

Diverse Molecular Targets for Therapeutic Strategies in Alzheimer's Disease

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Received: 27 February 2014

Accepted: 12 June 2014

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Funding: This work was supported by grants from the NRF (2012R1A2A1A01002881), the KNIH ROAD R&D Program Project (A092058 to I. M-J) and KIST ORP program.

Alzheimer's disease (AD) is the most common form of dementia caused by neurodegenerative process and is tightly related to amyloid β (A β) and neurofibrillary tangles. The lack of early diagnostic biomarker and therapeutic remedy hinders the prevention of increasing population of AD patients every year. In spite of accumulated scientific information, numerous clinical trials for candidate drug targets have failed to be preceded into therapeutic development, therefore, AD-related sufferers including patients and caregivers, are desperate to seek the solution. Also, effective AD intervention is desperately needed to reduce AD-related societal threats to public health. In this review, we summarize various drug targets and strategies in recent preclinical studies and clinical trials for AD therapy: Allopathic treatment, immunotherapy, A β production/aggregation modulator, tau-targeting therapy and metabolic targeting. Some has already failed in their clinical trials and the others are still in various stages of investigations, both of which give us valuable information for future research in AD therapeutic development.

Key Words: Alzheimer Disease; Amyloid Beta-Peptides; Tau Protein; Immunotherapy; Glucose Metabolism; Aggregation

INTRODUCTION

Alzheimer's disease (AD) is a pernicious neurodegenerative disease which is incurable with remedies developed up to date. The number of patients increases every year worldwide and 5.2 million people in the United States are suffering from AD. This number is expected to expand to 36 million to 115 million in worldwide around the year of 2050 and the estimated economic cost and suffering is increasing greatly (1, 2). Therefore, AD is seemingly insurmountable disease and the increasing numbers of patients produce diverse societal concerns in different aspects.

Senile plaques of amyloid β (A β) in the brain parenchyma have been regarded as not only the main pathological phenomena (3, 4) but also the culprit of this disease according to amyloid cascade hypothesis based on molecular information found in AD study (5). Abnormal production and accumulation of A β in brain parenchyma result in AD pathologies through sequential events by aggregated forms of this protein and the amyloid plaque. A β is generated as a consequence of sequential cleavages of amyloid precursor protein (APP) by β - and γ -secretases (6-8). APP is first cleaved by either α - or β -secretase, and then, the remaining remnants of C83 or C99, respectively, are vulnerable to intramembrane proteolysis by γ -secretase. Amyloidogenic process of γ -secretase cleavage followed by β -secretase produces aggregation-prone A β which are in the center of AD

etiology (4, 9). Disrupted synaptic plasticity, reduced dendritic spine density and memory impairment were proven in rodent model by extraction of A β oligomers from human patients (10).

Along with A β , microtubule-associated protein tau is another major factor of AD pathogenesis as a component of neurofibrillary tangles (NFTs) (11). Tau stabilizes microtubule protein and microtubule-associated processes in normal condition. During AD pathogenesis, tau becomes hyperphosphorylated, aggregated and finally accumulated as neurofibrillary tangles (12). Tau hyperphosphorylation and NFT formation is tightly related to the existence of excessive A β and plaques, proving the tau pathology in AD (13, 14). Not only as axonal protein but also as regulator of dendritic function, tau plays a pivotal role, especially mediating early A β toxicity during AD progress (15). Therefore, inevitably, A β and tau became the main targets in drug development. Many clinical trials aiming these two proteins have been performed whereas several lines of targets are still under investigations.

Up to date, only a few AD medications have been proved as improving AD symptoms, but none of them modify disease progress or pathological cascades (16). Researchers and clinicians suspect that the reason for many drug target candidates to fail in their clinical trials reside in the improper time of drug treatment, in fairly late stage of AD progression where irreversible damages have already occurred, including excessive A β deposits, neuronal impairment, death and blood brain barrier

