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Review of Alzheimer' Disease from Pathology, Diagnosis and APOE Targeted Treatment

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Abstract.: Alzheimer's disease (AD) is the main cause of dementia worldwide. Although the pathology of AD is not yet completely understood, it has been widely acknowledged that the biomarkers including beta-amyloid plaques, neurofibrillary tangles and the neuroinflammation induced by these hallmarks contributed to the AD's pathology. Different from the conventional diagnosis of AD which is based on clinical criteria, the modern methods relay on biomarkers imaging and fluid test. Currently, although the treatment of AD is targeted to symptomatic therapy, advanced therapies that target the hallmarks of AD such as anti-beta-amyloid and APOE related therapies are under developing. Here, we will first review the major hypothesis of AD's pathological mechanism and then discuss the methods of diagnosis and treatment of AD which is developed in progress.

Keywords: Alzheimer's disease, amyloid beta, tau, neuroinflammation, APOE, therapy.

1. Introduction

Dementia is a progressive syndrome that displays a deterioration in cognitive functions not causing by normal aging. The typical symptoms the patients can have are memory loss, language problems, and unpredictable behavior. There are more than 50 million people globally surfing from dementia according to WHO 2022 estimation. Due to the expensive medications and high payments to caregivers, Dementia places a huge financial burden on patients and their families. Among the various diseases and triggers that can cause dementia, Alzheimer's disease (AD) is the most common and fatal cause which accounts for more than 70% of all cases1,2. The AD's seriousness keeps growing due to the aging problems in global populations. Therefore, it is crucial to understand the neurobiological mechanisms of AD and to develop effective methods for diagnosis and treatment. It is now widely accepted that the extracellular deposition of amyloid beta-peptide (A β) plaque and intracellular neurofibrillary tangles of hyperphosphorylated microtubule-associated tau proteins (p-tau) are the hallmarks of AD. These features are toxic to neuronal cells and can induce neuronal dysfunction, resulting in neuronal damage, destruction of entire neural networks, and neuroinflammation3. Up to date, the major hypothesis of AD includes A β accumulation, tau hyperphosphorylation and neuroinflammation.

The traditional diagnostic approaches to AD relay on the patient's clinical criteria. However, traditional diagnostic methods have low sensitivity and specificity. To increase the diagnostic efficiency, advanced analyzing techniques such as position emission tomography (PET) and examination of A β

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and p-tau in cerebrospinal fluid (CSF) have been developed. Current treatments are designed to relief the symptoms, therefore, none of them can fundamentally terminate the AD pathological progress. Under the guidance of AD pathology hypothesis, new methods and medicine such as anti- Aβ antibody and APOE related genetic therapy are developed.

In this article, we will summarize and discuss the latest understanding of the pathology, diagnosis, and approved treatments of AD.

2. The amyloid hypothesis

Among the hypotheses associated with AD pathology, the Aβ extracellular accumulation is the principle one and has been generally accepted. This hypothesis states that AD is a consequence of pathological disturbance, involving the accumulation and deposition of Aβ which formed the amyloid plaques and the following synaptic and neuronal damage. Aβ is a highly insoluble peptide that is extremely difficult to undergo degradation. Amyloid 1-42 is considered to be the most hydrophobic isoform that has the greatest cellular toxicity. It usually presents as a β-pleated sheet-like structure with a high propensity to aggregate that form the main part of amyloid plaques 4–8. The Aβ amyloid is generated by the abnormal hydrolysis of amyloid precursor protein (APP) which is a transmembrane glycoprotein that wildly expressed on the membrane of human cells.

