

Deep Learning to Detect the Function of Calmodulin in Alzheimer's Disease

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Abstract. Alzheimer's disease (AD) is marked by losses in cognitive functions. Hallmarks of AD, amyloid beta (A β) plaques and neurofibrillary tangles, are intercellular structures that are highly associated with AD. The accumulations of these molecules could be seen as results of other events in prior stages of AD. The calcium hypothesis was first proposed to explain the forming of these hallmarks while the calmodulin (CaM) hypothesis was proposed to provide more improved insight later. The CaM hypothesis states that the uncontrolled flux of calcium ions in the neurons is the cause of neurodegeneration. In addition, the critical role of CaM and CaM-binding proteins (CaMBPs) are identified in the hypothesis. In this project, the regulating role of CaM and CaM-related protein in AD are discussed, including CaMBPs, critical receptors and risk factors. Besides, the potential therapeutic target related to CaM are also mentioned to provide a promise direction of treatment for AD.

Keywords: Alzheimer's Disease, Calcium, Calmodulin.

1. Introduction

As a dominant form of dementia, AD is characterized by losses in cognitive functions [1]. There are approximately 50 million AD patients [1]. The mechanism of AD prevention is ambiguous when the disease is associated with both age and genetics. Lifestyles and other environmental factors could contribute to AD as well, making targeting the disease's causes challenging [1]. Naturally, the dysfunctions of proteins in brains are highly involved in the pathogenesis of AD. Multiple proteins had been identified as risk factors for AD, helping accumulate the extracellular hallmarks of AD and leading to neuron death and toxicity. Amyloid beta (A β) plaques and phospho-tau neurofibrillary tangles are typical hallmarks of AD [1]. They are extracellular structures accumulating with the progress of AD. The current studies of potential treatment for AD aimed at preventing the accumulation of A β amyloid beta peptides and hyperphosphorylated tau-proteins. However, no clinical treatment is currently available.

Many different hypotheses reveal the cause of AD in various ways. The amyloid hypothesis and the tau hypothesis propose that the accumulation of hallmarks is partially involved in the pathogenesis of AD, when other factors are widely considered as the cause of AD. The dysregulated calcium typically occurs in early-onset of AD. The calcium hypothesis of AD assumes that the uncontrolled influx of calcium ions, Ca²⁺, in the brain leads to the accumulation of AD pathological hallmarks, which then results in the neurotoxicity and, eventually, neurodegeneration [2]. Calcium dysregulation is demonstrated to be highly associated with amyloidogenesis, tau-kinase phosphorylation, accumulation of the amyloid beta plaques, and intracellular tangles. Previous research on controlling calcium influx revealed the critical role of Calmodulin (CaM) instead of calcium in AD. CaM is a vital calcium sensor and plays crucial role in regulating the binding of calcium and other proteins. In the calmodulin hypothesis derived from the calcium hypothesis, scientists state that calmodulin is a potential target to prevent the formation of AD hallmarks and regulate AD risk factor proteins [1]. Increasing number of verified CaMBPs incorporated in production of hallmarks supports the importance CaM holds in the brain. In this review, we will deep in the role of CaM in regulating critical ion channels and receptors, risk factor proteins, and formation of AD hallmarks.

2. CaM hypothesis

In the calcium hypothesis for AD, which was purposed originally in the 1980s and refined in 1994 by Khachaturian, the function of calcium ions in the accumulation progress of hallmarks of AD was examined [1]. Calcium hypothesis states calcium ions as the focus of neuron degeneration. Ca^{2+} is essential for neurotransmitter synthesis and release to improve intercellular communication in the brain. Calcium signaling contributes as well to a vital role in energy metabolism by monitoring the function of mitochondria in neurons. The intracellular concentration of the calcium ion in neurons is generally between 50-100 nanomolar [3]. The disruption of calcium homeostasis and increase of this particular cation could cause neurodegeneration disorders and indicate an early stage of AD, when the production of amyloid- β oligomer alters the receptors signaling for calcium ion channels [3].

While Calcium hypothesis examines the part which calcium influx plays in the progression of AD and the production of A β plaques and neurofibrillary tangles, it fails to consider that calcium ions do not work alone without other calcium binding proteins. In 2004, O'Day and Myre first proposed the addition of CaM to modify the calcium hypothesis and illustrated that the activation of CaM is critical for AD [1]. The calmodulin hypothesis demonstrates how CaM serves as the therapeutic proteins to control calcium levels and the importance of CaM in amyloid pathways and neurofibrillary tangle accumulation [1]. In this review, we will deep in the role of CaM in regulating critical ion channels and receptors, risk factor proteins, and formation of AD hallmarks.