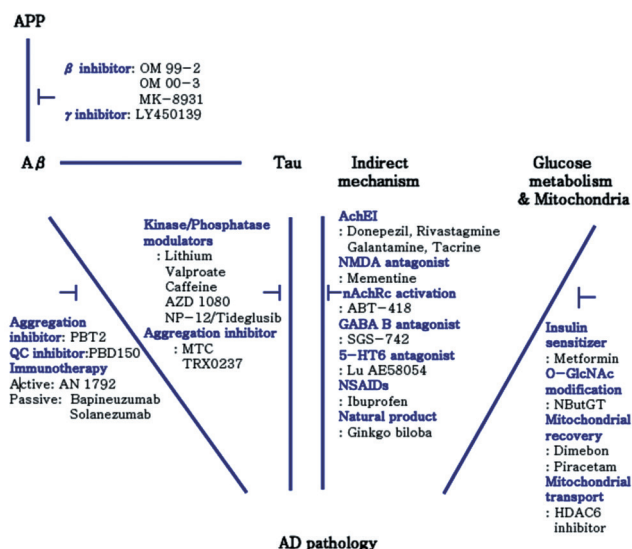


Fig. 1. Diverse AD therapeutic strategies and their example chemicals tested in pre-clinical or clinical studies. APP, Amyloid precursor protein; Aβ, Amyloid β; QC, Glutaminyl cyclase; AChEi, Acetylcholinesterase inhibitors; nAChRc, Nicotinic acetylcholine receptor; 5-HT6 Rc, 5-hydroxytryptamine 6 receptor; NSAIDs, Nonsteroidal anti-inflammatory drugs; O-GlcNAc, O-linked β-N-acetylglucosamine; NButGT, 1, 2-dideoxy-2'-propyl-α-D-glucopyranoso-[2,1-d]-Δ2'-thiazoline; HDAC, Histone deacetylation.

(BBB) disruption (17). Therefore, finding a diagnostic biomarker, especially for the early stages of AD pathology, is desperately needed for developing a valuable therapeutic target at the early stage and preventing progression of the disease.

Diverse approaches for AD therapeutic strategy have arisen along with better understanding of cellular and molecular mechanism of AD pathogenesis. In Fig. 1 and Table 1, we listed possible strategies of drug development target based on accumulated scientific findings. In this review, we summarize the recent status of some AD drug targets using different strategies among them from published reports and ongoing clinical studies. In each category, we stated the representative drug targets, preclinical and clinical trials.

ALLOPATHIC TREATMENT FOR AD

In spite of better understanding for molecular mechanism during AD pathogenesis, the available medications for AD up to date provide only symptomatic benefit, not regulate or delay the progression of disease pathology. The US Food and Drug Administration (FDA) has approved only five medications for AD, including acetylcholinesterase inhibitors (AChEIs: donepezil, rivastigmine, galantamine and tacrine) and N-methyl-D-aspartate (NMDA) receptor antagonist, such as memantine (18-21). These medications enhance cognitive function via increased acetylcholine level or glutamatergic receptor blocking, respectively. Combined administration of these two medications accelerates symptomatic improvement, representing slow

Table 1. Diverse strategies for Alzheimer's disease therapeutic target. This table was modified from the box table by Grill and Cummings (26)

Strategy	Target
Aβ production	α-secretase activation β-secretase inhibition γ-secretase inhibition γ-secretase modulation APP modulation
Aβ degradation	Neprilysin activation Insulin-degrading enzyme activation ApoE
Aβ removal	Vaccination Passive immunization General immune system modulation Microglial activation Receptor-mediated removal from CNS to periphery Prevent entry from periphery to CNS Modulation of Aβ balance between CNS and periphery
Preventing Aβ toxicity	Aggregation inhibition by binding Aβ Oligomerization inhibition through metal protein attenuation Prevent formation of pyroglutaminyl Aβ
Tau	Tau aggregation inhibition Prevent tau hyperphosphorylation Facilitate tau phosphatase Microtubule stabilization Increase tau clearance
Neuroprotection	Neuronal growth factor Anti-apoptotic agents Mitochondrial improvement Anti-inflammation BBB integrity Neuronal metabolism

Aβ, Amyloid β; APP, Amyloid precursor protein; ApoE, Apolipoprotein E; CNS, Central Nervous System; BBB, Blood Brain Barrier.

progress of cognitive and functional impairment and delayed time for nursing home admission (22, 23). Besides, several lines of regulators for neurotransmission were suggested for symptomatic therapies in AD, including neuronal nicotinic acetylcholine receptor activation (ABT-418), GABA_B receptor antagonism (SGS-742) and serotonergic modulation (Lu AE58054). However, they were insufficient to show significant efficacies of symptomatic improvement in clinical trials (24-27). Recently, high affinity 5-hydroxytryptamine (HT) 6 receptor antagonist Lu AE58054 ([2-(6-fluoro-1H-indol-3-yl)-ethyl]-[3-(2,2,3,3-tetrafluoropropoxy)-benzyl]-amine) was reported to improve novel object recognition task in a rat model with cognitive impairment induced by phencyclidine (28). The combined treatment of Lu AE58054 and donepezil is under phase III clinical trial (ClinicalTrials.gov identifier: NCT01955161). Additionally, even drugs with uncertain mechanism were reported to be effective in symptomatic improvement and protection against neurotoxicity by Aβ, including ethanolic extract of *Angelica gigas* (INB-176) and *Ginkgo biloba* (EGb761) respectively, however, none of which showed successful effectiveness in their preclinical and clinical trials (29-31).

