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New APOE-related therapeutic options for Alzheimer's disease FREE

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New APOE-related Therapeutic Options for Alzheimer's Disease

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Abstract. Alzheimer's disease is the most common type of dementia among humans. However, no commercial drug is available to fully cure the disease or reverse the progress. *APOE4* is recognized as the greatest risk factor of a sporadic AD. *APOE* is thought to alter the metabolism of Aβ and tau, influence lipid metabolisms (especially for cholesterols), glial cell inflammation, and synapse, which may contribute to neuronal degeneration. Here we reviewed the current update on the functions of *APOE* that was related to AD pathology. We also presented and evaluated the potential clinical options based on *APOE* including immunotherapies, gene therapy, and chemical structure correctors.

INTRODUCTION

Alzheimer's disease (AD) is an irreversible, progressive neurodegenerative disease, which is the most prevalent dementia in the world (constituting 80% of all cases). Its clinical symptoms include memory deficiency, delayed response, deficiency of generalized cognitive ability etc. (National Institute on Aging, 2016; Alzheimer's Disease and Dementia, n.d.).

The hallmarks of AD are amyloid-β plaques and neurofibrillary tangles. Alzheimer's disease can be classified into two distinct classes, autosomal dominant familial AD (FAD) and sporadic AD (SAD). FAD happens in less than 3% of AD, due to mutations in three genes: APP, PSEN1 and PSEN2. (Liu, Kanekiyo, Xu & Bu, 2013). Whereas the exact genetic cause of sAD is unclear, the APOE gene has been identified to constitute the greatest genetic risk factor, in an isoform- and dosage- dependant manner. (Liu et al., 2013).

Currently, cholinesterase inhibitors and NMDA receptor antagonists are the only available drug variants that are approved by the FDA for AD-related memory deficit. Such drugs aim to maintain acetylcholine, a neurotransmitter important for memory. Studies have shown some indication that such drugs achieve their intended goal of delaying the process to a limited degree and moderately improving cognition and behavior, but none achieved a cure (Farlow, Miller & Pejovic, 2008). Also, over half of the patients in either treatment failed to show any benefit (Kumar, Singh & Ekavali, 2015). Some of the newly developed therapy was amyloid-β (Aβ) based, by immunotherapy, preventing Aβ formation, inhibiting Aβ aggregation and etc. While preclinical trials worked on mouse models, it failed to show any benefits or severe side effects on human clinical trials (Giacobini & Gold, 2013). As a conclusion, a new therapy is needed to develop for a better solution.

In the previous studies, the APOE gene was shown to be a strong genetic factor of sAD as it affected Aβ metabolism (Huynh, Davis, Ulrich & Holtzman, 2017). While accumulating studies figured out APOE isoforms were related to tau-mediated neurodegeneration (Strittmatter et al., 1994; Kim, Basak & Holtzman, et al., 2009). Besides, Apolipoprotein E (ApoE) was found playing role in lipid metabolism, inflammation, and etc (Hara et al., 2002; Guo, LaDu & Van, 2004). All of them are closely connected with Alzheimer's disease. Therefore, APOE can be a powerful target for AD's treatment. In this review, we will briefly discuss APOE genetics, the process of the AD progression, different APOE functions, and evaluate potential APOE based therapies to alleviate the AD pathology.

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MAIN BODY

APOE Genetics, APOE & Sporadic / Familiar AD relationship

APOE gene codes for Apolipoprotein E, a fat-binding protein. ApoE has an N-terminal domain and a C-terminal domain, which are the receptor-binding region and fat-binding region respectively. There are three different isoforms, APOE2, APOE3 and APOE4. The structural differences between them are located at amino acid residues 112 and 158. ApoE2 has cysteine112 and cysteine158; ApoE3 has cysteine112 and arginine158; ApoE4 has arginine112 and arginine158 (Mondal, Wang, DeKoster, Baban, Gross & Frieden, 2016). The changes in R-group of amino acids result in structural and functional (i.e. binding ability) differences between them. In general worldwide population, e2, e3, e4 alleles frequency ratio was 8.4: 77.9: 13.7; However, e2, e3 and e4 alleles frequency in AD patients was 3.9: 59.4: 36.7, e4 homozygous carriers had 91% chance of developing AD, compared with 47% in heterozygotes and 20% in non-carriers (Liu et al., 2013). Carriers with one APOE4 allele would accelerate onset for about 5 years; one with two APOE4 alleles would accelerate for 10 years; whereas one APOE2 allele could push the time of onset 5 years later in sAD (Noguchi et al., 1993; Pastor et al., 2003). Although recent Genome-wide association study (GWAS) had identified other genetic loci in SAD linked with Aβ- or tau- pathology, none of them had a greater effect than APOE4 (Deming et al., 2017). Therefore, APOE4 is still the most powerful risk factor to investigate.

