs are synthesized from amyloid precursor protein (APP) by β- and γ-secretase and then cleared by several mechanisms including enzyme-mediated degradation.

UPTAKE BY MICROGLIAL PHAGOCYTOSIS
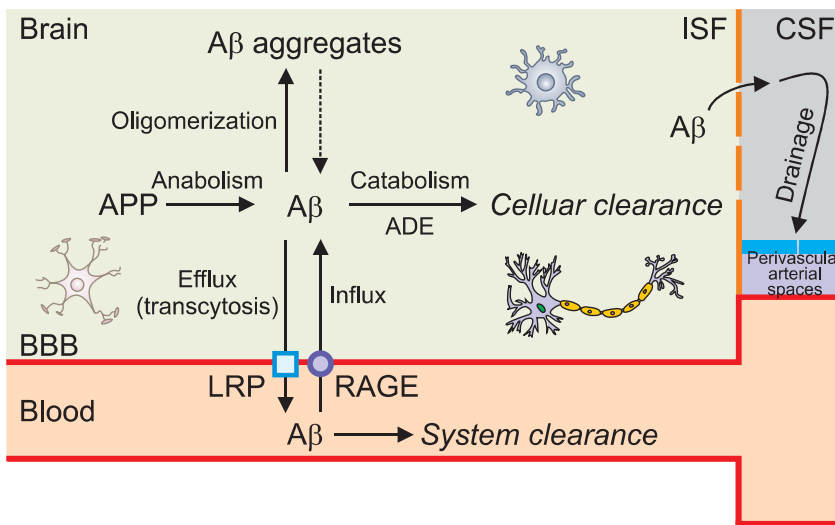
Microglia, brain's resident mononuclear phagocyte is found

Fig. 2. Schematic presentation showing the brain and blood vessel compartment and the ways of Aβ clearance pathways. The blood-brain barrier (BBB) is a separation of circulating blood from the brain interstitial fluid (ISF) in the central nervous system (CNS). It is composed of thin and flat endothelial cells in the capillaries. This barrier also includes a thick basement membrane, smooth muscle cells and astrocytic endfeet. This barrier restricts the diffusion of most materials into the cerebral ISF. See text for details. APP: Amyloid precursor protein, ADE: Amyloid-β degradation enzyme, LRP: Low-density lipoprotein receptor-related protein, RAGE: Receptor for advanced glycation end product, CSF: Cerebrospinal fluid.

within the core of amyloid plaques both in human brain and in rodent transgenic (Tg) AD models. Although the precise role of microglia in AD still remains unclear, microglia play an essential role in Aβ clearance through their ability to take up and degrade soluble and fibrillar forms of Aβ (Rogers *et al.*, 2002).

Microglia cells are activated by Aβ and secrete neurotoxic molecules. In contrast, they have neruroprotective actions by producing neurotrophic factors and by eliminating Aβ from the brain by phagocytosis. In early stage of AD, microglial activation delays disease progression by promoting clearance of Aβ by phargocytosis (Frautschy *et al.*, 1998; Wyss-Coray *et al.*, 2003; Wyss-Coray, 2006) before formation of senile plaques. In contrast, with aging microglia tends to be over-activated in response to stress such as amyloids and instigate an inflammatory reaction, which cause neuronal damage. In addition, the ability of microglia to uptake Aβ appears to be dependent on age. Exosome secretion from neurons was reported to enhance Aβ uptake into microglial cells and significantly decreased the extracellular levels of Aβ (Yuyama *et al.*, 2012). However, microglial cells prepared from neonates demonstrated phagocytic ability but this was lost by 6 months (Floden and Combs, 2011). Thus, it is critical to understand the state of microglia activation in different AD stages to determine the effect of potential anti-inflammatory therapies.

