Neurosci Bull February 1, 2013, 29(1): 111–120. http://www.neurosci.cn
DOI: 10.1007/s12264-012-1287-6

·Review·

Combination of Aβ clearance and neurotrophic factors as a potential treatment for Alzheimer's disease

Lian-Feng Lin[1,#], Min-Jing Liao[1,4,#], Xiao-Yan Xue[5], Wei Zhang[1], Li Yan[1], Liang Cai[1], Xiao-Wen Zhou[1], Xing Zhou[1], Huan-Min Luo[1,2,3]

[1]*Department of Pharmacology,* [2]*Institute of Brain Sciences, School of Medicine, Jinan University, Guangzhou 510632, China*

[3]*The Joint Laboratory of Brain Function and Health, Jinan University and The University of Hong Kong, Jinan University, Guangzhou 510632, China*

[4]*Department of Laboratory Medicine, Medical College of Hunan Normal University, Changsha 410013, China*

[5]*Ganzhou People's Hospital, Ganzhou 341000, China*

[#]These authors contributed equally to this review.

Corresponding author: Huan-Min Luo. E-mail: tlhm@jnu.edu.cn

There is no effective drug to treat Alzheimer's disease (AD), a neurodegenerative disease affecting an estimated 30 million people around the world. Strongly supported by preclinical and clinical studies, amyloid-beta (Aβ) may be a target for developing drugs against AD. Meanwhile, the fact that localized neuronal death/loss and synaptic impairment occur in AD should also be considered. Neuronal regeneration, which does not occur normally in the mammalian central nervous system, can be promoted by neurotrophic factors (NTFs). Evidence from clinical trials has shown that both Aβ clearance and NTFs are potentially effective in treating AD, thus a new approach combining Aβ clearance and administration of NTFs may be an effective therapeutic strategy.

Keywords: Alzheimer's disease; amyloid-beta; neurotrophic factors; treatment

Introduction

Alzheimer's disease (AD) is an age-related, progressive and irreversible neurodegenerative disease that causes serious cognitive dysfunction. Its pathological hallmarks are extracellular deposits of amyloid-beta (Aβ) and intracellular neurofibrillary tangles, together with drastic synaptic and neuronal reduction or loss[1]. It has been estimated that AD affects 30 million people around the world[2], and the past two decades have witnessed an explosion in research into the underlying mechanisms as well as potential therapeutic strategies. Although it was first described by German psychiatrist Alois Alzheimer in 1906, there is still no cure for AD, partly because the cellular and molecular mechanisms underlying it are far from clear.

The Food and Drug Administration in the United States has approved a limited range of drugs to treat AD, including acetylcholinesterase inhibitors (AChEIs) and N-methyl-*D*-aspartate receptor (NMDAR) antagonists[3], although their effects are merely symptomatic and memory-improvement occurs within a limited period. Donepezil, galantamine, rivastigmine and tacrine are AChEIs that prevent the breakdown of acetylcholine released from presynaptic terminals, increasing the residence time of the neurotransmitter within the synapse and thereby prolonging the duration of its interaction with postsynaptic receptors[4]. Memantine, an NMDAR antagonist, has been approved for the treatment of moderate and severe AD; it works by preventing the excitatory neurotoxicity induced by excessive glutamate[5].

Currently, no available pharmacological agents cure or reverse the progression of this devastating neurological disorder. It is therefore urgent to improve our understanding of its pathogenesis, and then develop an effective treatment strategy. This review aimed to provide insights

into the design of potentially effective pharmaceutical treatments for AD by targeting two seemingly separate but tightly connected components, Aβ and neurotrophic factors (NTFs).

Aβ as a Promising Target for AD Treatment

Aβ is a peptide of 36–43 amino-acids produced from amyloid precursor protein (APP) through aberrant cleavage by β- and γ-secretases. According to the Aβ cascade hypothesis proposed by Hardy and Selkoe, the accumulation of extracellular Aβ aggregates or senile plaques is the key causative factor in the pathological or neurodegenerative changes associated with the development of AD, including inhibition of long-term potentiation, impairment of synaptic function, and eventual neuronal death/loss leading to cognitive decline[6-8].

Of the AD-related factors listed in Table 1, ~50% are associated with Aβ. Although many factors are also known to be associated with AD (Table 1), the Aβ hypothesis remains the focus of research. This is strongly supported by the observation of abundant deposits of Aβ in the brain in both sporadic AD (~95% of cases) without a clear inheritance pattern, and familial AD (~5% of cases) with autosomal dominant inheritance caused by mutations of APP and the homologous presenilin genes, presenilin 1 and 2. These mutations inevitably lead to increased levels of Aβ which is considered to be the molecule that initiates neuronal degeneration[47]. Evidence for this hypothesis comes from APP transgenic mice that show correlations between elevated Aβ, Aβ plaques and cognitive deficits. Clinical studies of AD also support this hypothesis. Rovelet-Lecrux *et al.* reported a duplication of the *APP* locus on chromosome 21 in five families with autosomal dominant early-onset AD[48]. Barthel *et al.*, using positron emission tomography (PET) images of florbetaben (an ^{18}F-labeled Aβ-targeted PET tracer), demonstrated that nine of ten mild-moderate probable AD participants (DSM-IV and NINCDS-ADRDA criteria) were Aβ-positive, compared to only one of ten healthy controls[49]. Furthermore, in a global phase 2, open-label, non-randomized, multi-center study recruiting a total of 81 men and women with probable mild-to-moderate AD and 69 cognitively unimpaired healthy volunteers aged 55 years and older, florbetaben scans indicated a sensitivity of 80% (95% CI 71–89) and a specificity of 91% (84–98) for discriminating participants with AD from healthy controls[50].

In addition, Aβ has recently been reported to alter tau phosphorylation, which is associated with the other cardinal lesion of AD, neurofibrillary tangles composed primarily of hyperphosphorylated tau[51]. Zeng *et al.*[52], for example, reported that Aβ-induced neurotoxicity can be attenuated by inhibiting tau protein hyperphosphorylation. It is conceivable that the two pathological hallmarks of AD, Aβ deposits and neurofibrillary tangles, may actually reflect two distinct patterns of impairment caused by Aβ.

