

The role of Amyloid precursor protein (APP) in Alzheimer's disease (AD)

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Abstract. Alzheimer's disease (AD) is a neurodegenerative disease, which endangered human life expectancy. Since 2020, approximately 50 million people worldwide had got AD. With the increase of longevity of the whole population, the potential threat of AD Also increase. Although the pathogeny of AD is still not entirely clear, there have developed several hypotheses for AD. One of them is A β hypothesis. It suggests the deposition of A β plaques outside the neuron cells. This may cause a series of problems. It may induce ER stress, oxidative stress, neuro-inflammation and inhabited LTP in patients' hippocampus. These all stimulate the neuron cell death. The loss of neuron cells will further induce these symptoms and then triggered the loss of memory. Although there are still a lot of debates on the now existing drugs about AD, the further studies on the role of A β on AD is needed.

Keywords: Alzheimer's disease (AD), A β peptide, ER stress, Oxidative stress, Neuro-inflammation.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease, which will be getting worse by time. The death of the neuron will cause progressive irreversible memory decline, cognitive impairment and language loss. Because this disease is progressive, the symptom of this disease is mainly discovered in groups above 65 years old [1]. Nowadays, population aging is one of the main social problems. With the increase of longevity of the whole population, the potential threat of AD Also increase. Since 2020, approximately 50 million people had got AD worldwide [2]. The symptom of AD is broken to three parts: early, middle and late stages. Because AD is caused by neurodegenerative, nearly all the patients in late stages experience a brain shrinkage, till now, the medicine designed is useless for patients in that stage.

It is discovered that A β plaques may lead to the oxidative stress. This cause reactive oxygen species to be over produced and damage the macromolecules in organism like lipids, proteins, DNA and RNA.

Also, it will cause the oxidation of free radicals, which post effect on the neon death [3]. What's more, the accumulation of A β proteins will cause neuro-inflammation. A β proteins will stimulate the neuron cell to create inflammatory cytokines. Also, it will increase the release of pro-inflammatory cytokines. Then, long term of inflammation will induce the activity of toll like receptor 4 (TLR4), which will lead to the gradual death of the cell. Also, it will lead to the long-term potentiation in hippocampus (LTP). When A β Protein deposit, the proteins that act on LTP will be affected, thereby inhibiting its induction process and reducing the learning ability and memory of patients.

Till now, the pathogeny of AD is still not entirely clear, so there are a lot of difficulties on discovering AD in the early stage. There are several hypotheses for AD. Among them, Amyloid β protein (A β) hypothesis is really popular. At first, some plaques were discovered in the brain section of AD patients. Then, there are hypothesis that these plaques effect greatly on these patients' neurons death. These ex-cellular plaques made form A β proteins. There is a lot hypothesis on how these plaques are made and a lot of researches of the effect of these plaques. However, no-one combines them together to give a holistic view on this hypothesis.

Therefore, this Paper is going to combine the hypothesis of how the A β plaque exist with the effect of it. This may help to show a whole picture of the A β hypothesis and may give some new thoughts on the pathogeny of AD or even the medicine design of AD. The first part of the essay is the

hypothesis of how these plaques are formed. The second part is about the effect of A β plaques to the human body.

What's more, the accumulation of A β proteins will cause neuro-inflammation. A β proteins will stimulate the neuron cell to create inflammatory cytokines. Also, it will increase the release of pro-inflammatory cytokines. Then, long term of inflammation will induce the activity of TLR4, which will lead to the gradual death of the cell. Also, it will lead to the Long-term potentiation in hippocampus (LTP). When A β Protein deposit, the proteins that act on LTP will be affected, thereby inhibiting its induction process and reducing the learning and memory ability of patients.

Lat but not least, A β proteins will damage the activity of A β degradation enzyme like enkephalinase (NEP) and insulin degrading enzyme (IDE). This will further increase the accumulation of A β proteins and cause damage to the neuron cells.

2. App Hypothesis

APP is a membrane molecule of the neuron cells. This molecule is coded by the gene APP and controlled by substrate presentation. APP gene is a is an ancient protein which is highly conserved. It is located on the number 21 chromosome. It contains 18 exons spanning 290 kilobases.