The link between APOE and fAD is less clear. Some researchers suggested that APOE4 could increase the risk and decrease the time of onset in the early-onset-fAD compared to other isoforms (Corder et al., 1993). Furthermore, E280A PS1 heterozygous mutation in human showed that APOE4 did accelerate the time of onset (Pastor et al., 2003). Other result indicated that ApoE2 was sufficient to delay the time onset of early-onset fAD with APP V717I mutation (Nacmias et al., 1995). While some researchers found no link between ApoE4 allele and fAD phenotype (Nacmias et al., 1995). Data from a general study of early-onset-fAD indicated that APOE4 allele wasn't sufficient to be identified as a risk factor of it (Sorbi et al., 1994). These controversial results may due to the small sample size in the fAD researches and the differences in genetics.

Alzheimer's disease Pathology

Alzheimer's disease is a neurodegenerative disease that is marked by brain atrophy as well as two hallmarks of protein aggregation in the brain -- Amyloid-β plaque and neurofibrillary tangle (NFT). Aβ plaque is formed by the aggregation of Aβ 42 or 40, that is cleaved from an amyloid precursor protein (APP). The familiar AD is caused by mutations in either APP or gamma-secretase genes -- PSEN1 or PSEN2, which are codes for enzymes that cleave APP to form Aβ (Musiek & Holtzman, 2015). In the GWAS, the gene loci with a low Aβ42 level in CSF was linked with elevation in AD risk and in pathology rate (Deming et al., 2017), suggesting Aβ was correlated with AD pathology. Even though Aβ hypothesis proposed the causation between Aβ and AD, current effort on Aβ-based treatment was not optimistic. Some researches indicated that the decrease of Aβ plaques in brain and plasma alone didn't reduce the detrimental recognition. Moreover, many clinical failures would still occur that even if cognition improvement was shown on mice (Giacobini & Gold, 2013). The NFT is formed by hyperphosphorylated tau, which normally functions as a protein that stabilizes microtubules. Data indicated that 3-4 fold more tau was phosphorylated in AD patients, compared with normal people (Iqbal, Liu, Gong & Grundke-Iqbal, 2010). Meanwhile, Aβ plaque could enhance the formation of NFT in many animal models (Nisbet, Polanco, Ittner, & Götz, 2014), although the exact mechanism is unclear.

Synapse loss and neuronal degeneration can be identified as the key stages in AD pathology as well. As known, cognitive deficiency is one of the symptoms of AD. It is widely accepted that synapse loss is highly related to cognitive impairments on AD patients (see review in Koffie, Hyman & Spires-Jones, 2011)

Other factors may also contribute to AD pathology. Active microglia and reactive astrocyte were found associated with Aβ plaque (Itagaki, McGeer, Akiyama, Zhu & Selkoe, 1989). The gliosis induced by β mediated the release of chemokine and further activation of inflammation (for details, see review Tuppo & Arias, 2004). Moreover, from a postmortem study, glial cell inflammation was positively correlated with NFT and the duration of dementia in AD patients (Ingelsson et al., 2004). These studies indicated that glial cell inflammation might participate in AD pathology.

A Basic Function of Apoe in Lipid Metabolism

ApoE is mainly expressed in the liver and the brain. In the liver, ApoE binds with lipid forming lipoprotein in order to transport lipid among cells or tissues via ApoE receptor (Huang & Mahley, 2014). ApoE4 slows the rate of transporting cholesterol away from blood, thus can lead to coronary heart disease (Xu et al., 2016). In the brain, ApoE is mainly expressed and secreted from astrocytes (Huang & Mahley, 2014). It forms lipoprotein complexes and gets recognized by lipoprotein receptors, thus, it is taken up by other cells. Besides, the neurons and microglia are able to generate ApoE to some extent (Zhang & Liu, 2015).

Impairment of cholesterol metabolism has been shown in ApoE deficient animals. Total cholesterol level in plasma was abnormally higher in ApoE homozygous deficient mice than heterozygote and wild-type mice, when feeding with either low-fat or high- fat diet (Plump et al., 1992).