In health human brain, the degradation of APP recruits an enzyme called ADAM that hydrolyzes the APP at α site, producing a free peptide (APPsα) and a second peptide fragment within membrane. The second peptide is then breakdown further by γ secretase into small pieces of peptides called p3 and AICD. All the peptides degraded by ADAM are soluble and nontoxic9,10. In an AD patient brain, the APP is hydrolyzed by a different enzyme called β-site APP cleaving enzyme (BACE 1) which cut the APP peptide at its β-site forming a free peptide APPsβ and a second peptide fragment within membrane (C99) 11. The APPsβ is further hydrolyzed of APPsβ γ secretase which produces the β amyloid and AICD. The β-amyloid can aggregate into plaques easily and as a result the connection between neurons and neuron network is disrupted, finally cause AD.

Although Aβ plaques play an important role in the pathogenesis of AD, the underlying mechanism remains unclear and requires further investigation. Recent studies have shown that the Aβ oligomers also have cellular toxicity in the way of affecting the cellular components such as nucleus that damages the genomic information and penetrating cell membranes that leads to imbalance of membrane potential. Immune studies also show that Aβ aggregation is able to trigger immune cells in brain secreting different kinds of inflammatory mediators which is harmful to neurons, suggesting that Aβ accumulation could cause inflammation in brain.

3. The tau hypothesis

Despite of the accumulation of Aβ, neurofibrillary tangles is also one of the factors that causes AD. The tau hypothesis suggests that the hyperphosphorylation of the tau proteins may aggregates and form the tangles that causes neurodegeneration.

Tau proteins are soluble, hydrophobic, and mostly find in the CNS and associated with maintaining the stability of the microtubules in the neurons12,13. However, in AD brain the tau protein undergoes an abnormal phosphorylation process, which generated the hyperphosphorylated tau proteins. The hyperphoshorylated tau protein has very low affinity and is easy to detach from microtubules. This will result in the dysfunction and instability of microtubules and cytoskeletons, which can cause synapses death and finally lead to neurodegeneration.

4. Neuroinflammation

Although Aβ plaque and hyperphosphorylated tau protein are toxic and can cause damage to nerve cells directly, recent study suggested that they could also be able to cause inflammation in brain through activating immune cells such as microglia and astrocytes.

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Neuroinflammation is defined as the inflammatory responses in the brain. In AD brain, the neuroinflammation process includes the Aβ or p-tau triggered microglia and astrocytes activation followed by releasing of inflammatory mediators such as cytokines, chemokines, neurotransmitters and reactive oxygen species (ROS)14. These mediators are able to recruit monocytes and lymphocytes crossing the blood brain barrier (BBB) 15,16 and activate resting microglia cells. This process is a positive feedback control that more and more microglia cells will be get involved in and release even more inflammatory factors to accelerate the pathology of AD.

Microglia are the immune cells in CNS and play critical roles in maintaining the general functions of neurons which is to clear the unnecessary plaques and damaged neurons. Microglia are activated through the receptor for advanced glycation end products (RAGE) and macrophage colony-stimulating factor (M-CSF) 17. When the presence of Aβ plague is detected by the receptors, microglia will gather around the plague, but fail to engulf the hydrophobic Aβ plagues due to the plagues' size, therefore more mediators like cytokines and chemokines such as cytokines IL-1/6/8 and TNF-α 117,18 are secreted to activate more microglia to promote the clearing progress17,19. However, during the clearance progress, microglia release toxic substances like ROS and free radicals which are harmful to neural cells. As a result, numerous cells come to apoptosis and further neuroinflammation occurs.

Astrocytes are star-shaped cells with functions of maintaining the ion balance and repair the damage inside the brain and spinal cord. Like microglia, astrocytes are also activated by Aβ plagues and converge around them. Astrocytes also release mediators like prostaglandins, thromboxane, complement factors, ILs, leukotrienes, proteases, protease inhibitors and coagulation factors to stimulate more astrocytes to be involved in the clearing process. In return, extra astrocytes produce a surplus amount of ROS and cytokines that could cause further neuronal inflammation.