Aβ and NTF Deficiency as a Toxic Feed-Forward Loop Leading to AD

To date, various mechanisms underlying the impairments by Aβ have been proposed, including decreased brain-derived neurotrophic factor (BDNF) expression, activated inflammatory cells, generation of reactive oxygen species, inhibition of long-term potentiation, impairment of synaptic structure and function, and acceleration of neurofibrillary tangle formation (Table 1).

Aβ Downregulates NTFs

As neurotrophic effects are directly correlated with neuronal survival and the maintenance of synaptic plasticity, neurotrophic imbalance is a likely mechanism of Aβ impairment. NTFs, such as BDNF and nerve growth factor (NGF), play critical roles in neuronal survival and regeneration as well as synaptic plasticity. More importantly, Aβ has been found to contribute to the downregulation of NTFs.

Peng *et al.* demonstrated that transgenic mouse models of AD harboring mutations in APP resulting in Aβ overproduction, show a significant reduction in cortical BDNF mRNA expression in comparison with wild-type mice[9]. The cortex and hippocampus, regions involved in learning and memory, exhibit both increased Aβ production and reduced BDNF levels in AD[53-55]. Aβ may impair neurotrophic signaling by decreasing NTF levels. NTF deficiency has the same presentation as the end-point of AD, neuronal loss and synaptic dysfunction. Regionalized neuronal death/loss and synaptic impairment in the process of AD have been demonstrated in many diagnostic, clinical, and pathological studies[56-59]. For example, West *et al.*[60] found that neurons in the CA1 region of the hippocampus decline from 14.08×10^6 neurons in an age-matched control group to 4.40×10^6 neurons in an AD group. Similarly, significant neuronal loss

Table 1. Proposed pathogenesis of Alzheimer's disease (AD)

Causative/risk factors	Consequences
Aβ	Down-regulation of BDNF[9], activation of inflammatory cells[10], generation of reactive oxygen species[11], inhibition of long-term potentiation[6], impairment of synaptic structure and function[7,8], acceleration of neurofibrillary tangle formation[12]
Altered function of blood-brain barrier	Decreased clearance of Aβ[13], leakage of serum-derived components into brain leading to neuronal dysfunction[14], promotion of aluminum accumulation in brain[15]
Brain trauma	Increased risk of AD[16,17]
Chronic stress	Induction of abnormal hyperphosphorylation of tau[18] , accelerated impairment of cognition[19]
Decline in protein synthesis	Further neuronal impairment caused by other factors[20,21]
Decline in stimulation and acetylcholine	Impaired memory circuitry[22,23]
Decreased levels of neurotrophic factors	Deficient support for neuronal survival[24,25]
Depression	Chronic inflammation, impairment in the signaling of neurotrophins[26]
Diabetes	Apoptosis of neurons, defects of long-term potentiation, changed synapse plasticity[27,28]
Downregulation of neprilysin	Promotion of Aβ deposits[29-31]
Enhanced reactive oxygen species levels and calcium overload	Contributes to mitochondrial dysfunction[32,33]
Glutamate increases	Neuronal excitotoxicity leading to neuronal death[34,35]
Hyperphosphorylated tau	Formation of neurofibrillary tangles[36,37]
Inflammation	Exacerbation of tau pathology[38], induction of Aβ release from neurons[39], attenuation of long-term potentiation[40], retraction of synapses[41]
Metal ion dyshomeostasis	Promotion of Aβ aggregation[42,43]
Mutations in genes for presenillin-1 and -2, and amyloid precursor protein; ApoE 4 allele genotype	Increased Aβ levels, linked to Aβ accumulation[44-46]

in AD was confirmed by Bonbinski *et al.*[61] who demonstrated that the neuronal count in CA1 in AD patients is 14% of that in a normal elderly control group. Masliah *et al.* found that mild AD cases show a 25% loss of synaptophysin, a synaptic protein[62]. These findings provide evidence that neuroregeneration might be an approach to treat AD.

NTF Deficits Cause Aβ Overload

Recent evidence suggests that Aβ neurotoxicity may be a consequence of NTF deficiency. This is supported by the finding that the AD11 mouse model, which expresses the recombinant monoclonal antibody αD11 that specifically neutralizes NGF thus causing NGF deprivation, shows AD-like pathology including Aβ accumulation and hippocampus-dependent memory deficits[63,64]. Based on the report that activation of the amyloidogenic route by NGF deprivation induces apoptotic death in PC12 cells[65], Matrone and colleagues further found that APP and presenilin 1 N-terminus (which is the active component endowed with γ-secretase activity) levels were increased in hippocampal neurons that were previously exposed to NGF or BDNF for 48 h followed by deprivation by anti-NGF (or -BDNF) antibodies[66]. These findings suggest that Aβ and NTF deficits cause a feed-forward loop that accelerates the toxicity of Aβ sufficient to cause neuronal death.

Moreover, p75 neurotrophin receptor (p75NTR), a pan-receptor for neurotrophins including BDNF, NGF, neurotrophin-3 and neurotrophin-4/5, has been reported to correlate with Aβ production. Knockout of p75NTR in APP-transgenic mice reduces the Aβ production in the brain, suggesting that activation of p75NTR in Aβ-related pa-

thologies leads to increased Aβ production[67]. This finding may well explain the sporadic AD which does not involve mutations of genes implicated in Aβ generation. In the AD brain, p75NTR expression is increased while neurotrophin levels are decreased, resulting in activation of the neurodegenerative pathway (triggered by proNGF and Aβ) that leads to Aβ overproduction, neuronal death, and finally cognitive decline, rather than activation of the neurotrophic pathway (triggered by neurotrophins) that promotes neuronal survival and improves cognition[68]. Moreover, AD11 transgenic mice with chronic NGF deprivation (that is, the transgenic anti-NGF antibodies bind to mature NGF much more strongly than to proNGF, leading to a decrease in mature NGF availability with a lower NGF/proNGF ratio) show Aβ accumulation and cognitive decline, for which the "neurotrophic deficit" hypothesis was recently refined as "neurotrophin imbalance"[63]. Interestingly, by crossing anti-NGF mice to $p75NTR^{exonIII(-/-)}$ mice, Capsoni reported a full rescue of anti-NGF mice with the Aβ phenotype by p75NTR signaling abrogation[69]. It would thus be reasonable to enhance the neurotrophin/p75NTR/Trks pathway by introducing exogenous NTFs, switching the neurodegenerative pathway of p75NTR to the neurotrophic pathway and reducing Aβ load in the brain.