There is still debate regarding the maintenance of the microglial cell population in the CNS. There are two different types of microglia; which are the resident microglial cells and the newly differentiated cells derived from the bone marrow. Although they are both seen near Aβ plaques, bone-marrow derived microglia (BMDM) have been shown to delay or stop the progression of AD (Naert and Rivest, 2011) due to more efficient phagocytic properties compared to their resident counterparts (Simard *et al.*, 2006) and secretion of growth factors (example, glial cell line-derived neurotrophic factor). In this regard, it was demonstrated that transplantation of BMDM or their modified cells reduced Aβ accumulation by enhancing the expression of NEP in microglia to prevent synaptic dysfunctions and improve cognitive functions in AD mouse model (Kim *et al.*, 2012). All together, the recruitment of endogenous stem cells or transplantation of stem cells facilitates Aβ clearance and thus considered as a potential therapeutic strategy

for AD.

TRANSPORT ACROSS THE BLOOD VESSEL WALLS INTO THE CIRCULATION

For a long time, many investigators have paid attention to intrinsic neuronal components to understand the causes of neurodegenerative diseases including AD. However, many studies have shown that dysfunction in the blood brain barrier (BBB) rather than in neuronal components contributes to the accumulation of neurotoxic materials. Astrocyte, a component of BBB has been paid attention for its role in plaque maintenance and Aβ clearance. Cultured human astrocytes indeed bind to and internalize Aβ (Nielsen *et al.*, 2009). Animal experiments demonstrated that astrocytes internalize Aβ by the scavenger receptors, such as low density lipoprotein receptor-related protein 1(LRP1), scavenger receptor class B member 1 (SCARB1) and the macrophage receptor with collagenous structure (MARCO). In addition, albeit there was a controversy whether the prion protein is a receptor for amyloid (Hildebrandt *et al.*, 2009; Laurén *et al.*, 2009), it was found that cellular prion protein participates in Aβ transcytosis across the BBB (Pflanzner *et al.*, 2012).

Among Aβ scavenger receptors, LRP1 has been most extensively studied. LRP1 was originally known to play a role in the transport and metabolism of cholesterol. Later, LRP1 was characterized as a multifunctional scavenger receptor that binds to more than 40 structurally different ligands and has a function to transcytose ligands across BBB. It also serves as a transducing transmembrane cell signaling receptor. A series of evidence suggested that LRP1 expressed in astrocytes regulates brain Aβ levels through endocytic uptake of Aβ (Shibata *et al.*, 2000). LRP1 expressed in brain capillary endothelium (Deane *et al.*, 2004; Bell *et al.*, 2007) and the liver also plays a functional role in systemic Aβ clearance. Earlier studies revealed that LRP1 expressed in neurons were shown to have capability to uptake Aβ bound to alpha-2-macroglobulin or ApoE, ligands for LRP1 (Narita *et al.*, 1997; Bu *et al.*, 2006). In contrast, the recent surface plasma resonance study demonstrated the direct interaction between LRP1 and Aβ in the

abluminal side of the brain capillaries (Deane *et al.*, 2004; Bell *et al.*, 2007). Other lipoprotein receptors such as low-density lipoprotein receptor (LDLR), VLDLR and LRP2 are not likely to play a role in the transport of free Aβ across BBB (Deane *et al.*, 2009).

Interestingly, $Aβ_{1-40}$ is cleared rapidly across the BBB via LRP1 while $Aβ_{1-42}$ is removed across the BBB at a slower rate (~50%) than $Aβ_{1-40}$. Furthermore, mutant form of Aβ, $Aβ_{1-40}$ (Dutch), is also cleared less efficiently than $Aβ_{1-40}$ (Monro *et al.*, 2002). These findings suggest an isoform or mutant form-specific degradation mechanism. In addition to the enhanced toxicity by the mutant Aβ peptides, the mutations in APP resulting in production of aberrant Aβ may increase the accumulation of Aβ due to the slow LRP1-mediated degradation.