A β PRODUCTION/AGGREGATION MODULATOR

Abnormal A β production and accumulation in brain parenchyma have been regarded as the central etiological hypothesis in AD pathogenesis (5, 10, 32). Therefore, the first line of strategy was inhibition of A β generation processes to prevent or cure the disease. The tight relevance of α -, β - and γ -secretases to A β production made researchers to discover modulating drugs for these enzyme activities in order to reduce intracellular and extracellular A β level. Whereas effective α -secretase activator was rarely identified, several types of β -secretase inhibitors were discovered and tested, starting with first-generation potent inhibitor OM99-2, OM00-3 (33, 34). Since then, numerous reports and patents of β -secretase inhibition were published, however, finding drug candidate with desirable potencies and efficacy has been fairly challenging (35). Recently discovered MK-8931 (Merck) is a promising β -secretase inhibitor whose result of phase I clinical trial was released in April, 2012. MK-8931 is now under phase II/III trial which was initiated in 2012 (ClinicalTrials.gov identifier: NCT01739348).

Gamma-secretase plays the critical role in A β generation, in charge of the rate-limiting cleavage of APP into A β . However, modulating this enzyme activity may cause diverse side effects because of its multiple cleavage actions on diverse substrates which are physiologically important, including notch receptor signaling. For this reason, modulating γ -secretase activity seems to be greatly complicated, requiring restricted substrate specificity for APP to reduce A β only, not affecting other substrate processing such as notch signaling (36, 37). Consequently, substrate specificity is the critical issue in the development of AD therapy using γ -secretase inhibition. Semagacestat (LY450139, Eli Lilly) was a promising drug candidate targeting γ -secretase inhibition (38), tested in two Phase III clinical trials. Even though both trials finished with a disappointing result of insufficient efficacy it showed a breakthrough for possible utilization of γ -secretase modulation in AD therapeutic development.

Mostly, A β elicits its toxicity by aggregated forms (10, 39, 40). Therefore, the inhibition of A β aggregation is one of the most effective strategies in order to inhibit A β toxicity. Therefore, diverse candidates for inhibition of A β aggregation have attracted attention. Curcumin and β -sheet breaker such as RS-0406 were discovered to inhibit polymerization of A β into oligomer and fibril forms (41, 42). Compound D737 showed the most effective inhibition of A β aggregation among a collection of 65,000 small molecule candidates and elicited increased lifespan in a *Drosophila melanogaster* model of AD as well as reduction of A β toxicity in cell culture system (43). Indirect inhibition of A β aggregation was suggested by metal hypothesis of AD (44). Copper/zinc ionophore, PBT2, which target the copper and zinc ions that mediate A β aggregation was proven to facilitate the

aggregated A β clearance in the cortex, to lower A β level of cerebrospinal fluid (CSF) and to restore the cognitive impairment in AD patients (44-46). PBT2 completed phase II clinical trial (ClinicalTrials.gov identifier: NCT00471211) and are now under phase II clinical trial for Huntington disease as well. Additional large-scale clinical tests and high throughput screening for candidates of A β aggregation inhibitor are strongly encouraged in further investigation.

Various modifications of A β peptide have influence on its aggregation and toxicity. Especially, pyroglutamyl modification in N-terminus of A β is critical alteration because pyroglutamated A β (pGlu-A β) species readily accumulated into senile plaque and vasculature deposit due to increased stability and aggregation velocity (47-49). Glutaminy cyclase (QC) was demonstrated as the main catalytic enzyme responsible for this pyroglutamyl modification of A β and intracortical microinjection of QC inhibitor, PBD150, significantly decreased pGlu-A β formation (50, 51).

