Neurovascular Pathways and Alzheimer Amyloid β-peptide

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According to the prevailing amyloid cascade hypothesis, the onset and progression of a chronic neurodegenerative condition in Alzheimer disease (AD) is initiated by the amyloid β-peptide (Aβ) accumulation in brain and consequent neuronal toxicity. Recent emphasis on co-morbidity of AD and cerebrovascular disease and the recognition that cerebrovascular dysregulation is an important feature of AD, has shed new light on neurovascular dysfunction as a possible contributor to cognitive decline and Alzheimer neurodegeneration. In the same time, this association has raised a question as to whether there is a causal relationship between cerebrovascular dysregulation and Aβ-initiated pathology, and whether influencing targets in the neurovasculature may prevent different forms of Aβ brain accumulation and/or lower pre-existing accumulates in a later stage of the disease. Pathogenic cascades which operate to dissociate normal transport exchanges between central and peripheral pools of Aβ, and decreased vascular competence leading to brain hypoperfusion and impaired Aβ clearance are discussed. We suggest that there is a link between neurovascular dysfunction and elevated brain Aβ which provides a new scenario for therapeutic interventions to control Alzheimer mental deterioration.

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INTRODUCTION

A central feature of Alzheimer disease (AD) is a chronic neurodegenerative process which according to the prevailing amyloid cascade hypothesis is initiated by the amyloid β-peptide (Aβ) accumulation in brain and consequent neuronal toxicity (24). The neurofibrillary tangle (NFT) resulting from hyperphosphorylated tau is also a neuropathological feature of AD (55). The study of these pathological traits of AD has dominated the field for the last 3 decades. Indeed, much of the therapeutic armamentarium for AD is directed at developing strategies to control Aβ-related and tau-related pathology in hopes of improving cognitive decline associated with AD (24, 51). It is important to stress, however, that recent emphasis on co-morbidity of AD and cerebrovascular disease (21), the link between AD and atherosclerosis (6, 44), and the realization that cerebrovascular dysregulation is an important feature of AD (3, 26), has shed new light on neurovascular dysfunction as a likely contributor to dementia and a chronic neurodegenerative condition in AD (60).

Based on a growing body of literature demonstrating that vascular risk factors add to cognitive decline in AD (21), that brain microvascular system is significantly altered in AD (3, 20), and that disrupted cerebral blood flow (CBF) and hypoperfusion to the brain parenchyma are seen in both animal models of AD, as well as in human patients with AD (5, 22, 26, 54), it has been proposed that at least some forms of AD may develop primarily due to cerebrovascular changes and should be classified as vasocognopathies (15). Since the neurovascular unit maintains tightly controlled chemical composition of neuronal internal "milieu" by regulating local CBF and blood-brain barrier (BBB) molecular transport, pathological changes in the vasculature are likely to undermine the normal physiological function of this critical unit (60). However, this also raises a question of whether there is a causal relationship between Aβ accumulates and Aβ-initiated pathology and neurovascular dysfunction, and whether influencing neurovascular pathways may regulate Aβ brain homeostasis to ultimately lower brain Aβ?

IS CEREBRAL β-AMYLOIDOSIS DISORDER OF Aβ CLEARANCE OR Aβ PRODUCTION?

There is little evidence that normal brain aging results in local overexpression of the Aβ precursor protein (APP) and overproduction of Aβ (37). In addition, a relatively small number of AD patients have increased Aβ production in the CNS which is limited to familial forms of the disease caused by inherited mutations in the APP gene nearby the Aβ coding region (ie, Swedish mutation) or presenilins 1 or 2 genes (47). The majority of patients with so-called late-onset "non-genetic" AD, as well as patients with familial forms of cerebrovascular β-amyloidoses, do not have increased Aβ production or increased APP expression in the CNS. Therefore, these patients are likely to exhibit a failure in Aβ clearance from the CNS that could be either due to dysregulated transport mechanisms that control vascular efflux of Aβ across the BBB (61, 63) and/or due to its faulty degradation in the CNS (47). Alternatively, an increased influx of circulating Aβ into the CNS across the BBB may result in accumulation of soluble neurotoxic Aβ in brain interstitial fluid (ISF) and/or its subsequent aggregation and deposition in the CNS (11, 59). In sum, it is likely that cerebrovascular β-amyloidosis in AD develops slowly as a result of a progressive Aβ clearance disorder rather than due to its uncontrolled production from APP. It is also likely that decreased vascular competence associated with reduced CBF and diminished brain capillary surface area available for Aβ efflux from the brain may further amplify the failure of the vascular system to filter Aβ from the brain into the

blood for its systemic clearance and excretion through the liver, kidney and possibly some other excretory organs.