Sagare *et al.* have shown that a soluble form of LRP1 (sLRP1) binds to 70-90% of plasma Aβ, preventing its access to the brain (Sagare *et al.*, 2007). Thus, increased sLRP1 expression at the BBB and/or enhanced peripheral Aβ sink activity of sLRP1 has a significant potential to reduce brain Aβ accumulation (Deane *et al.*, 2009). In support of this result, deficient sLRP1-Aβ binding due to the increased level of oxidized sLRP1 which can not bind Aβ showed a failure to reduce AD progression (Sagare *et al.*, 2011). Similarly, dysfunction of LRP1 by antisense of LRP1 reduces BBB clearance, thereby increasing brain Aβ levels and impairing cognition (Jaeger *et al.*, 2009). Other studies demonstrate the role of LRP in AD pathogenesis; a semipurified extract of the root of Withania somnifera (withanolides and withanosides) improved AD pathology by enhancing LRP expression in the liver (Sehgal *et al.*, 2012).

DEGRADATION BY Aβ-DEGRADING ENZYME

Multiple Aβ-degrading enzyme family

The proteolytic machinery in the brain certainly contributes to the degradation of Aβ. For the last decade, multiple proteases of Aβ-degrading enzymes (ADE) implicated in this clearance process have been identified. Identification of these enzymes is rather perplexing because of their diversity. These enzymes belong to 1) zinc metalloendopeptidase [NEP-1 and NEP-2, endothelin-converting enzyme (ECE)-1 and -2, angiotensin-converting enzyme (ACE)], 2) thiol-dependent metalloendopeptdiase [insulin-degrading enzyme (IDE)], 3) serine proteases [plasmin, myelin basic protein (MBP), acylpeptide hydrolase], 4) cystein proteases [cathepsin B, D and S], 5) matrix metalloproteinase [MMP-9, MMP-2], and 6) others [GCPII, aminopeptidase A, mitochondrial peptidasome]. Most of them have endopeptidase activity cleaving the amino acid inside Aβ sequences while some (GCPII, MMP-9) contain carboxypeptidase activity cleaving amino acids from the carboxyl teminus. They cleave either at a single site or at multiple sites within Aβ. Specificity for cleaving sites within Aβ and for different Aβ aggregate forms has been well summarized in a recent paper (Nalivaeva *et al.*, 2012) and thus this review will not list the aspects in their cleavage functions.

The enzymes produce smaller-sized enzymatic products. *In vitro* studies showed that they cleave the full-length Aβ, producing fragments that are less neurotoxic and more easily cleared. However, it can't be certain whether the variety of Aβ products cleaved by ADE are beneficial to the cells or not; some products such as $Aβ_{25-35}$ and $Aβ_{22-35}$ have similar toxic-

ity and aggregation property as the full-length $Aβ_{1-40}$ or $Aβ_{1-42}$ monomer (Pike *et al.*, 1995). In fact, Aβs exist in systemic equilibrium of many heterogeneous Aβ forms, including soluble monomeric, oligomeric, protofibrillar, and fibrillar forms. Studies have suggested that toxicity of the Aβ fragments is probably attributable to the topology of the cleaved Aβ products. In this regard (Numata and Kaplan, 2010), demonstrated that a linking region between the two sheets in Aβ is the key determinant. Therefore, the toxicity of various Aβ forms and their cleaved products is dependent on what they are composed of and/or how they are assembled. Recently, Aβ oligomer structures have been reported by nuclear magnetic resonance spectroscopy (Ahmed *et al.*, 2010).