Experiments from different cell types indicated that ApoE isoforms influence the efflux ability of cholesterol. ApoE bound to ATP Binding Cassette Transporter A1 (ABCA1) and induced ABCA1-mediated cholesterol efflux. ABCA1 depletion caused lipid secretion deficiency in both astrocytes and microglia (Hirsch-Reinshagen et al., 2004). Gong's work indicated that the release of lipid in APOE3 astrocyte was about 2.5-fold more than APOE4 (2002). Meanwhile, ApoE3 was better at binding to extracellular cholesterol. Similarly, ApoE3 and ApoE2 showed greater efficiency in reducing cholesterol level than ApoE4 in macrophage cell line as well (Hara et al., 2002). Primary neurons could also release lipids when exposure to ApoE, whose activity was isoform-dependent (ApoE2 >> ApoE3 > ApoE4) (Michikawa, Fan, Isobe & Yanagisawa, 2000). In addition, the uptake of cholesterol in the brain was cell types and isoforms dependent as well. ApoE4-bounded cholesterol uptake was lower than other isoforms in hippocampal neurons; whereas in hippocampal astrocytes, the ApoE4 bounded cholesterol uptake was greater than ApoE2 and ApoE3 (Rapp, Gmeiner & Huttinger, 2006). Although ApoE4 in astrocytes showed some controversy, generally, ApoE4 was a less efficient isoform on lipid utilization.

Cholesterol is needed for multiple functions in the neuron, including synaptic integrity (Mauch et al., 2001), axons (Hayashi, Campenot, Vance & Vance, 2004) and dendrites (Goritz, Mauch & Pfrieger, 2005). So poor ability of lipid metabolism of ApoE4 may impact the health condition of a neuron.

Effect of APOE Gene on Aβ Pathology

In 1991, Namba's group found that ApoE was co-localised with senile plaques in AD's patients, which was the first evidence implied that ApoE might be involved in Aβ metabolism (1991). The link had been found in several types of research by different mice models. When ApoE was knock out under APP mutant transgenic (TG) background, a great reduction of Aβ load was observed that the control (Bales et al., 1997; Holtzman et al., 2000; Fagan, Watson, Parsadanian, Bales, Paul & Holtzmann, 2002). ApoE4 knock-in mice resulted in a greater load of Aβ burden (Holtzman et al., 2000), compared to ApoE3 knock-in mice; Whereas human ApoE2 expressing mice did the opposite (Fagan et al., 2002).

The exact mechanism between APOE isoforms and Aβ metabolism is unclear yet. Current studies found that ApoE could influence Aβ via altering the production, fibrillization and clearance. First, Huang's group identified that ApoE could activate DLK-MKK7-ERK1/2 cascade, which stimulated transcription factor AP-1 that eventually increased APP transcription and, therefore elevated Aβ production in the neuron cultures and mice model. ApoE4 group had the highest activation of this cascade and in turn, generated more Aβ than APOE3 and APOE2 (2017). Second, Aβ exhibited accelerated fibrillization when incubating with ApoE4, compared with ApoE3 in vitro (Castano et al., 1995). Moreover, extracellular ApoE positively affected the targeting of lipid rafts with Aβ (Fagan et al., 2002), where A was converted to an oligomer (Kim, Yi & Ko, 2006). Therefore, ApoE4 assisted the formation of Aβ. Third, ApoE4 was less efficient to clean up Aβ42 than ApoE3 and ApoE2 knock-in mice with Aβ injection (Sharman et al., 2010). The dysfunction of clearance might result from multiple pathways. Lipidated-ApoE could bind to soluble Aβ and increase the chance of uptake by ApoE receptors, then Aβ was sent to the lysosome for degeneration (Lee, Tse, Smith & Landreth, 2011). However, as previously described, ApoE4 was less lipidated than ApoE2 and ApoE3, thus, resulted in ApoE4 reduced rate of Aβ clearance than other isoforms; ApoE4 also impaired the role of ApoE on endocytic degradation of Aβ by proteases in microglia (Jiang et al., 2008); ApoE4 group was less capable of transporting Aβ42 by transcytosis via blood-brain barrier (BBB) (Bachmeier et al., 2013). This might be caused by the increased shedding of lipoprotein receptor on ApoE4 endothelial cells, which lost the normal ability to internal Aβ (Bachmeier et al., 2014); Moreover, APOE4 TG displayed an altered cerebrovascular basement membrane, which trapped more Aβ deposit from clearance (Hawkes et al. 2012).