The Status quo of AD Treatment Using Aβ Clearance

Aβ clearance has shown potential effectiveness in treating AD. Treatment with bapineuzumab, a humanized anti-Aβ monoclonal antibody, decreased the cortical Aβ load in patients with mild-moderate AD in a phase 2 clinical trial[70]. Exploratory analyses in another phase 2 trial of bapineuzumab in mild-moderate AD showed that potential treatment difference was found for 79 APOE ε4 noncarriers (47 bapineuzumab *vs* 32 placebo) and for completers (defined as patients who completed all bapineuzumab treatment and a week 78 efficacy assessment in this trial, 36 bapineuzumab *vs* 21 placebo)[71]. Bapinneuzumab is now undergoing phase 3 clinical trials. However, many failures have occurred in clinical trials for Aβ-lowering agents. Results obtained from a phase 1 trial on the long-term effects of $Aβ_{42}$ immunization using AN1792 (a synthetic form of the 42 amino-acid Aβ peptide) showed that the immunization cleared amyloid plaques composed primarily of Aβ in patients with AD but failed to prevent progressive neurodegeneration[72]. Alternative approaches that included targeting β- and γ-secretases, crucial enzymes for Aβ generation from APP, have been far from successful. Clinical trials using Semagacestat, the most studied γ-secretase inhibitor, have shown no promising improvement as far as cognitive function is concerned. Similar disappointing results have been obtained in two large phase 3 studies of a γ-secretase modulator (tarenflurbil) in patients with mild AD[73-75], and for tramiprosate, which targets the production of Aβ[76].

Therefore, a question arises as to why Aβ clearance for AD treatment seems not as effective as predicted by the Aβ hypothesis. One could argue that AD researchers should think outside Aβ box[77]. However, these failures do not predict the future, as proposed by Selkoe. On one hand, the failures of these experimental drugs may be due to their intrinsic drawbacks. Tarenflurbil is a weak γ-secretase modulator (~250 μmol/L half-maximal inhibitory concentration) with poor brain penetration[78]. Semagacestat is a non-selective γ-secretase inhibitor with a therapeutic index of <3; its half-maximal inhibitory concentration for Aβ reduction is only two to three times lower than that for inhibiting Notch cleavage[79], a receptor involved in regulating cell-fate decisions. Deficient Notch signaling is a severe side-effect of γ-secretase inhibitors; it can cause abnormalities in the gastrointestinal tract, thymus and spleen[80]. Besides, none of these experimental drugs showed enough potential disease-modifying of AD in phase 2 clinical trial but they were nevertheless advanced to phase 3 clinical trial[81], which could in part account for this status quo of Aβ clearance treatment. On the other hand, anti-Aβ therapies may be highly effective in preventing or slowing the progress of AD in asymptomatic patients with very early signs of AD pathology (that is, presymptomatic phases of AD are likely to benefit from anti-Aβ therapies)[82].

Other Therapeutic Approaches to AD

In addition to Aβ, microtubule-associated protein tau, the hyperphosphorylation of which contributes to axonal transport disruption and the accumulation of neurofibrillary tangles[83], is also an important therapeutic target for AD. As dysregulation of tau processing causes neuronal degeneration correlated with AD[84], researchers have made substantial efforts to search for targets to reverse abnormal

tau processing. To date, several kinases have been identified as key players in abnormal tau phosphorylation, particularly glycogen synthase kinase 3 (GSK-3) and protein phosphatase 2A[85]. The role of GSK-3 in the development of AD-like cognitive decline was supported by Liu *et al.*[86] who found that inhibition of phosphoinositol-3 kinase and protein kinase C results in overactivation of GSK-3, leading to tau hyperphosphorylation and eventually impaired spatial memory. Lithium, a GSK-3 inhibitor, has been shown to reduce the amount of altered tau protein in animal studies and improve cognitive and biological outcomes in participants with amnestic mild cognitive impairment in a phase 2 clinical study[87].

Besides, since inflammation may participate in the neurodegenerative process of AD, the use of non-steroidal anti-inflammatory medications such as naproxen may reduce AD incidence in vulnerable subjects[88]. Oxidative stress is also reported to play a role in the process of AD, as strongly proposed by Smith and Perry[89,90], and antioxidants may be associated with a lower risk of AD[91]. These treatment strategies for AD are still under way and more investigations and trials are needed to test their efficacy in its prevention or treatment.

Perspectives

Currently, a major problem we have to combat is that patients with AD do not receive interventional treatment until it has already developed to a level at which neurons have already been so disrupted that, we think, targeting Aβ alone is insufficient to cure the disease. Previous attempts using the approach of Aβ clearance decreased Aβ levels in the brain, as demonstrated by both preclinical studies in cellular or animal models and clinical trials. However, injured neurons cannot repair themselves. Neurons and synapses are the basic units of the nervous system and neural circuits. In the peripheral nervous system, injured neurons are capable of regenerating. Neuronal regeneration, however, does not occur normally in the mammalian central nervous system (CNS), a phenomenon that has been well documented[92]. Under normal circumstances, very few, if any, axons in the CNS can regenerate past a lesion site where distinctive large endings (retraction bulbs) are found[93].