Validation of ADE function in cleaving Aβ

The biological function of the enzymes in clearance of Aβ was validated by many *in vivo* studies. Although gene deletion of a few ADE such as plasmin, urokinase plasminogen activator, or tissue plasminogen activator caused no alteration in the endogenous Aβ levels (Tucker *et al.*, 2004; Eckman *et al.*, 2006), knockout experiment in mice or rats of the specific ADE clearly demonstrated the increased steady-state levels of Aβ in the brain (Iwata *et al.*, 2001; Eckman *et al.*, 2003; Farris *et al.*, 2003; Miller *et al.*, 2003; Hafez *et al.*, 2011). For example, deletion of NEP gene in hAPP mice increased Aβ oligomers and impaired hippocampal synaptic plasticity and cognitive dysfunction before the appearance of amyloid plaque load (Huang *et al.*, 2006; Madani *et al.*, 2006). Delivery of NEP inhibitors into hippocampus also caused an accumulation of Aβ and impairment of learning and memory (Mouri *et al.*, 2006; Zou *et al.*, 2006). Mice lacking other ADE such as ECE-1, ECE-2 or IDE gene also showed a significant but modest increase in endogenous Aβ amounts, suggesting that they are physiologically involved in Aβ metabolism.

The role for ADE in Aβ degradation is also ascertained by overexpression studies. Overexpression of the ADE gene in the Tg AD model mice showed reduction of Aβ level in the brain and improved cognitive function. In this regard, hNEP gene-overexpressed AD model mice (APP Swed/Ind) showed a reduced cerebral Aβ level and plaque formation, and significantly improved life expectancy (Leissring *et al.*, 2003; Poirier *et al.*, 2006), although this result was not reproducible in another separate experiment using the same double Tg mice (Meilandt *et al.*, 2009). Nevertheless, other studies demonstrated the evidence of clinical benefit of intracerebral NEP increase. Viral delivery of hNEP into the hippocampus of hAPP Tg mice reduced both intracellular and extracellular Aβ levels and plaque pathology, oxidative stress, inflammation, and synaptic and dendritic damage as well as improved behavior and memory (Marr *et al.*, 2003; Iwata *et al.*, 2004; El-Amouri *et al.*, 2008; Spencer *et al.*, 2008). Therefore, agents which are able to selectively increase ADE levels and activities have the potential as a candidate for AD treatment.

Paradox of increased ADE expression or activity in AD

The rise of brain Aβ levels has been widely accepted as an important pathogenic factor for development of AD. Thus, it was assumed particularly in late-onset AD brain, that age-related decline of ADE activity would contribute to Aβ accumulation and this decline should be sharper in AD. Studies of ADE expression levels or activity with aging in human and mouse brains have been undertaken from various laboratories but no

conclusive results have been obtained. Earlier studies supported the reduction of mRNAs and proteins of NEP and IDE in AD brain (Akiyama et al., 2001; Russo et al., 2005; Miners et al., 2006). Other laboratories also reported the reduction of NEP protein in AD and with age (Hellström-Lindahl et al., 2008). However, in those studies, the immunohistochemical analysis of NEP with human brain sections and its quantification in brain tissue homogenates was poor and less specific to the particular enzyme, raising a question that this reduction may result from the secondary phenomena of neuronal death rather than a primary cause of the disease development. To this end, a highly sensitive fluorescence immunocapture method was developed using a specific enzyme inhibitors to measure the specific enzyme activity which can discriminate between closely related enzymes, for example NEP and IDE-1 (Miners et al., 2008a, 2008b). The results showed that NEP

and IDE activities rather increase, but not decrease, with normal aging (Miners et al., 2010a), this rise progressively with increasing disease severity (Miners et al., 2009). In addition, those of NEP were also found to be elevated in brains of Down syndrome patients (Miners et al., 2011); the levels increased with disease progression.