CEREBROVASCULAR FACTOR IN Aβ ACCUMULATION

The exact physiological role of Aβ in the brain and in the body during development and aging still remains a mystery. Is Aβ just a waste product of APP metabolism with powerful cellular toxic actions which require its immediate removal from brain, or alternatively, is Aβ a regulatory molecule of neurogenesis (57) and cerebral angiogenesis (41, 42) with important roles in development and neurovascular repair? Nevertheless, a number of pathways exist to maintain Aβ homeostasis precisely in the body and within the CNS. These pathways seem to be well orchestrated during normal physiology, but are completely unsynchronized in diseases associated with dementia and storage of Aβ in the CNS. It is also interesting to note that amyloid lesions and NFTs can both be triggered by vascular mechanisms such as hypertension and ischemic brain injury (3, 15, 26), which frequently affect elderly individuals with Alzheimer-type dementia (53). It is possible that ischemic brain episodes in AD patients and "mini strokes" develop because of chronic brain hypoperfusion due to atherosclerotic changes in their cerebral arteries (6, 44). These novel findings raise a possibility that a cerebrovascular disorder may precipitate and/or amplify the Aβ- and tau-related pathology, and therefore could be an important pathogenic mechanism contributing to cognitive decline and neuropathology in AD.

CNS AND SYSTEMIC PATHWAYS REGULATING BRAIN Aβ

The CNS Aβ homeostasis is controlled by numerous pathways which interact with each other and may play important roles in early and later stage of the disease process resulting in Aβ accumulation and/or deposition in brain. These include: *i)* systemic and brain Aβ production, and its systemic clearance mechanisms; *ii)* rapid regulation of Aβ soluble brain interstitial fluid (ISF) pool by receptor-mediated transport across the BBB from brain to blood via the low density lipoprotein receptor related protein-1 (LRP1) (10, 16, 48), or under certain pathological conditions from blood to brain via the receptor for advanced glycation end products (RAGE) (12); *iii)* control of soluble Aβ pool sizes in the extracellular body fluids and plasma, and in brain ISF and cerebrospinal fluid (CSF) via Aβ binding proteins, eg, apolipoprotein E (ApoE), apoJ, α2-macroglobulin (α2M), transthyretin, and albumin (18, 19, 51), which may also regulate transport exchanges of their respective complexes with Aβ across the BBB and blood-CSF barrier (51, 62); *iv)* Aβ metabolism by different