IMMUNOTHERAPY

Since inflammation response and activation of phagocytic cells such as microglia and astrocytes had been appreciated as a pivotal contributor to AD pathogenesis, immune system became one of the most prominent targets in the aspect of AD therapeutic invention (52). Cytokines and other neurotoxic adducts secreted by immune-related cells were suspected as possible mediators of neuronal degeneration and cell death (53, 54). Furthermore, data analysis using genome wide association study (GWAS) supported this idea by proving that specific overrepresentation of genes related to immune pathway linked to AD risk (55). The protection effect of non-steroidal anti-inflammatory drugs (NSAIDs), especially ibuprofen, against AD proved that the suppression of immune response should be beneficial in AD (56). Many factors seemed to be tightly related to the protective effect of NSAIDs against AD, including age of cohort, apolipoprotein E (APOE) genotype, the duration of NSAIDs usage and NSAIDs types, showing significant effect in APOE ϵ 4 allele carrier (56-59).

Unfortunately, diverse clinical trials with different types of NSAIDs concluded not only beneficial effects but also insufficient efficacies and negative results (57, 60-65). Narrowing down the target along with amyloid hypothesis, immunotherapy against A β peptide attracted a great deal of attention because it is direct resolution of the seemingly main cause of pathogenesis and progression of disease in AD. Several strategies for A β peptide immunotherapy has been tested, including passive immunization with monoclonal antibody against different regions of A β 42 as well as active immunization using synthetic A β 42 (66). Because A β peptide, the major component of senile plaques in AD brain, is regarded as the critical contributor in AD patho-

genesis, enhanced clearance of A β via the administration of anti-A β monoclonal antibody, including bapineuzumab and solanezumab (passive vaccination), or A β antigen with adjuvant such as AN1792 (active vaccination) seemed fairly promising (67, 68). Preclinical trials for both active and passive immunotherapies against A β represented diverse beneficial effects of ameliorated brain A β burden, prevention of memory loss and improved cognitive function in different animal models of AD (67, 69). Preliminary test on human patients exerted promising outcomes of reduced plaque burden and cognitive benefit (70, 71), suggesting multiple mechanisms of actions including modulation of A β equilibrium balance between the central nervous system and plasma (72) or improved peripheral clearance and sequestration of brain A β (73). However, unavoidable side effects found in clinical trials hindered the further clinical development into AD therapeutic treatment on human, including meningoencephalitis, microhemorrhages and vasogenic edema (68, 74). Also, clinical evaluation in human AD patients failed to replicate the identical results as in the animal AD model, showing unsynchronized phenomena between reduced A β plaque and rescue of neurodegeneration during AD progression (75). Immunotherapy is regarded as one of the most promising therapeutic strategies in AD and some immunotherapeutic drug candidates are still under clinical trials, including the first monoclonal antibody for A β protofibril (BAN2401, ClinicalTrials.gov identifier: NCT01767311) and immunoglobulins combined with albumin by means of diverse application methods (ClinicalTrials.gov identifier: NCT01561053) (68).

TAU-TARGETING THERAPY

Intracellular neurofibrillary tangle (NFT) is another hallmark in AD pathogenesis, cytoskeletal inclusions consisted of hyperphosphorylated microtubule associated protein tau with paired helical filament structure (14). Especially, modulating endogenous tau level in APP-overexpressing mice halted A β -induced behavioral deficit in spite of maintaining high A β level, suggesting the relevance of tau during AD pathogenesis and implying the possibility for tau-targeting immunotherapy in AD (76, 77). Moreover, tight relationship between A β and tau pathologies during AD strengthened the rationale for tau-aiming therapeutic strategy in AD. It was proven by that A β immunotherapy reduced not only the extracellular A β plaques but also intracellular A β accumulation which resulted in the absence of early tau pathology (78). Different mechanistic approaches of tau-targeting therapies were tried, including reducing tau level itself, preventing tau hyperphosphorylation and inhibiting the aggregation (26). Many researches have focused on preventing hyperphosphorylation of tau, earlier event that cause detachment of tau protein from microtubule. Kinases responsible for tau phosphorylation (glycogen synthase kinase, GSK-3 β , cyclin-de-

pendent kinase-5, cdk5 and microtubule affinity-regulating kinase) and phosphatase (protein phosphatase 2A, PP2A) are the possible targets to achieve tau-aiming therapeutics, altering tau phosphorylation by modulating activity of the enzymes (79-81). Especially, GSK-3 β inhibition is implicated in both A β and tau pathway concomitantly and is greatly appreciated in AD therapeutic development. GSK-3 β inhibition by lithium, valproate, caffeine were also tested in preclinical and clinical studies and their efficacies were needed to be confirmed in further study because of inconsistent outcomes in different studies (82-85). AZD 1080 (AstraZeneca) and NP-12/Tideglusib (Noscra) were the most promising GSK-3 β inhibitor, however, AZD 1080 was withdrawn from AD therapeutic development due to the nephrotoxic side effect in phase I clinical trials (68, 86, 87). Since then, NP-12/Tideglusib has been recognized as an effective GSK-3 β inhibitor and completed not only pilot clinical study using small sample with the result of positive trends in mini-mental state examination (MMSE), Alzheimer's disease assessment scale-cognitive subscale (ADAS-cog), Global deterioration scale (GDS) and Global cortical atrophy (GCA) (88) but also phase II clinical trial (ClinicalTrials.gov identifier: NCT01350362).