APOE Affect Tau

The relationship between ApoE and tau is less known. Tau could bind to ApoE3 but not ApoE4 in vitro, however, the binding was inhibited by tau phosphorylation. One possible mechanism suggested that the binding between ApoE3 and tau could reduce the chance of tau phosphorylation and stabilize the microtubule, in turn alleviated AD pathology. Whereas ApoE4 that lost the binding ability did the opposite. (Strittmatter et al., 1994). Additionally, overexpressing C-terminal ApoE fragment in the neuron exhibited hyperphosphorylated-tau, astrogliosis, neuronal loss and memory deficit in vivo (Tesseur et al., 2000; Harris et al., 2003). APOE4 carriers tended to generate more ApoE C-terminal fragment in AD patients (Wang & Turko, 2013), which implied the role of ApoE on tau pathology.

Recently, data showed APOE effect on P301S tau TG mice model. ApoE4 knock-in mice had a higher insoluble tau level, more brain atrophy and glia activation despite equivalent tau synthesis and the absence of Aβ aggregation. While ApoE knock-out mice showed the opposite – with decreases in neuronal loss, brain atrophy and ventricular dilatation. (Shi et al., 2017). Thus, this suggests that APOE isoforms have the Aβ-independent role on tau aggregation in vivo.

The association between ApoE and tau is more exclusive in a human until recently (Kim et al, 2009). Multiple groups used GWAS demonstrated total tau and phosphorylated tau in cerebrospinal fluid (CSF) was correlated with APOE4 in large cohorts of both normal seniors and AD patients (Kim et al., 2010; Cruchaga et al., 2013; Han, Schellenberg & Wang, 2010). Moreover, the association still existed after accounting for Aβ42 level, which might affect tau level in CSF (Cruchaga et al., 2013; Deming et al., 2017). That evidence supports ApoE could influence tau pathology independent of Aβ. Since tau was temporally and anatomically closer to cognitive dysfunction than Aβ (Serrano-Pozo, Frosch, Masliah & Hyman, 2011; Arriagada, Growdon, Hedley-Whyte & Hyman, 1992; Gomez-Isla et al., 1997), ApoE became a more valuable target for AD.

APOE and Inflammation

ApoE can act as an anti-inflammation agent. Aβ is sufficient to induce glial cell activation, which leads to the release of proinflammatory cytokines (LaDu et al., 2001), which may contribute to neurodegenerative diseases (Barcia, 2013). However, Aβ induced inflammation can be reversed by the addition of ApoE. Exogenous ApoE succeeded in alleviating the inflammation, while glia cell culture of ApoE KO mice enhanced pro-inflammation marker (LaDu et al., 2001). The similar anti-inflammation effect was seen in astrocyte culture. And it was unaffected by ApoE isoforms (Hu et al., 2002).

However, ApoE4 itself may act as a pro-inflammatory agent. With the exposure of lipopolysaccharide (LPS) instead of Aβ, APOE3/APOE4 human subjects had a higher response on body temperature and a panel of increasing cytokines including TNFα, IL-6, IL-1β and IFNγ (Gale et al., 2014), suggesting the inflammation activation. The result was recapitulated in APOE-TR mice, e2, e3, e4 alleles knock-in mice showed glial cell inflammation in the ascending order (Gale et al., 2014; Zhu et al., 2012). Besides, the APOE4 TR mice had comparable inflammation load as the ApoE knock-out mice (Zhu et al., 2012). The converging evidence supports ApoE4 works as a pro-inflammation agent. Furthermore, the use of LPS indicates that ApoE has Aβ independent role on neuroinflammation.

Recent evidence supported that ApoE4, but not ApoE2 and ApoE3, was able to activate pro-inflammatory pathway cypA-NF-kB/MMP-9 in the pericyte (Bell et al., 2012; Halliday et al., 2015). Data supported that there were more pericyte degradation in APOE4 carriers than APOE3 carriers (Halliday et al., 2015). As a component of BBB, the pericyte was strongly correlated to BBB integrity (Armulik et al., 2010; Franco, Roswall, Cortez, Hanahan & Pietras, 2011). MMP-9 secretion in the brain plasma was associated with promoting the migration of pericyte and astrocyte away from their normal location and BBB disruption (Takata et al., 2011). Therefore, hyperactivation of cypA-NF-kB/MMP-9 pathway could be detrimental to BBB. In endothelial BBB model, APOE4 and APOE knock-out groups got greater losses of tight junction and permeability of BBB (Nishitsuji, Hosono, Nakamura, Bu & Michikawa, 2011; Halliday et al., 2015). The same phenomena were shown in traumatic brain injury mice model, with ApoE knock-in mice exhibiting the activation of NF-κB/MMP-9 and the loss of tight junction and BBB integrity (Teng et al., 2017). The BBB leakage might cause accumulation of neurotoxic factors occurrence, faulty transport, red blood cell extravasation, inflammatory response, immune response, autoantibodies and microbial pathogens, and eventually led to neurodegeneration (for details see review Sweeney, Sagare & Zlokovic, 2018).