5. Modern diagnosis

Symptom observation of the patients is the most conventional method used on diagnosing AD. In 2011, the National Institute on Aging-Alzheimer's Association (NIA-AA) has published the clinical criteria for symptom observation of AD patients based on the original 1984 vresion. However, the accuracy and specificity of conventional method is low. For example, it is very difficult to distinguish the symptoms between early stage AD and memory loss that due to normal aging. In order to address these issues, modern diagnosis method with higher accuracy are developed.

Positron emission tomography (PET) is a technique that requires radiotracers to locate and picture the changes in certain metabolism. When patients getting examined by this technique, a radiotracer that travels through blood stream will be injected to the patient and binds specifically to the Aβ plagues. Then the patient will undergo a PET scan to detect the presence and mounts of Aβ plague. The sensitivity and specificity of this diagnosis method was 96% and 100% respectively23. However, the high cost of amyloid PET imaging is not affordable for many people's income, which limited its practice.

Another less costly but more-invasive method is the analysis of cerebrospinal fluid (CSF). This method is able to detect the content of Aβ and p-tau protein in CSF which is collected thorough a lumbar puncture procedure. The CSF analysis has slightly lower accuracy compared to PET scan, about 85%-90%.

In spite of PET and CSF tests, a serum test could be carried out to detect the quantity of certain proteins which participated in AD. This method is less invasive than the others.

6. Current treatment

As mentioned in above, in AD patient's neuron system, Aβ plagues and NFTs (neurofibrillary tangles) will affect the neuron signal transaction. Neuron signal transaction rely on neurotransmitters (eg Acetylcholine), in this case neurotransmitter will be blocked by the NFTs and the plagues. To improve the signal intensity, currently there are two available groups of pharmaceutical therapies.

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The first one is the cholinesterase inhibitor such as donepezil, galantamine and rivastigmine. The cholinesterase inhibitors functioned by inhibiting the enzyme that degrade the neurotransmitter, such as acetycholine enabling them to remain in synpase for longer time and keep the extent of stimulation at a relative high level. However, cholinesterase inhibitors are only effective for patients who are in early stage of AD.

The second group is N-methyl-D-aspartate (NMDA) receptor antagonist such as memantine which is another medicine that is used to treat AD. Memantine works by obstruct the passage of calcium ions (Ca2+) so fewer or no calcium ions can flow inside the cell. This creates a greater difference in the charge balance and increased the strength of signal transmission. All the five drugs are approved to have good effect on ease the symptoms of AD. Donepezil, galantamine, and rivastigmine are effective for all AD and Parkinson patients24 while the Memantine is suitable for moderate or severe stages AD patients 25,26.

Certain scientific reports shows that patients who are diagnosed with AD are normally lack of vitamin D, so regular supplementation is suggested for people who has insufficient vitamin D. Additionally some research have shown that omega-3 supplements such as fish oil helped some AD patients to improve their cognitive behavior. Also being physically active would help to prevent getting cardiovascular disease and ensure peoples ‘cognitive functions are correctly working.

Furthermore, it is reputed that people who have Mediterranean diet are less likely to suffer from AD. Mediterranean diet often produce food with sea products, fresh primary products, and plant-based products (olive oil), at the same time this diet controls the use of processed food, (especially red meet and oil) and refined grains. The main reason this diet can lower the risk of getting AD is that olive oil and fish lipids will block the receptor on human immune cells, so these cells cannot be activated and release more ROS and chemokines.

7. Future treatment

From all the hypothesis of AD, Aβ plagues are the most dominant factor of AD. Anti-Aβ antibodies could be developed and injected into patients’ body to hydrolyze only Aβ plagues. In 2014, two monoclonal antibodies were developed, however none of them shows significantly improve in patients who are in moderate stage. Later, some research indicated that this medication is only beneficial towards patients who are in early or mild stages of AD.

Another way to prevent Aβ plagues from forming is to inhibit the production of beta-secretase. From the above, it is said that beta-secretase is the main component in the abnormal way of processing APP. Without the presence of beta-secretase, Amyloid β will not be present and neither do the plagues.