In addition to tau phosphorylation, several agents were also suggested to prevent tau aggregation. Methylthioninium chloride (methylene blue, MTC) was the first tau aggregation inhibitor discovered and reduced version of MTC, TRx0237, is now in the process of phase III clinical trial (89) (ClinicalTrials.gov identifier: NCT01689246). Also, diverse possible candidates were suggested as tau aggregation inhibitor, including anthraquinones, aminothienopyridazines, polyphenols and phenothiazines (68, 90-92). These compounds, however, need more verification because they failed to show consistent efficacies in *in vivo* studies.

METABOLIC TARGETING

Since type 2 diabetes mellitus (DM2) was found to be related to AD, glucose metabolism has emerged as a new interest in AD research. It was widely known that glucose metabolism and insulin signaling are impaired in AD brain (93-95). Insulin-degrading enzyme (IDE) was revealed to be responsible for A β degradation as well, more efficiently intracellular A β than extracellular form (96-98). Even though AD and DM2 share IDE as the key metabolic enzyme for their main etiological proteins, A β for AD and insulin for DM2, it is not enough to explain all the AD-mimic pathological phenomena found in diverse mechanism-driven diabetes mouse model with insulin resistance (98-100). Insulin has effect on cerebral function per se and is also tightly involved in inflammation and oxidative stress, representing enhanced inflammatory response and markers of oxidative stress by hyperinsulinemia (101). In other studies, increased autophagosome was suggested to accelerate the amy-

loidogenic APP processing in insulin-resistant condition (102). Thereby, insulin itself represented tight relevance to AD and became a new therapeutic target in AD. Metformin, a peripheral insulin sensitizer drug approved by the FDA, was reported to sensitize brain insulin action and prevent AD-associated pathological alteration in in vitro AD model (103). Also, other diverse insulin sensitizers are needed to be tested because they may have possibility to show valuable efficacy in AD as well. MSDC-0160 (mTOT modulator; Metabolic Solution Development Company, MSDC) was recently performed successful phase IIa clinical trial for type 2 diabetes (104). MSDC-0160 was demonstrated to elicit its insulin-sensitizing effect through newly discovered mitochondrial target of thiazolidinedione (mTOT) located in the mitochondrial inner membrane (104, 105).

Mitochondria are one of the most devastated organelle in the process of AD development. During AD pathogenesis, mitochondrial impairment occurs in various brain areas, representing not only morphological alteration but also physiological dysfunction (106). A β was detected inside mitochondria compartment of AD mouse model expressing human mutant amyloid precursor proteins (APP), mostly within membrane and membrane-associated region (107, 108). A β seems to accumulate within mitochondria precedent to extracellular A β deposition. Study using mitochondria-specific targeting A β proved that mitochondrial accumulation of A β induced not only morphological alteration but also physiological dysfunction which was fatal enough to induce neuronal apoptosis (109, 110). Other findings also showed morphological alteration of mitochondria and physiological dysfunction, especially electron transport chain through cytochrome C oxidase and increased oxidative stress in in vitro and in vivo AD-mimic system and patients (110-112). Therefore, mitochondrial dysfunction by A β is a critical contributor during AD pathogenesis, and in the same line of thought, mitochondrial protection becomes a new strategy in AD treatment. Also, mitochondrial dysfunction and oxidative stress is more evidently the common linker between AD and abnormal glucose metabolism (113, 114). Therefore, mitochondrial recovery drug was proposed as a new concept for AD therapy and treatment of insulin-resistance. Dimebon (Pfizer), originally allergy-treating drug used in Russia, was known to improve ATP generation and energy metabolism in mitochondria and was tested its effectiveness in clinical trial for AD therapeutics (115). Unexpectedly, phase III trial performed with 598 patients ended up as failure with the lack of improvement. This failure leads researcher to suspect the novel mitochondrial mechanism of action of Dimebon, rather than promiscuous clinical effects, including inhibition of histamine H1 and serotonin receptors. In extended thoughts, other medicines with the effect of mitochondrial rejuvenation have been investigated as probable candidates for AD therapeutics. Piracetam is a nootropic drug and has effect on cognitive impairment during aging and dementia