APOE, Synapse and Cognition

ApoE isoforms are correlated to the synapse integrity and neuron survival. It was found that ApoE4 decreased dendritic spine density more than ApoE3 and ApoE2, in both primary hippocampus neurons and cortices of APOE target replacement (TR) mice (Dumanis et al., 2009; Klein, Acheson, Mace, Sullivan & Moore, 2014; Neustadtl, Winston, Parsadanian, Main, Villapol & Burns, 2017). The similar phenomenon was seen in the neurons when co-cultured with human iPSC-derived astrocytes. ApoE4 homozygotes showed a reduced dendrite length and a lower ability of synapse maintenance, compared to ApoE3 (Zhao et al., 2017). Meanwhile, APOE knock-out displayed the loss of pre-synaptic marker, suggesting physiological dysfunction (Lane-Donovan et al., 2016). As a result of this, the excitatory activities were declined in the lateral amygdala of the aged human ApoE4 TR mice, indicated the reduced the synaptic transmission (Klein et al., 2014). Besides, ApoE could also influence the synaptogenesis, since the synapse development could be induced by glia-derived cholesterol/ApoE complex in cultured CNS neurons (Mauch et al., 2001). The lipidation states of ApoE isoforms were different, thus ApoE isoforms would alter synaptogenesis.

ApoE isoforms also affected long-term potentiation (LTP), spatial learning, and memory ability in an age-dependent manner. As mentioned above, synapse loss is associated with cognitive deficient on AD patients (Koffie, Hyman & Spires-Jones, 2011). APOE knock-out mice resulted in impaired LTP (Lane-Donovan et al., 2016), which was known to be linked with synaptic loss (Cooke & bliss, 2006). In Morris water maze test, ApoE protein level was positively associated with spatial learning and memory performance (Oitzl, Mulder, Lucassen, Havekes, Grootendorst & Kloet, 1997; Johnson et al., 2014; Lane-Donovan et al., 2016). Whereas the correlation between memory ability and e4 alleles was age-dependent in term of the human. Amongst the human aged from 35 to 60, there was no clear relationship between cognition ability with ApoE alleles (Lancaster, Tabet & Rusted, 2017). Batterham's study found the significant impairment on word recognition on APOE4 carriers in their 80s (2012). This was also verified by the cross-sectional data, the association between APOE4 and verbal episodic memory was appeared only on the group of the age of 69, but not any time before it in the adulthood (Rawle, Davis, Bendayan, Wong, Kuh & Richards, 2018). This implies the APOE4 is a strong factor but not sufficient for cognitive dysfunctions. Notably, in Lane-Donovan's experiment, the brain ApoE knock-out mice found that there were lost in synapse and LTP, however, the spatial learning was unaffected. However whole-body ApoE knock-out mice resulted in a spatial learning deficiency (Lane-Donovan et al., 2016). This implies that the amount of both plasma ApoE and brain ApoE contribute to the AD pathology.

CURRENT CLINICAL THERAPIES TARGETING TO APOE

Small Molecules That Correct Apoe4 Structure

The structural difference between ApoE isoforms is at amino acid residuals 112 and 158 as described above. It is wildly accepted that Arg 112 substitution in ApoE4 induces a salt bridge between Arg61 on N-terminal domain and Glu255 on the C-terminal domain (Chen et al., 2011; William Il, Convertino, Das & Dokholyan, 2017; Xu, Brecht, Weisgraber, Mahley & Huang, 2004). The extra intramolecular interaction can bring two domains closer (Xu et al., 2004) and may interfere with the normal function of ApoE (for detailed see review Zhong & Weisgraber, 2009). This hypothesis was supported by R61 or E255 mutation in APOE4. By abolishing the salt bridge, the mutation could correct the conformational change (Xu et al, 2004), and rescued many detrimental effects of ApoE4 such as lipid binding preference (Dong et al., 1994), phospholipid-binding capacity (Xu et al, 2004), Mitochondrial respiration in neuron (Chen et al., 2011), intracellular trafficking (Mahley & Huang, 2012) and etc. In addition, in the R61T mutant model of ApoE4, loss of movement of the protein increased the dynamics in hinge region, which located between N-terminal and C-terminal domain. This change of dynamics might alter the phospholipid-binding capacity and cholesterol level (William Il et al., 2017).