There is a metaphor that vividly elucidates the failure of targeting Aβ to treat AD. Suppose a mouse runs into a house and chews through the electrical circuit for lighting the house, then the light bulbs associated with this circuit will be off due to the failure of power. A mousetrap is used to catch the mouse to prevent further impairment of the circuit. If the broken circuit is not repaired, the light bulb is still off even though the mouse has been captured. Not only should the mouse be captured, but the broken circuit should be fixed in order to make the light work. In this image, we metaphorically take the mouse to be Aβ, the mousetrap to be Aβ clearance, the electrical circuit as neuronal networks, and the light bulb as cognitive function. From the point of view of this metaphor, treatment of AD by combining Aβ clearance with neuroregeneration may be beneficial.

NTFs Promote Neuroregeneration

There is ample and encouraging evidence that NTFs stimulate neuronal growth, increase synapse number and promote the survival of mature mammalian CNS neurons[94-96]. A study by Nagahara *et al.*[97] showed that BDNF prevents the lesion-induced death of entorhinal cortical neurons in aged rats and reverses the synaptic loss in APP transgenic mice. It has also been demonstrated that NGF has a trophic influence on the basal forebrain cholinergic neurons whose pronounced loss is responsible for the cognitive decline in AD[98]. In view of the potential therapeutic effects of NTFs on degenerating neurons, efforts have been made to use them to treat AD[99]. Since the first discovery of NGF in the 1950s, a variety of chemical compounds that possess neurotrophic activity, such as magnesium fructose 1, 6-diphosphate[100], magnolol and honokiol[101], have been identified. The known ability of these compounds or factors to promote nerve regeneration and neuronal survival has made them a highly attractive group of targets for developing drugs for AD.

There are also encouraging outcomes from clinical trials using neurotrophic agents to treat AD. Cerebrolysin is a peptide preparation acting in a way similar to endogenous NTFs. In randomized controlled clinical trials, patients with moderate to moderately severe AD receiving cerebrolysin, compared with those assigned to receive placebo, had significant improvement in initiation of activities of daily living and cognitive performance scaled by the Alzheimer's Disease Assessment Scale Cognitive Subpart Modified[102,103].

Combination of Aβ Clearance and NTFs to Treat AD

As illustrated above, neuronal loss and synaptic dysfunction are the consequence or end-point of multiple but convergent pathways, and Aβ is the leading causative factor for AD. Aβ causes neuronal death in AD by decreasing NTF expression, whereas NTF deficits are associated with Aβ overproduction. Deficits need compensation; thus it is a promising approach to use NTFs for preventing neuronal degeneration. Here, we propose that a combination of Aβ clearance and administration of NTFs may be an effective way to treat AD.

First, Aβ clearance is supposed to clear senile plaques and decrease the levels of neurotoxic Aβ to prevent further neuronal death caused by Aβ. Second, NTFs are capable of protecting neurons against risk factors and may even promote axonal regeneration to restore impaired neuronal networks and synaptic plasticity; NTFs can even reverse Aβ overload in the brain caused by NTF deficits and open the feed-forward toxic loop constituted by Aβ and NTF deficiency. Last but not least, NTFs may increase the efficacy of Aβ clearance in the treatment of AD in the advanced stage. The severity of neuronal/synaptic loss varies at different stages of AD. Synaptic loss in CA1 in mild AD plummets to 55% that of groups with mild cognitive impairment (MCI) or no cognitive impairment (NCI), while the synaptic value in CA1 of the MCI brain decreases by 18% of that in NCI brain[105]. Rossler *et al.* found that, according to the Braak staging system, neuron density in CA1 is reduced by 33% at stage IV (P <0.02) and 51% at stage V (P <0.000 2) in comparison with stage I during AD[104]. These data suggest a strong correlation between neuronal/synaptic loss and severity of decline in cognition. Such loss may influence the efficacy of anti-Aβ on treating AD. This might well explain why anti-Aβ therapies have a high efficacy in preventing and delaying the development of AD in early stages of the disease, but are much less effective in advanced stages[106]. Thus, neuroregeneration agents may enable anti-Aβ to have high efficacy in the advanced stage.

Ideally, these two approaches can be combined to ameliorate the pathological process of AD or better still to improve cognitive function in AD patients. Different from the specific reduction of Aβ neurotoxicity by Aβ clearance, NTFs target and act through various receptors on a range of injured or degenerating neurons. It is therefore likely that the protective or rescuing effects of the two mechanisms can be synergistic, and may be more effective for the treatment of AD. This indeed has been reiterated by Chopra *et al*.[107]: "a synergistic combination of agents will have the capacity to alter the neurodegenerative cascade and the major aim should be to design ligands with pluripotent pharmacological activities".

ACKNOWLEDGEMENTS

We are grateful to Professors LS Jen and W Pan for critical reading of the manuscript. This review was supported by the National Natural Science Foundation of China (30672450) and the National Basic Research Development Program (973 Program) of China (2011CB707500).

Received date: 2012-04-17; Accepted date: 2012-06-12

REFERENCES

[1] Lin LF, Luo HM. Screening of treatment targets for Alzheimer's disease from the molecular mechanisms of impairment by β-amyloid aggregation and tau hyperphosphorylation. Neurosci Bull 2011, 27: 53–60.

[2] Holtzman DM, Morris JC, Goate AM. Alzheimer's disease: the challenge of the second century. Sci Transl Med 2011, 3: 77sr71.

[3] Winslow BT, Onysko MK, Stob CM, Hazlewood KA. Treatment of Alzheimer disease. Am Fam Physician 2011, 83: 1403–1412.

[4] Aisen PS, Cummings J, Schneider LS. Symptomatic and nonamyloid/tau based pharmacologic treatment for Alzheimer disease. Cold Spring Harb Perspect Med 2012, 2: a006395.

[5] Olivares D, Deshpande VK, Shi Y, Lahiri DK, Greig NH, Rogers JT, *et al.* N-Methyl D-Aspartate (NMDA) receptor antagonists and memantine treatment for Alzheimer's disease, vascular dementia and Parkinson's disease. Curr Alzheimer Res 2012, 9(6): 746–758.