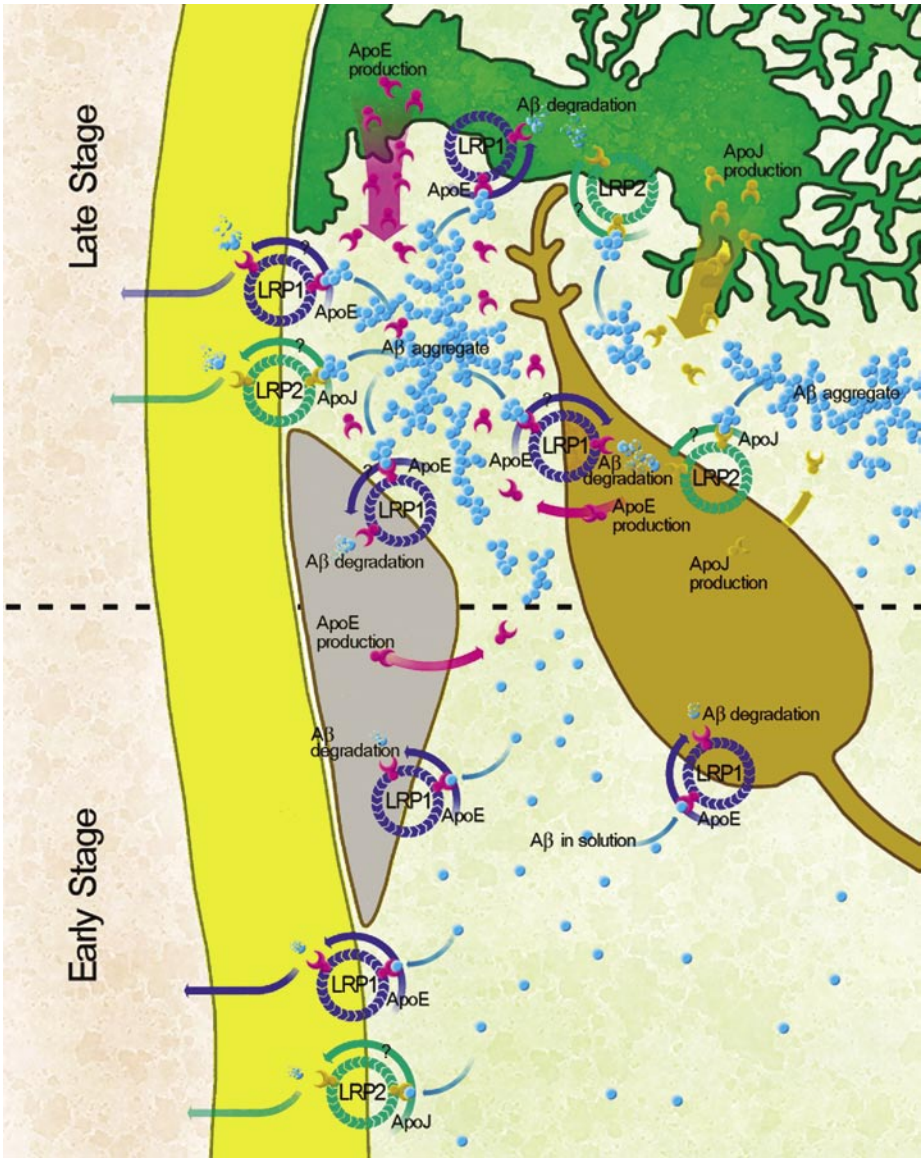

Figure 1. *The proposed role of liproprotein receptors LRP1 and LRP2 and of Aβ transport proteins ApoE and apoJ in controlling brain Aβ creates a new scene of potential therapeutic opportunities for AD.* Late stage. In the late stage of the disease LRP1 on astrocytes could mediate removal of diffuse Aβ aggregates (30). Astrocytes (*green*) produce ApoE, an LRP1 ligand, which is also produced to a lesser degree by neuronal cells (*brown*). Injured astrocytes and neurons in AD also express LRP2 and produce apoJ, an LRP2 ligand (32). Deletion of the apoJ gene has remarkable effect on development of Aβ-related pathology in APP mice lacking the ApoE gene (18) suggesting its probable major role in clearing Aβ. Whether ApoE secreted by astrocytic microdomains nearby the endothelial blood-brain barrier (BBB) (*yellow*) can co-localize diffuse capillary Aβ aggregates for clearance via LRP1 at the BBB, and/or whether apoJ may co-localize Aβ aggregates for clearance via LRP2 which is expressed at the BBB (62), is not known. There is also a possibility that vascular smooth muscle cells and pericytes (*gray*) which are in close proximity to the endothelial barrier, and express LRP1 and secrete ApoE and apoJ (25), may help clearing diffuse Aβ aggregates. The role of LRP1 and LRP2 on neurons with respect to clearing aggregated and/or oligomeric Aβ is not known, except for the fact that oligomeric Aβ species are extremely neurotoxic (24). Early stage. Before significant Aβ accumulates develop, LRP1 at the BBB can directly clear soluble Aβ(10), whereas LRP1 on neurons most likely regulates the intracellular vs. extracellular redistribution of soluble Aβ in brain and does not have an effect on net clearance from brain (58). How ApoE and apoJ binding to Aβ influences clearance of their respective complexes at the BBB still remains unknown.

systemic and brain enzymes including neprilysin (29), insulin degrading enzyme, coagulation-related enzymes (eg, plasmin, tissue plasminogen activator), or different matrix metalloproteinases (47); *v)* removal of deposited Aβ by different brain cell types including astrocytes (56) which may depend on ApoE-directed co-localization of deposits (30) and via microglia (1); *vi)* slow removal of Aβ via the ISF-CSF bulk flow (50); and *vii)* oligomerization and aggregation of Aβ alone and/or with its binding proteins (24, 51). Figure 1 summarizes a hypothetical diagram on some selected lipoprotein receptors-mediated pathways in different brain cells and of their ligands/Aβ binding proteins, which could potentially be involved in removal of aggregated Aβ and/or soluble Aβ from the brain and across the BBB.

