INTRODUCTION

Alzheimer’s disease (AD) is a devastating 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 (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 Aβ1-42, 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 Aβ_{42} or of the ratio of Aβ_{42} to Aβ_{40}, Aβ_{42} is more hydrophobic and more prone to aggregate than Aβ_{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 Aβ accumulation and AD pathogenesis.

Previously, it has been generally accepted that amyloid plaques, fibrils, or much complicated forms synthesized from Aβ are the major pathological species causing impaired synaptic and neuronal dysfunctions. However, Aβ oligomers are highly toxic and cause synaptic dysfunction (Hardy and Selkoe, 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 Aβ oligomers to produce neurotoxicity. For example, Aβ binds to prion proteins (Laursen 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 Aβ more favorably than their corresponding receptors (Hong et al., unpublished data). This result suggests that the binding of neurotransmitters with Aβ might play an important role in AD pathogenesis through the disturbance of the normal signaling of neurotransmitters.

**Aβ CLEARANCE**

Aβ 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 Aβ and thus Aβ-mediated cellular toxicity. Recently, however, a new concept of Aβ accumulation has been emerged; Aβ clearance or degradation rather than its synthesis has been found to be more critical in accumulation of Aβ. Furthermore, another mechanism responsible for controlling the brain Aβ 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 Aβ represent a dynamic equilibrium between synthesis, re-uptake and clearance, any factors that result in the reduced rate of Aβ removal is likely to cause Aβ accumulation. Thus, Aβ clearance pathways including protease-mediated Aβ degradation have been emerged as a new therapeutic target for AD treatment, which are mostly handled in this review.

Aβ 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, Aβ is unlikely to accumulate in the normal brain. However, small defects in Aβ clearance could be sufficient to cause Aβ accumulation leading to cell toxicity. Many data clearly suggest that in the central nervous system (CNS), decreased Aβ clearance is more responsible for the development of AD rather than increased Aβ synthesis (Weller et al., 2000). In particular, defects in Aβ clearance process is also likely to be relevant for the accumulation of Aβ 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 Aβ 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 nephrilysin (NEP), insulin-degrading enzyme (IDE), matrix metalloproteinase (MMP)-9 and glutamate carboxypeptidase II (GCP II).

**INTERSTITIAL FLUID DRAINAGE PATHWAY**

In addition to accumulation within senile plaques, accumulation of Aβ in AD brain is also found in the walls of capillaries and arteries as characterized in CAA. Several lines of evidence suggested that Aβ deposit in the wall reflects a failure of the elimination of Aβ along the perivascular ISF drainage pathways of the brain (Weller et al., 2000). As shown in Fig. 2, increased production of Aβ or blockage of Aβ 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 Aβ 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.

**UPTAKE BY MICROGLIAL PHAGOCYTOSIS**

Microglia, brain’s resident mononuclear phagocyte is found
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 neuroprotective 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 phagocytosis (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
absolutal 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 Aβ are 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 metalloendopeptidase [insulin-degrading enzyme (IDE)], 3) serine proteases [plasmin, myelin basic protein (MBP), acylypeptide 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 terminus. 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_{32-35} have similar toxicity and aggregation property as the full-length Aβ_{1-42} 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 cause 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; Hafetz 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, tNEP 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 |
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 NPEP in AD Tg mouse brain after injection of Aβ1-42 (Mohajeri et al., 2002) in a dose-dependent manner. Similarly, the activity of ADE including GCPII is increased 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 SSSTR-4 (Saito et al., 2005). In AD brain, the reduction of somatostatin and SSSTR 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 abnormalities.

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 expression (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 Janaka, 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., 2003). 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 long time 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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