There are three kinds of secretase: α -secretase, β -secretase and γ -secretase. Normally, the α -secretase will be dissected APP molecule to sAPP α and CTF α these two parts and then, γ -secretase will cut p3 peptides out and release AICD. P3 peptides can dissolve to water, therefore, will causing no problem. However, in abnormal condition, β -secretase will alternate α -secretase and cut APP molecule to sAPP β and CTF β . The sAPP β is a long, insoluble amyloid. Then, γ -secretase cut CTF β to AICD and A β peptides.

A β peptides contain 37 to 49 amino acid and it cannot dissolve in water. Then, the imbalanced production and clearance rate of A β peptides will cause them to accumulates to plaques outside the neuronal cell of patients. The accumulation of A β peptides will cause several consequences, and these symptoms will in turn lead to larger number of A β plaques formation and deposition of proteins. This will ultimately lead to the death of neuron cell, which post irreversible damage to brain tissue.

There are several pathways that can help to clear the existence of A β Peptide. One of them is the receptor-Mediated Clearance of A β . The molecules participate in receptor-mediated flow of A β across the BBB are Low-density lipoprotein receptor-related protein (LRP) and the receptor for advanced glycation end products (RAGE) [4]. These two receptors located on cell surface can also mediate the clearance of A β . LRP mediate A β to floating out brain and float the periphery, while RAGE participate in the process for A β to influx back into the CNS.

Another clearance of A β pathway is peptidolytic removal. There are two major endopeptidases which help A β to degradation, are insulin degrading enzyme (IDE) and neprilysin (NEP). These are zinc metalloendopeptidases. There is once an experience of IDE knockout mice, who reveal the increase of A β and AICD level in the brain. NEP can also be referred as neutral endopeptidase, which is a membrane protein of type II. It can hydrolyze circulating biologically active peptides like IDE. NEP is rate-limiting enzyme for A β degradation, with the evidence from the knockout studies in mice [5].

There are several pathways for the deposition of A β . One of it is the vicious circle of blood-brain barrier (BBB) dysfunction and A β deposition. A β is neurotoxic which will harm the BBB. The dysfunction of BBB activates β -secretase and γ -secretase to secret. Theses secretase helps to cut the APP molecule, which increase the production of A β . What's more, BBB dysfunction harm the transport of A β , which leads to aggregation of A β . As what is listed before, LRP1, RAGE are the transporters which help to transport A β through BBB. The development of AD will cause the expression these transporters become abnormal and lead to disfunction, which will cause abnormal A β to transport and deposit. Therefore, BBB will be destruct, which will reduce A β clearance pathway. Hence, induce inflammation to cause A β deposition [6].

Normally, when amyloid plaques deposit, microglia will be recruited to change into phagocytosis and process A β and the A β will be digested. However, if this mechanism is dysregulated, it will cause Ab to accumulate. CD33 is a type-1 transmembrane protein. The signaling cascades mediated by CD33 are responsible for the innate immune responses. It is found that CD33 can inhibit the clearance mechanism of A β in BV-2 microglia and the removal of CD33 can then enhance the phagocytosis of Ab1–42. Therefore, CD33 is a negative regulator which will lead to AD [7].

The enzymes that are able to identify and decompose A β proteins are mainly enkephalinase (NEP) and insulin degrading enzyme (IDE). NEP can be highly expressed in activated microglia and astrocytes. When neurons are over activated, the secretion of NEP will be insufficient, resulting in A β Protein deposition. IDE is a cytoplasmic protease which can degrade A β protein. Its secretion is negatively correlated with age. With the increase of age of organism, the ability of the protein to disintegrate A β protein is not enough to maintain a balance with A β protein's production ability. This further causes the A β protein to condense.

3. The Effect of AB Peptide

A β imbalance of protein metabolism will lead to several consequences in organism. Among them are, oxidative stress, neuro-inflammation, LTP and the decrease of activity of A β degradation enzyme. Also, the above factors can induce the onset of AD or aggravate the disease.