3. CaM and CaMBPs:

CaM exists in all eukaryotic cells and participates the calcium signal transduction pathway as a messenger protein to serve as important regulators for cellular activities. CaM is a small protein with two separate globular domains which are located at the C-terminal and the N-terminal respectively [4]. Two high affinity calcium binding sites are located at each of the domains and the different amino acid sequence from the respective calmodulin binding domains builds the intricate designs and conformations of CaM proteins. These multiple binding domains ensure CaM can recognize different CaMBPs to improve the binding. The CaMBPs includes kinases, phosphatases, cytoskeletal proteins, and a wide range of other proteins. The binding between CaM and CaMBPs could happen with or without Ca^{2+} and CaMBPs are classified as Ca^{2+} -dependent or Ca^{2+} -independent according to this (Figure 1). CaM has the ability to bind to more than 300 different peptides totally and most of them are exist in the human brain [4]. The combination of CaM and Ca^{2+} can lead to the opening of the two terminal domains in CaM [5]. Hydrophobic surfaces are next revealed in order to bind with the complementary nonpolar section of CaMBPs [5]. As previous description, CaM has flexible recognition amino acids sequences to recognize multiply motifs which share some standard sequences. However, there is a dramatically difference between the motifs of Ca^{2+} -dependent and Ca^{2+} -independent CaMBPs. CaM mainly targets to motifs IQ ([FILV]Qxxx[RK]Gxxx[RK]xx[FILVWY]), IQ-like ([FILV]Qxxx[RK]Gxxxxxxx) and IQ motifs of Ca^{2+} -independent CaMBPs [1]. Several proteins engaged in the production and accumulation of Alzheimer's features were confirmed with calmodulin binding domains (CaMBDs) [1][6].

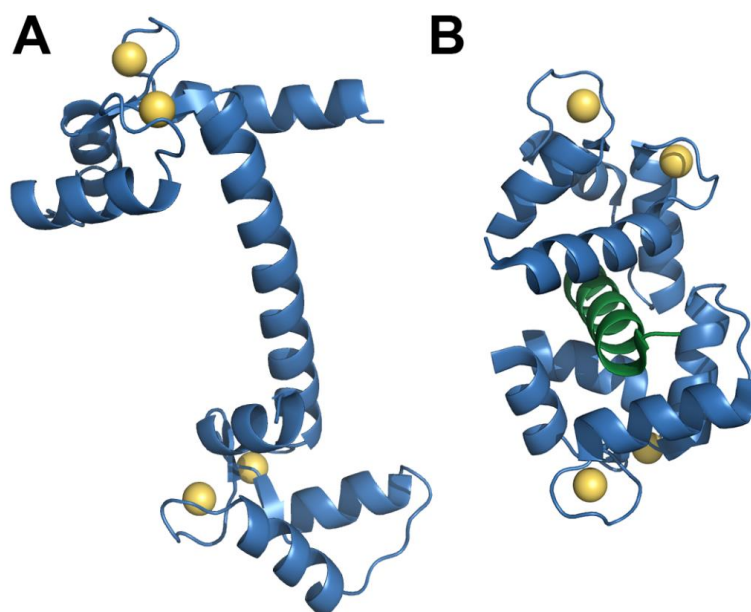


Figure 1. Diagram of CaM structure after binding [6]. (A) The CaM bind with four Ca^{2+} . (B) CaM bind with CaMBPs.

4. CaM and hallmarks of AD

In 1906, Alois Alzheimer firstly named two main hallmarks of AD as plaques and tangles. The hallmarks of AD were proven to be regulatable with the use of CaM on calmodulin binding proteins and be essential to the formation of AD and neurodegeneration [1].

Plaques forms and associates with CaM in AD. Amyloid plaques are produced by the buildup of around 40 amyloid beta peptides ($\text{A}\beta$), which related to the hydrolysis of the amyloid beta precursor protein ($\text{A}\beta\text{PP}$). After forming, the $\text{A}\beta$ experience oligomerizing and cluster together to become insoluble and hard lump surround neurons in the brains. These clusters, amyloid plaques, are highly toxic and can kill the nerve cells to cause multiply neurological disease, including AD.