These controversies extend to other ADE as well. The ACE activity was increased in AD (Savaskan et al., 2001; Miners et al., 2008c, 2010b). The levels of ECE-1 (Wang et al., 2009a), ECE-2 (Palmer et al., 2009), MMP-2 (Yan et al., 2006), MMP-3, MMP-9 (Bruno et al., 2009) and ACE-2 were reported to increase in AD, but other studies showed no alterations in those of MMP-2, MMP-3, and MMP-9 (Baig et al., 2008). Similarly, ADE activity was also found to be increased in the cortex of aged Tg2576 mice (Deb et al., 1999; Tucker et al., 2000; Leal et al., 2006; Palmer et al., 2009). We also found that GCPII,

Table 1. The regulatory molecules that are known to modulate the ADE level and or activity

ADEs	Regulator	Effects	References
Neprilysin (NEP)	Somatostatin	Upregulation of NEP activity in primary cortical neurons	Saito et al., 2005
	Minocycline	Prevention of toxic effects of Aβ (25-35) by enhancing NEP expression in rat temporal cortex	Burgos-Ramos et al., 2009b
	Intracellular domain of APP and APLP (AICD)	Upregulation of NEP expression by AICD	Pardossi-Piquard et al., 2005
	Gleevec (Tyrosine kinase inhibitor)	Elevation of NEP mRNA and protein levels	Eisele et al., 2007
	Valproate and trichostatin A (Histone deacetylase inhibitors)	Increase in NEP expression and activity in SHSY-5Y cell	Belyaev et al., 2009
	Estrogen	Regulation of NEP expression through physical interactions between estrogen receptor and estrogen response elements in the NEP gene	Xiao et al., 2009; Liang et al., 2010
	Ginsenoside Rg3	Promotion of Aβ degradation by enhancing gene expression of NEP	Yang et al., 2009
	Green tea extract (EFLA®85942)	Strong enhancement of cellular NEP activity without change of cellular ACE activity	Melzig and Janka, 2003
	GW742 (Selective PPARδ agonist)	Upregulation of NEP in 5xFAD mice	Kalinin et al., 2009
	Polyphenols (Epilobium angustifolium)	Induction of NEP activity in SK-N-SH and PC-3 cells	Kiss et al., 2006
	sICAM-1	Induction of NEP expression in BV2 cells and in wild-type mice brains Decrease of Aβ plaques by hUCB-MSC-derived sICAM-1 which induces NEP expression in microglia	Kim et al., 2012
	Erythropoietin	Enhanced metabolism of Aβ in MSCs by increasing their NEP content	Danielyan et al., 2009

Table 1. Continued

ADEs	Regulator	Effects	References
Endothelin-converting enzyme (ECE-1)	PKCε	Promotion of Aβ clearance and reduction of AD neuropathology through increased ECE enzyme activity	Choi *et al.*, 2006; Kim *et al.*, 2011b
	4-hydroxy-nonenal (HNE)	Upregulation of ECE-1 mRNA and protein	Wang *et al.*, 2009a,b
	Aβ	Elevation of endothelin-1 in AD and upregulated by Aβ	Palmer *et al.*, 2012
Insulin degrading enzyme (IDE)	Retinoic acid	Upregulation by retinoic acid, a well-known inducer of neuronal differentiation and/or programmed cell death	Melino *et al.*, 1996
	PPARγ	Activation of an IDE like Aβ degrading activity	Espuny-Camacho *et al.*, 2010
	HES-1 and Hey-1 (Notch signaling proteins)	Binding to IDE proximal promoter and repression of transcription and its activity	Leal *et al.*, 2012
	U0126 (ERK1/2 inhibitor)	Blocking of increased IDE protein level induced by fAβ	Leal *et al.*, 2006
Angiotensin converting enzyme (ACE)	Perindopril (ACE inhibitor)	Cognitive impairment and brain injury in a mouse model of AD induced by intracerebroventricular injection of Aβ	Dong *et al.*, 2011
MMP-2	Aβ	Enhancement of MMP-2 and membrane-type-MMP expression in U87 human glioma cell	Deb *et al.*, 1999
MMP-3 (Stromelysin-1)	Aβ	Increase of MMP-3 in enriched astrocytes and mixed hippocampal cultures	Deb and Gottschall, 1996
MMP-9	Yin Yang 1	Binding to MMP-9 promoter to repress MMP-9 transcription	Rylski *et al.*, 2008
	JunB	Repression of MMP-9 transcription in depolarized rat brain neuron	Rylski *et al.*, 2009
Plasmin	Small molecule inhibitor of PAI-1	Enhanced clearance of Aβ in brain by sustaining the plasmin proteolysis cascade	Jacobsen *et al.*, 2008
	PAI-1 (neuroserpin)	Inhibition of tissue plasminogen activator activity leading to reduced plasmin activity	Fabbro and Seeds, 2009