Small molecule structural correctors (SMSC) of ApoE4 were found to change the ApoE4 into an ApoE3-like structure, and reduced the detrimental effect of ApoE4 on AD pathology (Wang et al., 2018). Among them, PH-002 showed the best capability in blocking the domain interaction in ApoE4 and therefore ameliorating the detrimental effect in the neuronal culture (Chen et al., 2011). This effect of PH-002 was also efficient for human induced pluripotent stem cell (hiPSC) as well, with a decrease in GABAergic neuron degeneration, a decrease in phosphorylated tau, and the reduction in Aβ production or secretion (Wang et al., 2018).

The valid SMSC for ApoE4 can be designed as a drug in the future. Till now, there were already many SMSCs designed as drugs for other protein misfolding disease successfully, for instance, Cystic Fibrosis, Fabry, Gaucher and

Pompe disease (Mahley & Huang, 2012). SMSC treatment is promising as its topological polar surface area allows it to be absorbed by the human intestine and across though BBB easily (Goyal et al., 2013), so it is likely to have a great effectiveness.

ApoE2 Overexpression

ApoE2 allele is considered as the "protective allele" against AD pathology. The present of ApoE2 could delay the time of onset of AD pathology by 8.24 years on average (Velez et al., 2015). Human ApoE2 knock-in in APP/PS1 mutant mice had significantly lower Aβ plaque load and better memory ability than ApoE4 model (Pankiewicz et al., 2014). ApoE2 was more efficient on lipid utilization, induced less glia cell activation, compared to ApoE4, as mentioned above. In addition, a cohort study indicated that episodic memory was improved in e2/e2 and e2/e3 genotypes, worsen in e3/e3 genotype, and further worsen in e4/e3 and e4/e4 genotypes (Wilson et al., 2002).

So far, several preclinical studies using gene therapy to express ApoE2 showed benefits on AD pathology. Injection of lentivirus carrying ApoE2 to the hippocampus resulted in a successful local increase in human ApoE load. This injection on PDAPP mice could reduce the insoluble Aβ42 load, Aβ burden on the hippocampus, compared to the vehicle control and lenti-ApoE4 injection group (Dodart et al., 2005). This tested the possibility to use ApoE2 as treatment. However, lentivirus injection was less efficient due to its local distribution around the injection site (Dodart et al., 2005). Therefore, scientists started developed Adeno-associated virus (AAV) based particles. AAV8-GFAP-ApoE2 intracranial injection that conditioned expressed APOE2 in astrocytes displayed restored lipidation of APOE, normal cholesterol level and decreased Aβ40 load as the ApoE load increases, in apoE4 transgenic mice (Hu, Liu, Chen, Zhang, Xu & Bu, 2015). Later on, the AAVrh.10hAPOE2-HA hippocampal injection was tested to be sufficient to increase of APOE2 level in both neurons and glia, and led to significant reduction of the insoluble Aβ42 and Aβ40 in the PAPP mice. A similar effect was seen in the APP/PS1 TR4 model when hippocampal injection was performed right after Aβ pathology onset (2.5 months). However, the efficiency decreased if older mice were treated (Zhao et al., 2016). AAVrh.10hAPOE2 may be the closest to clinical therapy, since it is safer, wild-spreading, more targeted, and effective to pathological mice. The study found that intracisternal route of AAVrh.10h transfection could distribute broadly around the brain (Rosenberg et al., 2018). Bearing in mind, the therapy may limit to only early stage or middle stage, since the limited time window of efficiency on the mouse model. The AAV-based therapy looks promising in general. In 2017, AAV2-based treatment was approved by FDA to treat retinal dystrophy with the long effectiveness for four years (Smalley, 2017). Meanwhile, another AAVrh.10 vector clinical trial was currently in phase I / II on Metachromatic Leukodystrophy (Clinicaltrials.gov, 2013).