Apart from the Aβ plagues, hyperphosphorylated tau protein is also a very influential factor that cause AD. Some tau vaccines and anti-tau, a drug that is designed to clear the gathering of hyperphosphorylated tau protein are also in development. So far anti-tau and the vaccines have only being tested on animals.

G oscillation is a brainwave that relates to communication between the neurons. This brain wave has a high frequency, and its function might be determining the authenticity of certain memory pieces. Lately researchers at MIT figured out that G oscillation in AD mouse model, has reduced the number of Aβ aggregates and leads to improved cognitive function. For human, this method is only used for stimulating patients visual and auditory ability.

8. APOE therapy

Apolipoprotein E (APOE) is a protein combining lipids to form lipoproteins and in recent years it is also considered a significant influence on various diseases pathology. Among those diseases, the impact on Alzheimer’s disease pathology is the most notable one. APOE possess isoform dependency which means that different APOE isoforms have different functions. There are three isomers of APOE: APOE2, APOE3 and APOE4. APOE2 is thought to decrease the likelihood of getting AD, APOE3 is neutral and lastly, APOE 4 is believed to increase the likelihood of getting AD27–29. Between all three pathology

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cascades of AD, APOE is closely related and mainly affects the amyloid hypothesis via three aspects: clearance, fibrillization and production

8.1. Clearance

Among all clearance pathways to remove soluble Aβ from the brain interstitial fluid (ISF), transporting through the blood-brain barrier (BBB) and neuroglia are the most two common metabolisms30.

Transportation through the blood brain barrier relays on the receptors expressed at BBB. The APOE 2 – Aβ and APOE 3 – Aβ complexes can be recognized and transported by Very low density lipoprotein receptor (VLDLR) and low lipoprotein receptor 1 (LRP1) while APOE 4 – Aβ complexes can only be transported via VLDLR. As VLDLR mediates the internalization of the APOE–Aβ complex at a slower rate than LRP1, APOE 4 – Aβ complexes have higher chances to aggregate and forming fibrils and plagues in ISF31–33.

Neuroglia pathways is achieved by astrocytes and microglia, which are the two most important immune cells in the brain and their main functions are protecting and supporting the central nervous system (CNS)34,35. Normally when astrocytes and microglia detect the presence of Aβ, the Aβ will be engulfed by these cells. However, when APOE 4 exists, it competes with Aβ which implies that APOE 4 can also be engulfed by astrocytes and microglia35. The part of Aβ that is not engulfed will clump together to form oligomers and plagues and this will rapidly aggravate the patient's condition. In addition, astrocyte by itself in cellular uptake normally engulfs Aβ that are detected by the LRP1 cellular receptor, the presence of APOE greatly reduces the amount of Aβ that passes through LRP1 by competing with them36.

8.2. Fibrilization

Aβ fibrils are the major constituent of Aβ plagues, there are three kinetic stages of the Aβ fibrils formation: the lag phase, the growth phase, and the plateau phase37. During the very first phase, the lag phase, the monomers of Aβ clump together to form oligomers as seeds. The oligomers will then attract the monomers that are nearby to form fibrils. This phase has a relatively low paste compared to the others. In the growth phase, the paste of formation rapidly increases so the fibril can grow to a certain extent. Finally, in the plateau phase, the growth rate decreases until the fibrils stop extending. Above is the ordinary routine for the formation of Aβ fibrils. However, if APOE4 participates in this process, the speed of fibril growth rate will be raised. APOE4 mainly affects the lag phase by accelerating the chance of Ab oligomers formation from monomers to produce more Aβ fibrils. In the growth phase and the plateau phase, APOE 4 only has little effect on them38. This is because the growth phase has the highest speed of formation, any acceleration barely affects the process. For the plateau phase, the fibrils formed are mostly long enough, so increasing the rate doesn't act a big part overall.

8.3. Production of Aβ

Additionally, the presence of APOE 4 will boost the expression of APP (amyloid precursor protein) which leaves an increased probability of more APP being cut by beta secretase, forming more Aβ in the brain34.