[6] Jo J, Whitcomb DJ, Olsen KM, Kerrigan TL, Lo SC, Bru-Mercier G, *et al.* Abeta(1-42) inhibition of LTP is mediated by a signaling pathway involving caspase-3, Akt1 and GSK-3beta. Nat Neurosci 2011, 14(5): 545–547.

[7] Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, *et al.* Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat Med 2008, 14: 837–842.

[8] Shipton OA, Leitz JR, Dworzak J, Acton CE, Tunbridge EM,

Denk F, *et al.* Tau protein is required for amyloid {beta}-induced impairment of hippocampal long-term potentiation. J Neurosci 2011, 31: 1688–1692.

[9] Peng S, Garzon DJ, Marchese M, Klein W, Ginsberg SD, Francis BM, *et al.* Decreased brain-derived neurotrophic factor depends on amyloid aggregation state in transgenic mouse models of Alzheimer's disease. J Neurosci 2009, 29: 9321–9329.

[10] Van Muiswinkel FL, Veerhuis R, Eikelenboom P. Amyloid beta protein primes cultured rat microglial cells for an enhanced phorbol 12-myristate 13-acetate-induced respiratory burst activity. J Neurochem 1996, 66: 2468–2476.

[11] Hensley K, Carney JM, Mattson MP, Aksenova M, Harris M, Wu JF, *et al.* A model for beta-amyloid aggregation and neurotoxicity based on free radical generation by the peptide: relevance to Alzheimer disease. Proc Natl Acad Sci U S A 1994, 91: 3270–3274.

[12] Gotz J, Chen F, van Dorpe J, Nitsch RM. Formation of neurofibrillary tangles in P301l tau transgenic mice induced by Abeta 42 fibrils. Science 2001, 293: 1491–1495.

[13] Bell RD, Zlokovic BV. Neurovascular mechanisms and blood-brain barrier disorder in Alzheimer's disease. Acta Neuropathol 2009, 118: 103–113.

[14] Serlin Y, Levy J, Shalev H. Vascular pathology and blood-brain barrier disruption in cognitive and psychiatric complications of type 2 diabetes mellitus. Cardiovasc Psychiatry Neurol 2011, 2011: 609202.

[15] Yokel RA. Blood-brain barrier flux of aluminum, manganese, iron and other metals suspected to contribute to metal-induced neurodegeneration. J Alzheimer's Dis 2006, 10: 223–253.

[16] Plassman BL, Havlik RJ, Steffens DC, Helms MJ, Newman TN, Drosdick D, *et al.* Documented head injury in early adulthood and risk of Alzheimer's disease and other dementias. Neurology 2000, 55: 1158–1166.

[17] Lye TC, Shores EA. Traumatic brain injury as a risk factor for Alzheimer's disease: a review. Neuropsychol Rev 2000, 10: 115–129.

[18] Sotiropoulos I, Catania C, Pinto LG, Silva R, Pollerberg GE, Takashima A, *et al.* Stress acts cumulatively to precipitate Alzheimer's disease-like tau pathology and cognitive deficits. J Neurosci 2011, 31: 7840–7847.

[19] Tran TT, Srivareerat M, Alkadhi KA. Chronic psychosocial stress accelerates impairment of long-term memory and late-phase long-term potentiation in an at-risk model of Alzheimer's disease. Hippocampus 2011, 21: 724–732.

[20] Keller JN. Interplay between oxidative damage, protein synthesis, and protein degradation in Alzheimer's disease. J Biomed Biotechnol 2006, 2006: 1–3.

[21] Ding QX, Dimayuga E, Keller JN. Oxidative damage, protein synthesis, and protein degradation in Alzheimer's disease. Current Alzheimer Res 2007, 4: 73–79.

[22] Laxton AW, Tang-Wai DF, McAndrews MP, Zumsteg D, Wennberg R, Keren R, *et al.* A Phase I trial of deep brain stimulation of memory circuits in Alzheimer's disease. Ann Neurol 2010, 68: 521–534.

[23] Bentwich J, Dobronevsky E, Aichenbaum S, Shorer R, Peretz R, Khaigrekht M, *et al*. Beneficial effect of repetitive transcranial magnetic stimulation combined with cognitive training for the treatment of Alzheimer's disease: a proof of concept study. J Neural Transmission 2011, 118: 463–471.

[24] Levy YS, Gilgun-Sherki Y, Melamed E, Offen D. Therapeutic potential of neurotrophic factors in neurodegenerative diseases. BioDrugs 2005, 19: 97–127.

[25] Ji C, Song C, Zuo P. The mechanism of memory impairment induced by Abeta chronic administration involves imbalance between cytokines and neurotrophins in the rat hippocampus. Curr Alzheimer Res 2011, 8: 410–420.

[26] Caraci F, Copani A, Nicoletti F, Drago F. Depression and Alzheimer's disease: neurobiological links and common pharmacological targets. Eur J Pharmacol 2010, 626: 64–71.

[27] Ciobica A, Padurariu M, Bild W, Stefanescu C. Cardiovascular risk factors as potential markers for mild cognitive impairment and Alzheimer's disease. Psychiatr Danub 2011, 23: 340–346.

[28] Trudeau F, Gagnon S, Massicotte G. Hippocampal synaptic plasticity and glutamate receptor regulation: influences of diabetes mellitus. Eur J Pharmacol 2004, 490: 177–186.

[29] Miners JS, Van Helmond Z, Chalmers K, Wilcock G, Love S, Kehoe PG. Decreased expression and activity of neprilysin in Alzheimer disease are associated with cerebral amyloid angiopathy. J Neuropathol Exp Neurol 2006, 65: 1012–1021.

[30] El-Amouri SS, Zhu H, Yu J, Marr R, Verma IM, Kindy MS. Neprilysin: An enzyme candidate to slow the progression of Alzheimer's disease. American J Pathology 2008, 172: 1342–1354.