Aβ Antibody That Targets the Apoe Binding Site

ApoE naturally has a binding affinity to Aβ, binding of ApoE/Aβ was found at residues 12-28 of Aβ (Strittmatter et al., 1993). Although ApoE was thought to play the role in Aβ clearance, Aβ knock-out AD transgenic mice resulted in less plaque occurrence than ApoE4 knock-in model (Liu et al., 2017). This indicated that blocking ApoE could be beneficial for Aβ pathology. One explanation is that other Aβ receptors, such as Apolipoprotein J, has a higher efficient clearance mechanism (Bell et al., 2006), so blocking ApoE/Aβ might drive Aβ clearance to a more competent way. Thus, Aβ12-28p, a blood-brain barrier-permeable antibody that competes with ApoE to bind with Aβ at this region was designed as a therapeutic option of AD (Sadowski et al., 2004).

Co-cultured astrocytes experiment suggested that Aβ12-28p diminished Aβ accumulation and synaptic protein degeneration (Kuszczyk et al., 2013). In both APP/PS1, APP, APP/PS1 APOE2 TR, APP/PS1 APOE4 TR mouse models and in vitro studies, results all suggested that Aβ12-28p generally acts as a good Aβ aggregation inhibitor (Sadowski et al., 2004; Sadowski et al., 2006; Pankiewicz et al., 2014). In addition, the 3xTg mice, that had PS1, APP and tau mutations, showed that this antibody reduced Aβ, phosphorylated tau, astrogliosis and microgliosis (Liu et al., 2014). This might prevent possible astrocyte and microglia inflammation and increase neuron survival. Experiments indicated the use of Aβ12-28p had improved memory ability in APP and APP/E4mutant mice as well (Sadowski et al., 2006; Pankiewicz et al., 2014).

Deriving from the Aβ 12-28, the peptoid CPO_Aβ17-21p treatment could inhibit ApoE/Aβ interaction, thus, decrease Aβ aggregation load and Aβ neurotoxicity. It was demonstrated in APP/PS1 mouse model, injection of peptoid CPO_Aβ17-21p after the pathology onset was able to decrease the Aβ burden, and in turn alleviated glia activation, spatial and short-term memory were both improved by this peptoid antibody treatment (Liu et al., 2017). It may be a better solution than Aβ12-28p antibody. Compared with peptide, peptoid is protease resistance, less

immunogenic, and smaller in size, which means more diffusible. Also CPO_Aβ17-21p showed more efficient in binding with IC50 about 35 times less than Aβ 12-28 antibody. Of note, peptoid had no toxic effect on cells between 0.1nanoM to 10nanoM concentration, when cells were treated in vitro (Liu et al., 2017). Overall, CPO_Aβ17-21p treatment is safer and more efficient than Aβ12-28p.

ApoE Antibody

As previously described, ApoE isoforms interacted with different molecules, for instance, cholesterol, tau, and Aβ. As mentioned above, ApoE knock-out mice had a lower neuron loss and brain atrophy compared to ApoE4 knock-in mice through a tau-mediated pathology. ApoE4/Aβ complex led to increase of Aβ metabolism the most amongst three isoforms, and ApoE knock-out showed a reduction in Aβ metabolism, thus ApoE/Aβ binding antibody might be able to ameliorate the effect. ApoE antibody might improve AD pathology in different pathways. In that case it is highly valuable to be investigated.

HJ6.3 is one of the APOE antibodies targeting all forms of ApoE to prevent ApoE binding with Aβ (Kim et al., 2012). Continuous intraperitoneal injection of HJ6.3 both before and after the onset of pathology resulted in the decrease of cortical ApoE and cortical Aβ on APP/PS1 mice (Kim et al., 2012; Liao et al., 2014). In addition, most of the absent Aβ plaques were previously contacted with HJ6.3, and nearly half of the plaques that decreased in size were affected by HJ6.3. Notably, APP/PS1 mice showed improvement on the spatial learning after HJ6.3 injection (Liao et al., 2014). However, H6.3 injection group had a slower swimming speed than the vehicle control, even though the plasma ApoE and cholesterol level were comparable to the vehicle (Liao et al., 2014). This indicates that HJ6.3 may have a severe safety issue, so further analysis of the general health of the mice is needed.

Most recently, anti-human ApoE4 (HAE4) was developed that more favourable to non-lipidated ApoE4 and ApoE3. In APP/PS1 human APOE4 knock-in model, peripheral injection of HAE4 decreased Aβ plaque load, fibrillary plaque load, insoluble Aβ42 and Aβ40 in the cerebral cortex. HAE4 had a longer half-life than other HAE forms due to the low affinity to lipidated-ApoE (Liao et al., 2018). HAE4 treatment may overweight than HJ6.3 as it is more ApoE3, ApoE4 specific, thus, more efficient.