9. Therapies

Based on the mechanism that APOE interact with Aβ, the APOE therapies, the current strategies for the targeting of APOE to treat AD fall into three main categories: modulating APOE quantity and lipidation, targeting APOE structural properties and interactions with Aβ, and targeting APOE receptors.

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9.1. The quantity and the lipidation status of APOE.

Recent studies report that certain medications e.g Bexaroteneare are able to stimulate liver X receptor (LXR) and retinoid X receptor (RXR), those two stimulated nuclear receptors will increase the secretion of APOE-2 and APOE-3 which decline Aβ deposition39,40. However, the side effect that comes with bexarotene is the rise of hypertriglyceridemia which give the patient a higher risk of getting cardiovascular diseases41. Another way to increase the expression of APOE-2 is via a virus-mediated gene. This special gene aims to embed in patients' DNA so that more APOE-2 will be translated, less Aβ will form42–45. With the same idea, reducing the expression of APOE-4 can lead to a reduction of Aβ. This can be achieved by two pathways. One is injecting antibodies that are complementary to APOE-4 receptors into the patients and the antibodies will only clear APOE-4 specifically46,47. Another one is by gene editing, which knock out all the genes that produce APOE-4 expressions, this will also decrease the formation of APOE-448.

Modulating lipidation of APOE-4 is another therapy that helps with AD symptoms. Some studies show that the lower the lipidation level of APOE-4, the higher risk of forming more Aβ plagues48. ABCA1 which is an enzyme that is responsible for the lipidation of APOE4. According to recent studies, lowering the lipidation status cause an increase in the amount of Aβ deposition, theoretically enhancing the lipidation status by increasing expressions of ABCA1 will decrease Aβ gathering49. However, at current stage, there is only a hypothesis with insufficient evidence.

9.2. Structure of APOE

Basic amino acid contains amino group and carboxyl group, so as APOE-4. Some scientific studies imply that by blocking the amino and carboxyl group of APOE-4 will inhibit the interactions between APOE-4 and Aβ50,51. A special designed molecule that binds better with APOE-4 will compete with Aβ thus slowing down the formation of Aβ51. Aβ1-42 and APOE-4 complexes are the most toxic substances among all Aβ complexes. Aβ1-28, an exceptional artificial made nontoxic peptide will take the precedence of binding with APOE-4 which results in less toxic Aβ-APOE complexes forming and reducing the Aβ depositions52.

9.3. Genetic engineering

To achieve the target of inhibiting expressions of APOE-4, genetic engineering can also be applied to the gene that produce APOE-453. Not only deleting the APOE-4 gene but also transform it to the one that express APOE-2. This could also result in a reduction of Aβ deposition.

9.4. Targeting the receptors

LDLR and APOE receptors mediate partial clearance pathway of Aβ. Recent investigations show that the scarcity of LDLR may cause an over accumulation of Aβ deposition so it has been speculated that an enhancement of LDLR expressions may promote the clearance of Aβ54.

10. Conclusion

In this review, we summarized the main hypothesis of AD pathology including A β , p-tau and neuroinlfammasion. However, either of them could only partially explain the AD pathology. Therefore, more investigations are required in the future.

We also reviewed the development of AD diagnosis and treatment method. Compared with the symptom based clinical criteria, advanced imaging and chemical fluid test point out the new direction of diagnosis development. In terms of treatment, although the currently approved drugs can improve the memory and alertness of AD patients, the overall AD progression still remained uncured. To increase the efficiency of treatment, several advanced method based on the A β and p-tau theories including antibody and vaccines that targets A β plauqe, BACE1 enzyme and p-tau have been developed.

In addition, we reviewed the relation between APOE and A β in the aspects of A β clearance, fibrilization and production in ISF. The potential therapeutic strategies based on APOE is also

summarized in the three main categories: modulating APOE quantity and lipidation, targeting APOE structural properties and interactions with A β , and targeting APOE receptors.

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