3.1. ER stress

The A β deposition can trigger ER stress by stimulating the unfolded protein response (UPR). The pathway of UPR is a system for defending. It can handle the aggregation of mis-folded proteins. When this process is prolonged, the cell will switch to apoptosis. When GRP78/BiP these ER chaperone bind with the mis-folded proteins, UPR is initiated, which causes these ER chaperones to dissociate from PERK, ATF-6, and IRE-1, which causes PERK and IRE-1 to autophosphorylate and moves ATF-6 to the Golgi apparatus to activate. Among these pathways, PERK-eIF2 α pathway is initiated by A β . Activated PERK can phosphorylate eukaryotic translation initiation factor 2 subunit α (eIF2 α). After eIF2 α is phosphorylated, the protein translation of cell mRNA is stopped. In addition, it selectively stimulates the translation initiation of the transcription factor ATF4. Therefore, the initiation of the UPR leads to a total reduction in translation, an increase in degradation of protein and an increase in ER chaperones, like GRP78/BiP, thereby increasing ER's capacity of protein folding. Finally, the cell will come back to ER homeostasis or continue to go towards apoptosis. Since neurons are sensitive to AD aggregation antibodies, ER stress-mediated cell death may play a vital part in the pathogenesis of AD [8].

3.2. Oxidative stress

Oxidative stress is a process caused by imbalance of redox state. It includes the process when excessive reactive oxygen species (ROS) are produced or antioxidant system is dysfunctional. The combination of redox active metal ions with A β peptide, like copper, can catalyze the production of ROS. ROS can induce oxidative stress. However, when the concentration of ROS becomes uncontrolled, it may lead the free radical-mediated chain to react to target molecules like lipids, proteins, polysaccharides and DNA, which will damage these molecules. Under normal conditions, there are three different pathways to prevent ROS. But, of this equilibrium is disrupted in which these defenses become insufficient. Oxidative stress will occur and often leads to apoptosis, a mechanism for cell death [9].

To gain energy, organisms convert the ion gradient to chemical energy like ATP. The ion gradient was formed by the electron transfer reactions across the cell membrane. At the end of the electron transport chain, oxygen helps to accept the final electrons. The intermediate of the reaction sometimes are hydrogen peroxide, hydroxyl radicals and superoxide anion. These molecules are reactive. Therefore, they are difficult to control. Also, some of them can be precursors and can form reactive

species. Therefore, they can be classified as ROS. ROS are reactive oxygen-containing chemical [10]. Generally, ROS are at the low level. If ROS accumulate, it will then be defined as oxidative stress.

In amyloid plaques, there are ions such as iron ions, zinc ions and copper ions. Normally, in the synaptic cleft of some neurons Cu and Zn can be excreted [11]. With the existence of amyloid plaques, these ions cannot be removing normally. It will combine with the A β peptide, therefore, stimulate the oxidative stress.

Furthermore, oxidative stress may get into a vicious circle. ROS can damage bio-molecules, which will lead to more ROS to accumulate. This will increase the severity of oxidative stress, which finally lead to the death of neuron cells.

3.3. Neuro-inflammation

A β plaques are inflammatory stimulators which can stimulates nerve cells to produce inflammatory factors, leading to neuro-inflammation. They trigger the microglia in central nervous system (CNS) to activate. Hence different kinds of pro-inflammatory cytokines and chemokines will be secreted. Also, around the amyloid deposits in the brain of the patients who got AD, the clusters of reactive microglia and astrocytes can be found [12]. Cytokines includes IL-1b, IL- 6 and TNFa, while chemokines include CCL 2/4/11. These molecules will induce microglia as well as astrocytes to go the site where inflammatory occur. A β plaques also immune clearance mechanism. These molecular also add to the inflammation of the neuron and induce the reactivity of deleterious microglial and astrocytic. Protein deposition can induce the activation of TLR4 and further develop neuritis. TLR4 is signaling pathway which trigger the initiation of inflammatory responses. TLR4 will be triggered by A β plaques which will then drives the inflammatory responses and cause inflammation [13].

TLRs and Nod-like receptors (NLRs) can detect the aggregation of A β . These receptors cause the microglial-dependent sensing of A β . TLRs are used as pattern recognition receptors. They can help to defense organisms against the pathogenic infection and injuries on tissues. There are thirteen kinds of TLR subtypes. Among them, TLR2, TLR4, TLR6, and TLR9 are the subtypes which can detect Ab species.

