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

Lian-Feng Lin^{1, #}, Min-Jing Liao^{1, 4, #}, Xiao-Yan Xue⁵, Wei Zhang¹, Li Yan¹, Liang Cai¹, Xiao-Wen Zhou¹, Xing Zhou¹, Huan-Min Luo^{1, 2, 3}

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

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

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

⁵Ganzhou People's Hospital, Ganzhou 341000, China

[#]These authors contributed equally to this review.

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

© Shanghai Institutes for Biological Sciences, CAS and Springer-Verlag Berlin Heidelberg 2013

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 (A β) 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 A β clearance and NTFs are potentially effective in treating AD, thus a new approach combining A β 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 (A β) 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, A β and neurotrophic factors (NTFs).

A β as a Promising Target for AD Treatment

A β 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 A β cascade hypothesis proposed by Hardy and Selkoe, the accumulation of extracellular A β 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 A β . Although many factors are also known to be associated with AD (Table 1), the A β hypothesis remains the focus of research. This is strongly supported by the observation of abundant deposits of A β 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 A β 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 A β , A β 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 ¹⁸F-labeled A β -targeted PET tracer), demonstrated that nine of ten mild-moderate probable AD participants (DSM-IV and NINCDS-ADRDA criteria) were A β -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, A β 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 A β -induced neurotoxicity can be attenuated by inhibiting tau protein hyperphosphorylation. It is conceivable that the two pathological hallmarks of AD, A β deposits and neurofibrillary tangles, may actually reflect two distinct patterns of impairment caused by A β .

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

To date, various mechanisms underlying the impairments by A β 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).

A β Downregulates NTFs

As neurotrophic effects are directly correlated with neuronal survival and the maintenance of synaptic plasticity, neurotrophic imbalance is a likely mechanism of A β 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, A β 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 A β 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 A β production and reduced BDNF levels in AD^[53–55]. A β 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
A β	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 A β ^[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 A β 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 A β release from neurons ^[39] , attenuation of long-term potentiation ^[40] , retraction of synapses ^[41]
Metal ion dyshomeostasis	Promotion of A β aggregation ^[42,43]
Mutations in genes for presenilin-1 and -2, and amyloid precursor protein; ApoE 4 allele genotype	Increased A β levels, linked to A β 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 A β Overload

Recent evidence suggests that A β 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 α D11 that specifically neutralizes NGF thus causing NGF deprivation, shows AD-like pathology including A β 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 A β and NTF deficits cause a feed-forward loop that accelerates the toxicity of A β 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 A β production. Knockout of p75NTR in APP-transgenic mice reduces the A β production in the brain, suggesting that activation of p75NTR in A β -related pa-

thologies leads to increased A β production^[67]. This finding may well explain the sporadic AD which does not involve mutations of genes implicated in A β 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 A β) that leads to A β 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 A β 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 A β 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 A β load in the brain.

The Status quo of AD Treatment Using A β Clearance

A β clearance has shown potential effectiveness in treating AD. Treatment with bapineuzumab, a humanized anti-A β monoclonal antibody, decreased the cortical A β 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 ϵ 4 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]. Bapineuzumab is now undergoing phase 3 clinical trials. However, many failures have occurred in clinical trials for A β -lowering agents. Results obtained from a phase 1 trial on the long-term effects of A β ₄₂ immunization using AN1792 (a synthetic form of the 42 amino-acid A β peptide) showed that the immunization cleared amyloid plaques composed primarily of A β in pa-

tients with AD but failed to prevent progressive neurodegeneration^[72]. Alternative approaches that included targeting β - and γ -secretases, crucial enzymes for A β 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 A β ^[76].

Therefore, a question arises as to why A β clearance for AD treatment seems not as effective as predicted by the A β hypothesis. One could argue that AD researchers should think outside A β 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 μ mol/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 A β 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 A β clearance treatment. On the other hand, anti-A β 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-A β therapies)^[82].

Other Therapeutic Approaches to AD

In addition to A β , 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 amnesic 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 A β alone is insufficient to cure the disease. Previous attempts using the approach of A β clearance decreased A β 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 A β 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 A β , the mousetrap to be A β 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 A β 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 A β 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 A β is the leading causative factor for AD. A β causes neuronal death in AD by decreasing NTF expression, whereas NTF deficits are associated with A β overproduction. Deficits need compensation; thus it is a promising approach to use NTFs for preventing neuronal degeneration. Here, we propose that a combination of A β clearance and administration of NTFs may be an effective way to treat AD.

First, A β clearance is supposed to clear senile plaques and decrease the levels of neurotoxic A β to prevent further neuronal death caused by A β . 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 A β overload in the brain caused by NTF deficits and open the feed-forward toxic loop constituted by A β and NTF deficiency. Last but not least, NTFs may increase the efficacy of A β 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 ($P < 0.0002$) 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-A β on treating AD. This might well explain why anti-A β 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-A β 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 A β neurotoxicity by A β 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, Brumercier 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 P301 τ transgenic mice induced by A β 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, Wen-berg 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 A β 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 A β -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] Duchon 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. Icaritin 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, Wu 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] Ohnishi 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, Ras-kind 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-amyloid 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 phosphoinositide-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 amnesic 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.