Both HJ6.3 and HAE4 injections were associated with an increase in activated microglia level (Liao et al., 2018; Kim et al., 2012), this indicates that anti-ApoE antibody might reduce Aβ plaque load via a microglia inflammation associated pathway. Therefore, more experiments are needed for testing the suitable injection dose to avoid over-inflammation.

Antisense Oligonucleotides Positively Ameliorate AD Pathology

Antisense oligonucleotides (ASO) is a recurrent therapeutic choice in many neurodegenerative diseases including AD. Due to its pharmacokinetic property, ASO can be rapidly transported into neurons and glia after entering into the brain and alter the complementary RNA expression. Moreover, ASO cannot easily escape from BBB and be degraded by liver or kidney, therefore, it has a long half-life. So ASO injection can be less in frequency and amount compared with an antibody, which makes the treatment safer and less invasive (Evers, Toonen & Roon-mom, 2015; Geary, Norris, Yu & Bennett, 2015). Current clinical trials of ASO found no adverse effect of the treatment (Evers et al, 2015). Thus, anti-ApoE ASO may be a potential solution. Intracerebroventricular treatment of APOE ASO reduced the 50% of ApoE mRNA load in hippocampus and cortex, therefore depressed ApoE production and improved AD pathology. In APP/PS1 with APOE3/3 or APOE4/4 mice model, ASO decreased Aβ load and plaque-associated neuritic dystrophy when treated before the onset of plaque formation; whereas ASO could only decrease neuritic dystrophy without changing the Aβ level when mice were treated at the Aβ deposition stage (Huynh et al., 2017). This suggests that ASO treatment after onset may have some positive effects, but not as efficient. More investigations are needed for ASO treatment, especially the effectiveness after onset.

CONCLUSION REMARKS

APOE4 is found as the greatest risk factor of sAD. Among Alzheimer's patients, 36.7% of them contained one or two alleles of APOE4 (Liu et al., 2013). In general, APOE plays important roles not only on lipid and Aβ metabolism, but tau pathology, inflammation, neurite morphology and etc. Therefore, APOE can be a powerful target to moderate the AD patients in the late stage. Here, we discussed the potential therapeutic options based on APOE in AD, including SMSC, ApoE2 injection, Aβ antibody, ApoE antibody and ASO. Preclinical experiments of AAV-based ApoE2

injection, Aβ antibody and ApoE antibody did show some reduction in Aβ metabolism, improvement in the neuron and/or amelioration in recognition ability after the pathology onsets. However, there was some caveat for those methods. First, all studies were still under the preclinical stage, so more studies are demanding to optimise the treatment. For example, preclinical AAV-based ApoE2 injection and APOE ASO exhibited decreasing effectiveness treated after the onsets in the mouse model. Second, the preclinical studies were mostly executed under APP background, while the effect on tau pathology, which is more correlated with neurodegeneration, is still blank. Third, all of AAV-therapy, APOE ASO injection and APOE antibody are invasive to the body, and potentially are at risk of immune response. Therefore, more investigation on dosage, side effects and modification of the construct are necessary.

Finally, according to some data, APOE knock-out mice displayed a reduction in synapse integrity, the present of ApoE is essential for some function. As mentioned, APOE is a versatile protein involved in altering brain cholesterol level, Aβ metabolism, tau phosphorylation, inflammation and etc. Of note, plasma ApoE is essential for spatial learning ability and other non-neuronal degeneration disease, such as coronary heart disease. In addition, ApoE works as anti-inflammatory agent in Aβ induced inflammation. ApoE antibody has already shown the elevated risk of inflammation and risk of a slower motor response in the mice (Liao et al., 2018; Kim et al., 2012; Liao et al., 2014). Therefore, a broad depression of ApoE could be risky and the side effect of ApoE silencing should be treated with caution.

In this review, we also inspected the function of ApoE in AD pathology in both Aβ dependent and independent way. ApoE isoforms have been studied since discovered in 1993, however, we are still blinded in many aspects of ApoE in the brain, for example, the relationship between ApoE and sAD pathology, the exact mechanisms of ApoE in inflammation and tau-mediated pathology, and etc. Those issues are important to pin down the exact role of ApoE in AD, and therefore could be helpful for the drug development. Notably, APOE ASO and AAV-based ApoE2 injection only showed the limited effect when treated after the onset. Thus, more studies to improve the detection of early-stage AD or predict the AD rate in health human being is needed in the future, which could assist to treat patients on earlier stage or even before the onset.

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