There are several key mediators for neuroinflammation. Chemokines is one of them. It can help microglial to recruit in neuroinflammatory sites. Proinflammatory cytokines, IL-1b, IL-6 and TNFa are also mediators. If the genes controlling the production of these are up-regulated, polarization of microglia will be drive to the pro-inflammatory [14].

3.4. Long term potentiation inhibition in hippocampus

The proteins in the hippocampus that affect LTP will change as a result of A β protein deposition. LTP will be induced. When A β protein precipitates and enters the CA1 region of hippocampus, it will induce the loss of 70% of neurons and the deformation of pyramidal neurons, by inhibit the effect of G-protein-gated inward rectifier potassium channel (GIRK) channel in the hippocampal CA1 [15].

In the hippocampus of the patients who got AD and in iPSC-derived neurons, defective mitophagy were found. Compared with healthy controls, hippocampal neurons in exhibited mitochondrial morphological changes which includes the reduced size of mitochondrial and excessive damage. Dysfunctional mitochondrial cause energy production to be insufficient, leading to ATP depletion. This will stimulate AMPK. AMPK and MFF, its downstream effector, will be activate. When monitored by CO localization of the mitochondrial protein TOMM20 with Lamp 2 lysosomal proteins and quantification of mitotic phagocytosis, mitophagy levels in AD hippocampus are around 30-50 presents lower. These findings suggest that damaged mitochondria are aggregate in the hippocampus because defective mitophagy [16].

Even though the deposition of A β peptides is been studied so thoroughly, there still are a lot of doubts on this theory. Some people believe that it is only an important feature of AD, not the main cause of AD. Some study states that before the aggregation of extracellular amyloid protein,

autolysosome acidification has already declined, which means the deposition of amyloid protein may not be the main reason for AD but a side effect [17].

Recently, there are some Ab-targeted drugs or treatments invented. However, they don't have a statistically significant benefit at their prespecified clinical endpoints at the phase 3 clinical trials [18]. Aducanumab is the first drug indicated to treat AD that was approved for listing by FDA. Three advisers resigned from FDA because of this. It is an amyloid beta-directed antibody. It can directly grease the A β plaques in the patients' brains. But there are still a lot of criticize on it. It seems that this medicine is only effective for AD patients in early stage. Also, FDA's external experts concluded that there was no strong evidence for the efficacy of aducanumab. They listed several suspicious efficacy and multiple risk signals found in the data analysis. What's more, there are evidence that the drug is associated with health risks: brain swelling or brain bleeding occurred in 41% of patients in the studies [19]. Therefore, the further studies on the role of A β on AD is needed. This may help to develop more efficient drugs or treatments.

4. Conclusion

AD is a progressive neurodegenerative disease, which endangered human life expectancy. Although the pathogeny of AD is still not entirely clear, there have developed several hypotheses for AD. One of them is A β hypothesis. It suggests the deposition of A β plaques outside the neuron cells. This may cause a series of problems. It may induce ER stress. UPR signaling pathway may be stimulated, like Perk-eIF2a pathway. Activated PERK phosphorylates eIF2a, which repressed the translation of normal cellular mRNAs and initiate transcription factor ATF4. This can force the cell to go towards apoptosis. Oxidative stress may also be stimulated. Amyloid plaques can deposit with ions such as iron, zinc and copper. These ions cannot be removing normally, which means ROS is accumulated. This induces the oxidative stress. Neuro-inflammation is also one of the effects. TLRs and NLRs are receptors that can detect the aggregates of A β , which cause the initial microglial-dependent sensing. This triggers the initiation of the resident CNS microglia. Pro-inflammatory cytokines and chemokines will be secreted. Cytokines includes IL-1b, IL- 6 and TNFa to form. Chemokines includes CCL 2/4/11. These molecules will induce microglia and astrocytes to float to the inflammatory site. Furthermore, LTP can be inhabited in hippocampus. When A β protein deposition, the effect of GIRK channel in the hippocampal CA1 will be inhabited, which induce the loss of neurons. Even though, there are still a lot of problems unsolved about the A β hypothesis and the drugs about AD are still not have a clear result, the further studies on the role of A β on AD is needed.

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