[31] Miners JS, Kehoe P, Love S. Neprilysin protects against cerebral amyloid angiopathy and Abeta-induced degeneration of cerebrovascular smooth muscle cells. Brain Pathol 2011, 21(5):594–605.

[32] Nicholls DG. Mitochondrial calcium function and dysfunction in the central nervous system. Biochim Biophys Acta 2009, 1787: 1416–1424.

[33] Duchen MR. Mitochondria in health and disease: perspectives on a new mitochondrial biology. Mol Aspects Med 2004, 25: 365–451.

[34] Kim K, Lee SG, Kegelman TP, Su ZZ, Das SK, Dash R, *et al.* Role of excitatory amino acid transporter-2 (EAAT2) and glutamate in neurodegeneration: opportunities for

developing novel therapeutics. J Cell Physiol 2011, 226: 2484–2493.

[35] Scott HA, Gebhardt FM, Mitrovic AD, Vandenberg RJ, Dodd PR. Glutamate transporter variants reduce glutamate uptake in Alzheimer's disease. Neurobiol Aging 2011, 32: 553 e551–553 e511.

[36] Guillozet-Bongaarts AL, Garcia-Sierra F, Reynolds MR, Horowitz PM, Fu YF, Wang TY, *et al.* Tau truncation during neurofibrillary tangle evolution in Alzheimer's disease. Neurobiol Aging 2005, 26: 1015–1022.

[37] Pei JJ, Braak H, Gong CX, Grundke-Iqbal I, Iqbal K, Winblad B, *et al*. Up-regulation of cell division cycle (cdc) 2 kinase in neurons with early stage Alzheimer's disease neurofibrillary degeneration. Acta Neuropathol 2002, 104: 369–376.

[38] Lee DC, Rizer J, Selenica ML, Reid P, Kraft C, Johnson A, *et al*. LPS-induced inflammation exacerbates phospho-tau pathology in rTg4510 mice. J Neuroinflammation 2010, 7: 56.

[39] Nagele RG, Wegiel J, Venkataraman V, Imaki H, Wang KC, Wegiel J. Contribution of glial cells to the development of amyloid plaques in Alzheimer's disease. Neurobiol Aging 2004, 25: 663–674.

[40] Griffin R, Nally R, Nolan Y, McCartney Y, Linden J, Lynch MA. The age-related attenuation in long-term potentiation is associated with microglial activation. J Neurochem 2006, 99: 1263–1272.

[41] Farfara D, Lifshitz V, Frenkel D. Neuroprotective and neurotoxic properties of glial cells in the pathogenesis of Alzheimer's disease. J Cell Mol Med 2008, 12: 762–780.

[42] Ha C, Ryu J, Park CB. Metal ions differentially influence the aggregation and deposition of Alzheimer's beta-amyloid on a solid template. Biochemistry 2007, 46: 6118–6125.

[43] Zatta P, Drago D, Bolognin S, Sensi SL. Alzheimer's disease, metal ions and metal homeostatic therapy. Trends Pharmacol Sci 2009, 30: 346–355.

[44] Oakley H, Cole SL, Logan S, Maus E, Shao P, Craft J, *et al.* Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: Potential factors in amyloid plaque formation. J Neurosci 2006, 26: 10129–10140.

[45] Janssen JC, Beck JA, Campbell TA, Dickinson A, Fox NC, Harvey RJ, *et al.* Early onset familial Alzheimer's disease: Mutation frequency in 31 families. Neurology 2003, 60: 235–239.

[46] Leoni V. The effect of apolipoprotein E (ApoE) genotype on biomarkers of amyloidogenesis, tau pathology and neurodegeneration in Alzheimer's disease. Clin Chem Lab Med 2011, 49: 375–383.

[47] Hardy J, Orr H. The genetics of neurodegenerative diseases. J Neurochem 2006, 97: 1690–1699.

[48] Rovelet-Lecrux A, Hannequin D, Raux G, Le Meur N, Laquerriere A, Vital A, *et al.* APP locus duplication causes autosomal dominant early-onset Alzheimer disease with cerebral amyloid angiopathy. Nat Genet 2006, 38: 24–26.

[49] Barthel H, Luthardt J, Becker G, Patt M, Hammerstein E, Hartwig K, *et al.* Individualized quantification of brain beta-amyloid burden: results of a proof of mechanism phase 0 florbetaben PET trial in patients with Alzheimer's disease and healthy controls. Eur J Nucl Med Mol Imaging 2011, 38: 1702–1714.

[50] Barthel H, Gertz HJ, Dresel S, Peters O, Bartenstein P, Buerger K, *et al.* Cerebral amyloid-beta PET with florbetaben (18F) in patients with Alzheimer's disease and healthy controls: a multicentre phase 2 diagnostic study. Lancet Neurol 2011, 10: 424–435.

[51] Jin M, Shepardson N, Yang T, Chen G, Walsh D, Selkoe DJ. Soluble amyloid beta-protein dimers isolated from Alzheimer cortex directly induce Tau hyperphosphorylation and neuritic degeneration. Proc Natl Acad Sci U S A 2011, 108: 5819–5824.

[52] Zeng KW, Ko H, Yang HO, Wang XM. Icariin attenuates β-amyloid-induced neurotoxicity by inhibition of tau protein hyperphosphorylation in PC12 cells. Neuropharmacology 2010, 59: 542–550.

[53] Colangelo V, Schurr J, Ball MJ, Pelaez RP, Bazan NG, Lukiw WJ. Gene expression profiling of 12633 genes in Alzheimer hippocampal CA1: transcription and neurotrophic factor down-regulation and up-regulation of apoptotic and pro-inflammatory signaling. J Neurosci Res 2002, 70: 462–473.

[54] Garzon D, Yu G, Fahnestock M. A new brain-derived neurotrophic factor transcript and decrease in brain-derived neurotrophic factor transcripts 1, 2 and 3 in Alzheimer's disease parietal cortex. J Neurochem 2002, 82: 1058–1064.