The importance of Aβ exchanges between cerebral vascular compartment and brain ISF compartment has been well documented by several recent studies in animal models of AD-like brain β-amyloidosis. For example, it has been shown that Aß efflux across the BBB in AD mice can predict the volume of brain amyloid burden (16), whereas the development of senile plaques directs the Aβ transport equilibrium towards the brain (17). Similar findings have been reported in aged non-human primates which develop either cerebral β-amyloidosis (Rhesus monkey) (38) or cerebral β-amyloid angiopathy, CAA (Squirrel monkey) (2).

A recent study reported that the apoJ gene is essential for preventing Aβ accumulation and deposition in APP overexpressing mice (18). Deletion of the apoJ gene accelerated Aβ-related pathology in APP mice lacking the ApoE gene (18), raising the possibility that apoJ could be a major apolipoprotein mediating Aβ efflux from brain. The role of apoJ in blood-to-brain and brain-to-blood transport exchanges of Aβ is still unclear. An earlier report suggested Aβ complexed to apoJ in the circulation may utilize the lipopoprotein receptor at the BBB, gp330/megalin or LRP2, for its transport into brain ISF and/or CSF across the BBB and blood-CSF barrier of the choroid plexus epithelium, respectively (62). It has been noted that LRP2 at the BBB is completely saturated by relatively high levels of circulating apoJ (~1 μM); whereas, the in vivo determined half-saturation transport constants (K_m) at the BBB for Aβ40-apoJ complexes were in the range <1 nM (62). It remains unknown whether LRP2 can clear Aβ-apoJ complexes across the BBB and the blood-CSF barrier into the blood, nor whether apoJ is as effective in transporting the pathogenic Aβ42 across the biological membranes as it is in mediating Aβ40 trafficking. On the other hand, it is not clear whether LRP2 expressed on different brain cells in AD brains, including injured neurons and astrocytes (32), could be involved in the removal of aggregated Aβ by the apoJ-directed co-localization in a fashion similar to that reported for the ApoE/LRP1-mediated removal of aggregated Aβ via astrocytes (30). Whether vascular smooth muscle cells and pericytes which express LRP1 and secrete ApoE (25) have a role in removing aggregated Aβ, and can brain endothelial cells can be as effective in removing Aβ aggregates as they are in transporting soluble Aβ from brain to blood is not known (Figure 1).

It has also been suggested that P-glycoprotein at the luminal side of the BBB may reduce the levels of Aβ in the brain endothelial cells by promoting its efflux into blood (33). Our work based on Aβ40 transport kinetics has suggested that RAGE and LRP1 are dominant transport pathways creating almost instantaneous changes in the levels of soluble Aβ in brain ISF by regulating its rapid bi-directional trafficking across the BBB (11, 51).

RAGE, a multiligand receptor in the immunoglobulin superfamily, mediates transcytosis of Aβ across the BBB under pathologic conditions associated with a neuroinflammatory response, increased cytokine expression and oxidant stress, and neuronal co-localization of circulating Aβ (12).

LRP1, an endocytic and signaling multiligand receptor for several molecules associated with development of AD pathology including Aβ, ApoE, α2M and APP, is linked to AD genetically and biochemically through regulation of APP processing and metabolism and Aβ clearance (11, 25, 51). Originally, it has been suggested that LRP1 on neurons may clear Aβ, but more recent work in APP mice overexpressing the LRP1 mini-receptor on neurons demonstrated accumulation of soluble Aβ in brain (51), arguing against the role of neuronal LRP1 in Aβ clearance. Since LRP1 binds and internalizes free Aβ (10), it is possible that neuronal LRP1 plays a role in distributing soluble Aβ between the intracellular versus extracellular neuronal milieu, but without a net effect on Aβ clearance from brain. In contrast to neurons, LRP1 on brain capillary endothelium interacts directly with free Aβ and clears preferentially Aβ40 into the blood, whereas the affinity of other Aβ isoforms for LRP1 is greatly reduced with an increasing β-sheet content in Aβ compared to Aβ40 (10, 48). These findings suggest that low affinity of certain soluble Aβ species for LRP1 at the BBB may result in accumulation of these Aβ isoforms in brain, as recently demonstrated in the Dutch/Iowa transgenic APP model (9, 10). Whether LRP1 on brain endothelium may clear aggregated Aβ via an ApoE-dependent mechanism as shown for astrocytes (30), or via an apoJ-dependent co-localization to LRP2 on vascular endothelial cells, is not known. Whether brain endothelium can clear oligomeric forms of Aβ, and whether chaperone proteins can influence BBB clearance of oligomeric Aβ is not known.