(116). The mechanism of action for piracetam was controversial, including effects on glutamate receptors, GABA-mimetic action and activation of calcium influx into neuronal cells (116). Recently, mitochondrial relevance of this drug has been found to enhance the membrane fluidity in brain mitochondria and consequently improve membrane potential, ATP generation and decrease apoptotic vulnerability in aging and AD model (117). These findings suggested the possibility that this drug exerted therapeutic effect through mitochondrial recovery in AD. In addition to mitochondrial function itself, axonal transport of mitochondria through microtubule protein was observed to be impaired in AD (118). This could be another strategic target in AD therapeutic development. The acetylation status of α -tubulin by histone deacetylation (HDAC6) is highly related to cargo transport along microtubules (119). Restored α -tubulin acetylation by HDAC6 inhibitor improves both anterograde and retrograde motility of mitochondria and, furthermore, rescued mitochondrial morphology in hippocampal neurons under AD-mimic condition of A β -induced impairment of mitochondria function (120). These diverse mitochondria-targeting mechanisms of action are all likely to be probable therapeutic mechanism for AD treatment and deserve to be evaluated.

Besides the relevance to mitochondrial dysfunction, disturbed glucose metabolism could be directly connected to A β generation and accumulation by modulating the enzyme activity of A β -generating enzyme through post-translational modification. Addition of O-linked β -N-acetylglucosamine (O-GlcNAc) to protein is a glucose level-dependent post-translational modification and the specific inhibition of O-GlcNAcase, 1,2-dideoxy-2 γ -propyl- α -D-glucopyranosyl-[2,1-d]- Δ 2 γ -thiazoline (NButGT), ameliorated A β generation by modulating nicastrin activity, a component of γ -secretase, through S708 site O-GlcNAcylation (121). Different from other O-GlcNAcylation modulating drugs, such as O-(2-Acetamido-2-deoxy-D-glucopyranosylidene) amino N-phenyl carbamate (PUBNAc) and Streptozotocin (STZ), NButGT was specific to O-GlcNAcase and showed the lack of cellular toxicity nor insulin resistance (122). Additionally, O-GlcNAcase inhibitor was reported to reduce the tau phosphorylation and improve long-term potentiation (LTP) as well, which are possibly beneficial in AD (123, 124). O-GlcNAcase inhibitor seems to be effective not only in A β generation but also in memory impairment and taupathies, which is needed to be further verified in future clinical studies. In addition to O-GlcNAcylation, diverse post-translational modification could be another valuable target for AD therapeutics, especially specific modification for AD-related proteins, including γ -secretase and β -secretase.

CONCLUSION

Recent findings suggest that A β accumulation is fairly slow and

time-consuming process, likely to require more than two decades (125). During this long process, more than one physiological system seems to be linked each other to harmonize in order to induce pernicious AD pathology. Taken information together, it is unlikely that a single remedy could cure AD because of its complexity and intricate relationship among the multitude of pathological components during pathogenesis. For this reason, therapeutic targets for AD should include multiple strategies and combinational remedy, not single, for the maximum effectiveness and better consequences. Also, not only direct therapeutic treatment for pathological intervention but also delaying this long pathogenic process would contribute to reduce the number of AD patients and increase prognostic benefit (26).

Up to date, researchers are desperate to find new ways for AD treatment and tune the drug candidates for the maximum efficacies. For instance, in developing A β synthesis modulator as AD therapeutic target, researchers has to consider diverse questions and concerns, including the right margins of decreased A β production level, maintaining proper physiological level and off-target effects by influencing other substrates besides A β . Because it is hard to tune the enzyme activity within the right physiological catalytic range, the successful development of AD therapeutics with enzyme modulator is dependent upon its efficacy on aiming action, specificity and selectivity to target substrate. Also, as everyone agrees that the right timing of drug treatment is essential for the evaluation of efficacies for therapeutic targets, improvement in early diagnostic tool for AD have to be pursued along with AD therapeutic development.

ACKNOWLEDGEMENTS

This is an invited review a part of which has been presented at the Fourth Academic Forum of the National Academy of Medicine of Korea, held on September 26, 2013 in Seoul, Korea.

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