[55] Peng S, Wuu J, Mufson EJ, Fahnestock M. Precursor form of brain-derived neurotrophic factor and mature brain-derived neurotrophic factor are decreased in the pre-clinical stages of Alzheimer's disease. J Neurochem 2005, 93: 1412–1421.

[56] Selkoe DJ. Alzheimer's disease. Cold Spring Harb Perspect Biol 2011: Epub.

[57] Seeman P, Seeman N. Alzheimer's disease: beta-amyloid plaque formation in human brain. Synapse 2011, 65(12): 1289–1297.

[58] Ohyagi Y. Intracellular amyloid beta-protein as a therapeutic target for treating Alzheimer's disease. Curr Alzheimer Res 2008, 5: 555–561.

[59] Roberson ED, Mucke L. 100 years and counting: prospects for defeating Alzheimer's disease. Science 2006, 314: 781–784.

[60] West MJ, Coleman PD, Flood DG, Troncoso JC. Differ-

ences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease. Lancet 1994, 344: 769–772.

[61] Bobinski M, Wegiel J, Tarnawski M, Bobinski M, Reisberg B, de Leon MJ, *et al.* Relationships between regional neuronal loss and neurofibrillary changes in the hippocampal formation and duration and severity of Alzheimer disease. J Neuropathol Exp Neurol 1997, 56: 414–420.

[62] Masliah E, Mallory M, Alford M, DeTeresa R, Hansen LA, McKeel DW Jr, *et al.* Altered expression of synaptic proteins occurs early during progression of Alzheimer's disease. Neurology 2001, 56: 127–129.

[63] Capsoni S, Giannotta S, Cattaneo A. Beta-amyloid plaques in a model for sporadic Alzheimer's disease based on transgenic anti-nerve growth factor antibodies. Mol Cell Neurosci 2002, 21: 15–28.

[64] Houeland G, Romani A, Marchetti C, Amato G, Capsoni S, Cattaneo A, *et al.* Transgenic mice with chronic NGF deprivation and Alzheimer's disease-like pathology display hippocampal region-specific impairments in short- and long-term plasticities. J Neurosci 2010, 30: 13089–13094.

[65] Matrone C, Di Luzio A, Meli G, D'Aguanno S, Severini C, Ciotti MT, *et al*. Activation of the amyloidogenic route by NGF deprivation induces apoptotic death in PC12 cells. J Alzheimers Dis 2008, 13: 81–96.

[66] Matrone C, Ciotti MT, Mercanti D, Marolda R, Calissano P. NGF and BDNF signaling control amyloidogenic route and Abeta production in hippocampal neurons. Proc Natl Acad Sci U S A 2008, 105: 13139–13144.

[67] Wang YJ, Wang X, Lu JJ, Li QX, Gao CY, Liu XH, *et al.* p75NTR regulates Abeta deposition by increasing Abeta production but inhibiting Abeta aggregation with its extracellular domain. J Neurosci 2011, 31: 2292–2304.

[68] Zeng F, Lu JJ, Zhou XF, Wang YJ. Roles of p75NTR in the pathogenesis of Alzheimer's disease: a novel therapeutic target. Biochem Pharmacol 2011, 82: 1500–1509.

[69] Capsoni S, Tiveron C, Vignone D, Amato G, Cattaneo A. Dissecting the involvement of tropomyosin-related kinase A and p75 neurotrophin receptor signaling in NGF deficit-induced neurodegeneration. Proc Natl Acad Sci U S A 2010, 107: 12299–12304.

[70] Rinne JO, Brooks DJ, Rossor MN, Fox NC, Bullock R, Klunk WE, *et al.* 11C-PiB PET assessment of change in fibrillar amyloid-beta load in patients with Alzheimer's disease treated with bapineuzumab: a phase 2, double-blind, placebo-controlled, ascending-dose study. Lancet Neurol 2010, 9: 363–372.

[71] Salloway S, Sperling R, Gilman S, Fox NC, Blennow K, Raskind M, *et al.* A phase 2 multiple ascending dose trial of bapineuzumab in mild to moderate Alzheimer disease. Neurology 2009, 73: 2061–2070.

[72] Holmes C, Boche D, Wilkinson D, Yadegarfar G, Hopkins V, Bayer A, *et al.* Long-term effects of A beta(42) immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. Lancet 2008, 372: 216–223.

[73] Imbimbo BP, Giardina GA. Gamma-secretase inhibitors and modulators for the treatment of Alzheimer's disease: disappointments and hopes. Curr Top Med Chem 2011, 11: 1555–1570.

[74] Vellas B. Tarenflurbil for Alzheimer's disease: a "shot on goal" that missed. Lancet Neurol 2010, 9: 235–237.

[75] Imbimbo BP, Panza F, Frisardi V, Solfrizzi V, D'Onofrio G, Logroscino G, *et al.* Therapeutic intervention for Alzheimer's disease with gamma-secretase inhibitors: still a viable option? Expert Opin Investig Drugs 2011, 20: 325–341.

[76] Sabbagh MN. Drug development for Alzheimer's disease: where are we now and where are we headed? Am J Geriatr Pharmacother 2009, 7: 167–185.

[77] D'Alton S, George DR. Changing perspectives on Alzheimer's disease: thinking outside the amyloid box. J Alzheimers Dis 2011: Epub ahead of print.

[78] Golde TE, Petrucelli L, Lewis J. Targeting Abeta and tau in Alzheimer's disease, an early interim report. Exp Neurol 2010, 223: 252–266.

[79] Selkoe DJ. Resolving controversies on the path to Alzheimer's therapeutics. Nat Med 2011, 17: 1060–1065.

[80] Geling A, Steiner H, Willem M, Bally-Cuif L, Haass C. A gamma-secretase inhibitor blocks Notch signaling *in vivo* and causes a severe neurogenic phenotype in zebrafish. EMBO Rep 2002, 3: 688–694.

[81] Golde TE, Schneider LS, Koo EH. Anti-abeta therapeutics in Alzheimer's disease: the need for a paradigm shift. Neuron 2011, 69: 203–213.