THERAPEUTIC INTERVENTIONS FOR AD

The core of research for potential breakthrough therapeutic interventions to control cognitive decline in AD patients has been and is still centered on Aβ, yet we are awaiting for an approved Aβ-based therapy for this devastating brain disorder. Currently approved therapies for AD are mainly symptomatic directed at either compensating for the neurotransmitter deficit in brain resulting from neuronal and synaptic loss, or at effecting the blood flow to the brain, and/or at controlling certain symptomatic neurological features of the disease. However, no known treatment can arrest the progression of AD.

Approved therapies. In addition to Aricept, there are 4 other approved therapies for AD. For some people in the early and middle stages of the disease, the drugs tacrine (Cognex), rivastigmine (Exelon), or galantamine (Reminyl) may help slowing down the progression of symptoms for a limited time. While each of these drugs is regarded as commercially successful, it is difficult to distinguish between them in terms of efficacy since they all have a similar mechanism of action, ie, the inhibition of acetyl cholinesterase activity (15, 52). A re-

cently approved new drug memantine (Namenda) marketed by Forest Laboratories Inc, is an N-methyl D-aspartate (NMDA) antagonist. The main effect of memantine is to delay progression of the symptoms of moderate to severe AD which may allow some patients to maintain certain daily functions a little longer (52). Other drugs not initially targeted or labeled for AD patients may help controlling some behavioral symptoms of AD such as sleeplessness, agitation, wandering, anxiety, and depression. Treating these symptoms often makes patients more comfortable and makes their care easier. Again, these drugs do not arrest or alter the progression of the disease.

Aβ-based experimental therapies. The prevailing concept states deficient Aβ clearance has a major role in late onset, nongenetic AD, (>98% of AD cases) (11, 13, 45, 47, 51, 59). Aβ clearance strategies had remarkable results in preclinical immunization trials (46), but were less conclusive in humans (40), although improvements in cognitive decline have been reported. The Aβ clearance pathways delineate new therapeutic opportunities including activation of several Aβ-degrading enzymes (47, 51), which may help preventing Aβ accumulation. Strategies to directly enhance the binding of Aβ to LRP1 (10) or to its lipoprotein receptors ligands/Aβ chaperones (51), may offer promising therapeutic avenues in future giving the prevailing role of LRP1 in Aβ clearance across the BBB (10, 48) and on astrocytes (30). It has been shown that some agents, eg, statins (11) and proteasome inhibitors (10), may increase LRP1 expression at the BBB and perhaps enhance its activity. It has also been shown that blocking Aβ/RAGE interaction at the BBB by systemic treatment with soluble form of the receptor (sRAGE) prevents Aβ accumulation in PDAPP mice if treatment is initiated before 6 months of age (12). This effect of sRAGE is most likely created through a peripheral binding of Aβ in the circulation which in turn would allow efflux mechanisms to prevail. In addition, blocking Aβ/RAGE interaction may help increase LRP1 levels at the BBB (11). It is important to note that the efficacy of many Aβ peripheral binding agents, eg, immunoglobulins (16, 46, 49), sRAGE (12), sLRP1 (10), gelsolin (39), may critically depend on the availability of clearance mechanisms in the brain, which depend on the levels of LRP1 at the BBB. The LRP1 levels in brain capillaries, however, are substantially reduced in APP overexpressing animals at 9 to 12 months of age and in late-stage AD patients and Dutch patients with cerebrovascular β-amyloidosis (10). Therefore, early treatment with Aβ-binding agents may be a key to success of such peripheral Aβ sequestration-based therapies. Metal chelators may destabilize β-amyloid deposits and attenuate cognitive decline in AD patients (51).