Aβ: Amyloid-β peptide, APLP: Amyloid precursor-like protein, APP: Amyloid precursor protein, HUCB-MSC: Human umbilical cord blood-derived mesenchymal stem cell, MMP: Matrix metalloproteinase, PAI-1: Plasminogen activator inhibitor-1, sICAM-1: Soluble intercellular adhesion molecule-1.

a newly-identified ADE by our group (Kim *et al.*, 2010), was increased in the aged brain and further increased in AD model mice brain (APPswedish/presenilin exon 9 deletion mutant; unpublished data).

Although these unexpected findings are not yet to be fully understood, it is likely that ADE is increased in response to Aβ through a compensatory mechanism of the body; ADE induction by Aβ may reflect a protection of cells against the Aβ toxicity. This hypothesis is supported by other *in vitro* and in vivo results; the induction of NEP in AD Tg mouse brain after injection of Aβ$_{1-42}$ (Mohajeri *et al.*, 2002) in a dose-dependent manner. Similarly, the activity of ADE including GCPII is in-

creased in cells treated with aggregated Aβ (Deb *et al.*, 1999; Lee *et al.*, 2003; Jung *et al.*, 2003; Leal *et al.*, 2006; Mueller-Steiner *et al.*, 2006; Wang *et al.*, 2009a, 2009b; Palmer *et al.*, 2009; Miners *et al.*, 2010b). Taken together, these findings argue strongly against a notion that deficit of ADE is associated with AD.

THERAPEUTIC APPROACHES OF ADE IN TREATMENT FOR AD

Although it is too early to conclude that a decline in ADE ac-

tivity plays a major role in the accumulation of Aβ in AD brain (Miners *et al.*, 2009, 2010b), increase or over-expression of these enzymes at least could significantly reduce amyloid deposit and enhance cognitive function. The strategy to translate ADE into therapeutic applications is as follows: 1) The administration of compounds that enhance the ADE activity, 2) the gene therapy using the ADE genes, and 3) the cell therapies based on stem cell transplantation.

Compounds that enhance the ADE activity

Several agents have been identified to increase NEP expression. The neuropeptide somatostatin has been found to upregulate NEP activity through the concerted action with its receptors, somatostatin receptors (SSTR)-2 and SSTR-4 (Saito *et al.*, 2005). In AD brain, the reduction of somatostatin and SSTR levels were found and infusion of Aβ caused impairment of somatostatin signaling (Aguado-Llera *et al.*, 2005) and reduction of NEP expression (Burgos-Ramos *et al.*, 2009a). Although the exact mechanism involved in this signaling pathway is yet to be clarified, this result tells that Aβ levels may be associated with or even controlled by somatostatin agonists. Concurrently, studies with minocycline (Burgos-Ramos *et al.*, 2009b) and erythropoietin (Danielyan *et al.*, 2009) showed increased NEP expression, preventing AD abnormalties.

Another agent is APP intracellular domain which is reported to increase the NEP promoter activity in human neuroblastoma cells. A tyrosine kinase inhibitor, imatinib (Gleevec) increased both APP intracellular domain and NEP (Eisele *et al.*, 2007). Epigenetic regulators such as valproic acid also increased NEP activity (Belyaev *et al.*, 2009) as well as plasmin (Pulukuri *et al.*, 2007). Estrogen (Xiao *et al.*, 2009; Liang *et al.*, 2010), gensenoside Rg3, a major component of ginseng (Yang *et al.*, 2009), green tea extracts (Melzig and Janka, 2003; Ayoub and Melzig, 2006) and red wine (Melzig and Escher, 2002) have all been reported to increase NEP activity. Table 1 summarizes the regulatory molecules that regulate the ADE levels or activity.