[82] Lemere CA, Masliah E. Can Alzheimer disease be prevented by amyloid-beta immunotherapy? Nat Rev Neurol 2010, 6: 108–119.

[83] Berger Z, Roder H, Hanna A, Carlson A, Rangachari V, Yue M, *et al.* Accumulation of pathological tau species and memory loss in a conditional model of tauopathy. J Neurosci 2007, 27: 3650–3662.

[84] Iqbal K, Grundke-Iqbal I, Zaidi T, Merz PA, Wen GY, Shaikh SS, *et al.* Defective brain microtubule assembly in Alzheimer's disease. Lancet 1986, 2: 421–426.

[85] Liu R, Pei JJ, Wang XC, Zhou XW, Tian Q, Winblad B, *et al.* Acute anoxia induces tau dephosphorylation in rat brain slices and its possible underlying mechanisms. J Neurochem 2005, 94: 1225–1234.

[86] Liu SJ, Zhang AH, Li HL, Wang Q, Deng HM, Netzer WJ, *et al.* Overactivation of glycogen synthase kinase-3 by inhibition of phosphoinositol-3 kinase and protein kinase C leads

to hyperphosphorylation of tau and impairment of spatial memory. J Neurochem 2003, 87: 1333–1344.

[87] Forlenza OV, Diniz BS, Radanovic M, Santos FS, Talib LL, Gattaz WF. Disease-modifying properties of long-term lithium treatment for amnestic mild cognitive impairment: randomised controlled trial. Br J Psychiatry 2011, 198: 351–356.

[88] Breitner JC, Baker LD, Montine TJ, Meinert CL, Lyketsos CG, Ashe KH, *et al.* Extended results of the Alzheimer's disease anti-inflammatory prevention trial. Alzheimers Dement 2011, 7: 402–411.

[89] Smith MA, Rottkamp CA, Nunomura A, Raina AK, Perry G. Oxidative stress in Alzheimer's disease. Biochim Biophys Acta 2000, 1502: 139–144.

[90] Perry G, Cash AD, Smith MA. Alzheimer disease and oxidative stress. J Biomed Biotechnol 2002, 2: 120–123.

[91] Rutten BP, Steinbusch HW, Korr H, Schmitz C. Antioxidants and Alzheimer's disease: from bench to bedside (and back again). Curr Opin Clin Nutr Metab Care 2002, 5: 645–651.

[92] Hammarlund M, Nix P, Hauth L, Jorgensen EM, Bastiani M. Axon regeneration requires a conserved MAP kinase pathway. Science 2009, 323: 802–806.

[93] Case LC, Tessier-Lavigne M. Regeneration of the adult central nervous system. Curr Biol 2005, 15: R749–753.

[94] Yuen EC, Howe CL, Li Y, Holtzman DM, Mobley WC. Nerve growth factor and the neurotrophic factor hypothesis. Brain Dev 1996, 18: 362–368.

[95] Hu B, Nikolakopoulou AM, Cohen-Cory S. BDNF stabilizes synapses and maintains the structural complexity of optic axons *in vivo*. Development 2005, 132: 4285–4298.

[96] Grimes ML, Zhou J, Beattie EC, Yuen EC, Hall DE, Valletta JS, *et al.* Endocytosis of activated TrkA: evidence that nerve growth factor induces formation of signaling endosomes. J Neurosci 1996, 16: 7950–7964.

[97] Nagahara AH, Merrill DA, Coppola G, Tsukada S, Schroeder BE, Shaked GM, *et al.* Neuroprotective effects of brain-derived neurotrophic factor in rodent and primate models of Alzheimer's disease. Nat Med 2009, 15: 331–337.

[98] Fjord-Larsen L, Kusk P, Emerich DF, Thanos C, Torp M, Bintz B, *et al.* Increased encapsulated cell biodelivery of nerve growth factor in the brain by transposon-mediated gene transfer. Gene Ther 2011, 19(10): 1010–1017

[99] Huang EJ, Reichardt LF. Neurotrophins: roles in neuronal development and function. Annu Rev Neurosci 2001, 24: 677–736.

[100] Lin LF, Xue XY, Liao MJ, Xiao F, Lv RH, Luo HM. Neurotrophic effects of magnesium fructose 1, 6-diphosphate on cortical neurons. Int J Neurosci 2011, 122: 248–254.

[101] Matsui N, Takahashi K, Takeichi M, Kuroshita T, Noguchi K, Yamazaki K, *et al.* Magnolol and honokiol prevent learning and memory impairment and cholinergic deficit in SAMP8 mice. Brain Res 2009, 1305: 108–117.

[102] Alvarez XA, Cacabelos R, Sampedro C, Aleixandre M, Linares C, Granizo E, *et al.* Efficacy and safety of Cerebrolysin in moderate to moderately severe Alzheimer's disease: results of a randomized, double-blind, controlled trial investigating three dosages of Cerebrolysin. Eur J Neurol 2011, 18: 59–68.

[103] Muresanu DF, Rainer M, Moessler H. Improved global function and activities of daily living in patients with AD: a placebo-controlled clinical study with the neurotrophic agent Cerebrolysin. J Neural Transm Suppl 2002: 277–285.

[104] Rossler M, Zarski R, Bohl J, Ohm TG. Stage-dependent and sector-specific neuronal loss in hippocampus during Alzheimer's disease. Acta Neuropathol 2002, 103: 363–369.

[105] Scheff SW, Price DA, Schmitt FA, DeKosky ST, Mufson EJ. Synaptic alterations in CA1 in mild Alzheimer disease and mild cognitive impairment. Neurology 2007, 68: 1501–1508.

[106] Reiman EM, Langbaum JB, Tariot PN. Alzheimer's prevention initiative: a proposal to evaluate presymptomatic treatments as quickly as possible. Biomark Med 2010, 4: 3–14.

[107] Chopra K, Misra S, Kuhad A. Current perspectives on pharmacotherapy of Alzheimer's disease. Expert Opin Pharmacother 2011, 12: 335–350.