In addition to the clearance strategies, considerable efforts by major pharmaceutical companies in the past decade have been focused on developing inhibitors of γ-secretase and β-secretase, enzymes responsible for proteolytic cleavage of Aβ-precursor protein (APP) and formation of Aβ (43, 47). Studies with secretase inhibitors have demonstrated that inhibiting APP production in transgenic models designed to overproduce Aβ in brain will result in improved cognitive decline and reduced Aβ pathology (43). If these strategies were able to influence normal Aβ production at physiological APP expression levels in brain, they could also have the potential to reduce brain Aβ in late onset AD patients, who do not have increased APP levels in brain. However, as with all strategies for Aβ as discussed above, the side effects of the inhibition of secretases would still remain, at least for the present, an important concern.

Finally, studies have used small molecule libraries to screen for compounds that either interfere with assembly of Aβ particles into fibrils (14, 34) or disaggregate existing fibrils (4).

Tau-based experimental therapies. Since the microtubule-associated tau protein is hyperphosphorylated in NFT, efforts have been directed towards interfering with the phosphorylation of tau either by inhibiting various protein kinases or promoting phosphatase activities (28, 35). High-throughput screening assays are currently underway to define drugs that interfere with the hyperphosphorylated tau, as well as its associated sequestration of normal tau (27). A recent report described a small molecule (N744, <700 Da) that dose-dependently inhibited tau filament nucleation and fibrillization in vitro, making this a promising candidate to test in animal models (8).

Neurovascular protection strategies. We now appreciate that neurovascular dysfunction is a major hallmark of AD (15, 26, 60). Brain ischemia and athero-thrombotic disorder could therefore be important therapeutic targets in AD (6, 44). Agents which exhibit direct cytoprotective activity on stressed and ischemic brain endothelium and neurons, and have substantial anti-inflammatory and pro-angiogenic activity, such as the serine protease activated protein C (7, 23, 36), may hold the potential to protect the neurovascular unit from combined Aβ, hypoxic and excitotoxic injuries as seen in AD. Altered physiology of vascular smooth muscle cells and altered brain endothelium in AD could be important therapeutic targets for controlling arterial and brain capillary disorder in AD, but at present their role is still poorly understood.

CONCLUSIONS

The accumulating body of evidence suggests that neurodegenerative disorder in AD may have a complex multifactorial origin which may not necessarily be primarily centered on neurons and/or originate from a primary neuronal disorder. It became evident that in contrast to valuable animal models of AD-like cerebral β-amyloidosis, which are created by overexpressing APP in neurons associated with Aβ neuronal overproduction, the actual disease in patients does not mimic this situation in models since the accumulation of Aβ in late onset AD is not related to APP neuronal overproduction (47, 51, 59). Moreover, studies demonstrating that vascular dysregulation and brain hypoperfusion may result in increased APP expression/Aβ production and tau pathology (3, 26) further support the view that observed neuronal changes may be rather secondary to cerebrovascular problems associated with AD. Recent experimental studies in transgenic mice producing low levels of poorly cleared vasculotropic Dutch/Iowa Aβ mutants confirmed impaired vascular clearance of Aβ across the BBB leads to an increased Aβ brain capillary deposition and formation of amyloid lesions (9, 10). It has also been suggested that dense Aβ plaques in Alzheimer Tg2576 APP overexpressing mice and double transgenic PS1/APP Tg2576 mice

(31), as well as in AD brains (60), may have vascular origin.

At this point, we would argue that a more in depth understanding of multiple neurovascular cascades that operate to dissociate normal neurovascular coupling, induce brain hypoperfusion and reduce vascular competence resulting in impaired Aβ clearance from brain may open new avenues to link neurovascular dysfunction to Aβ brain accumulation and AD pathology. Although numerous pathways may initially precipitate the onset of the disease, at a later stage in the disease process, these cascades may work synergistically to form interrelated and irreversible pathogenic circles to devastate the brain. For example, Aβ oligomers are directly neurotoxic (24), whereas Aβ aggregates are anti-angiogenic (41, 42), synergistically destroying the neurovascular unit leaving brain without its important clearance mechanism. Therefore, combined therapies to enhance Aβ clearance, improve neurovascular repair and protect brain cells from different types of stress may hold potential to control cognitive dysfunction associated with AD.

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