Therapy for ADE gene delivery

Gene therapy approaches has employed viral-vector mediated transfection of ADE genes to increase their expression in AD model animals. Both *in vitro* and *in vivo* studies have shown that gene delivery of NEP is effective in reducing Aβ level; NEP expression using Sindbis viral vector in murine primary cortical neurons effectively reduced the Aβ levels (Hama *et al.*, 2001). Similarly, lentiviral NEP injected into the hippocampus of hAPP Tg mice reduced the plaque burden (Marr *et al.*, 2003) and improved memory performance (Spencer *et al.*, 2008). In addition, injection of adeno-associated viral NEP to NEP knock-out mice abolished the increase in Aβ levels in hippocampus, and led to efficient degradation of soluble and insoluble Aβ in hAPP AD mice (Iwata *et al.*, 2004). Recently the peripheral delivery of ADE, instead of the brain delivery was attempted and the results showed the promising results. Adeno-associated virus transfection of NEP into the hind limb of triple Tg AD model mice produced 60 % reduction in soluble Aβ and 50 % reduction in plaque deposit within the brain at 6 months (Liu *et al.*, 2010).

A technology called convection-enhanced delivery has been developed to improve the brain delivery of the proteins. This technique is a novel neurosurgical method of direct drug delivery to the brain through ultrafine microcatheters. The application of this technology might be effective for brain diseases showing local pathology, such as Parkinson's disease. Further studies demonstrated that drugs delivered with this method are accumulated near blood vessels and perivascular spaces (Krauze *et al.*, 2005), suggesting the possibility of using CAA treatment (Weller *et al.*, 2000; Carare *et al.*, 2008; Weller *et al.*, 2008).

Stem cell therapy

Stem cells have the potential to directly substitute damaged cells and as a vehicle for delivering ADE into the CNS. Studies demonstrated that transplantation of adult mesenchymal stem cells reduced the brain Aβ, which was mediated by increased NEP mRNA and protein levels (Miners *et al.*, 2011). In other study using with human umbilical cord blood mesenchymal stem cells (hUCB-MSCs) NEP expression in transplanted cells into the hippocampus of APP AD mice was increased and amyloid plaques in that region and other regions were decreased by the active migration of hUCB-MSCs toward Aβ deposits (Kim *et al.*, 2012). Although the exact mechanism is not clear yet, cytokines such as intracellular adhesion molecule-1 which is released from the transplanted mesenchymal stem cells were involved (Kim *et al.*, 2012).

CONCLUSIONS AND PERSPECTIVES

Understanding of Aβ metabolic pathway is uppermost to enlighten the pathogenesis and cure for AD. Although there are huge publications dealing with signaling pathways of Aβ synthesis and related enzymes, the identification of molecules responsible for Aβ clearance pathways and their mechanistic links to AD is still underway. In addition to nonenzymatic pathway, enzymatic pathway by ADE serves Aβ clearance. The results have suggested a pivotal role for ADE in reducing AD symptoms in both cell and animal models. Thus, the modulation of ADE expression and activity provides a simple strategy of whether clearance of Aβ offers the therapeutic potential for AD. It is of great interest to find that peripheral delivery of ADE gene gives a significant efficacy to reduce AD symptoms as compared to the direct delivery to the brain, which has been a longtime obstacle for curing brain diseases. In addition, it is valuable to develop modifiers of ADE as therapeutics for AD. However, several studies also found that, unexpectedly, ADE expression and activity increase with normal aging and rise progressively with increasing severity of AD, which is likely to occur through a compensatory mechanism against increased levels of Aβ. Thus further researches will answer a question of what the benefits of ADE overdose are.

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