$\text{A}\beta$ peptides are formed through the aspartyl membrane proteases beta-secretase and γ -secretase enzymes, leading to differences in size [6]. The most common products are peptides $\text{A}\beta$ -40 and $\text{A}\beta$ -42. $\text{A}\beta$ -42, for example, is extremely damaging towards one's ability to retain memory [7]. Research has shown that the increase of toxic $\text{A}\beta\text{PP}$ products can prompt cytosolic and organellular calcium levels, therefore further augmenting AD [7]. Many current treatments remain on inhibiting amyloid beta proteins. The amyloidogenic pathway twist the normal functions of calcium homeostasis [7]. The N-methyl-D-aspartate receptor (NMDAR) is a glutamate receptor present on the plasma membrane, can regulate calcium ion flow. $\text{A}\beta$ oligomers can activates the NMDAR receptors to produce signals for the increase of calcium [7]. Two central proteins involved in the production of plaques, $\text{A}\beta\text{PP}$ and beta-secretase (BACE1), are found to be CaM-regulatable as well [1]. Therefore, the regulation of amyloid- β plaques and regulation of calcium ion flux in the brain serves as a promising therapeutic for AD.

There are at least 24 CaMBPs associated with neuroinflammation were demonstrated [6]. Among these CaMBPs, two Kinases and one calcineurin were confirmed to be with neurofibrillary tangle formation [6]. Neurofibrillary tangles are accumulation of tau-proteins, which are extra-cellular proteins and are extremely toxic to the brain.

Tau is a phosphorylated protein with 85 phosphorylation sites. It is mainly present in the heart and brain [18]. Tau protein built up, which is the accumulation of neurofibrillary tangles, is regulated by the augmentation of tau-kinases. One typically example of tau-kinase is calcium/calmodulin-dependent kinases type 2 (CaMKII), which is one of the main targets of CaM [1]. CaMKII participates in the phosphorylation process of tau in AD patients' brains. The sustained and harmless level of tau

phosphorylation is a due to dynamic regulation of tau kinases [8]. In AD, tau protein is over-expressed and phosphatase, therefore continue producing neurofibrillary tangles [18]. The calmodulin binding domain of CaMKII is located within residues 290–309 [8]. While calmodulin itself doesn't bind to CaMKII, calcium bind calmodulin does automatically inhibiting the tau kinase [20]. When there is a lack of Ca^{2+} -CaM binding complex, the connections between the regulatory domain with the catalytic domain inhibits autophosphorylation of phospho-CaMKII (Thr286), achieving prevention of CaMKII auto-activation [8]. There's a negative correlation with the increase of CaM in brain and the decrease of tau-hyperphosphorylation. When calmodulin binds to CaMKII, the calcium binding affinity of individual CaM changes [8].

5. Critical receptors and channels

Muscarinic acetylcholine receptor (mAChR) and NMDAR are two identified receptors that are involved in AD through crosstalk with CaM [9].

mAChR is highlighted in the acetylcholine-associated excitatory process and has been considered as CaMBP. The dysfunctional mAChR and reduced mAChR expression are found in AD patients' brains. Research shows CaM plays a regulatory role in the neurotransmission induced by the combination of mAChR and acetylcholine [9].

As previous description, VGCCs are a key transmembrane channel protein to mediate intracellular Ca^{2+} concentration. Another critical gate for Ca^{2+} is NMDAR, which is in the ionotropic glutamate receptors (iGluRs) family. The structure of activated NMDAR shows as Figure 2. It is essential and irreplaceable to the brain in developing skills in memory and learning.

Insufficient NMDAR signaling causes neuron cell death, while an extensive number of activations leads to neurological trauma. In AD, the uncontrolled influx of calcium ions mainly associates with the NMDAR because of the higher permeability. CaM can regulate the open rate and time of NMDAR by combining with the C0 and C1 domains of NMDAR subunit NMDAR1.

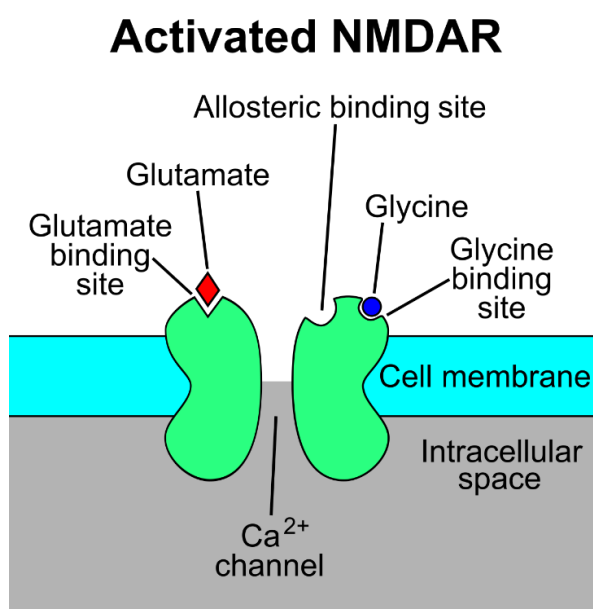


Figure 2. Schematic of activated NMDAR [10]. Glutamate can bind to the glutamate binding site when glycine binds to the other binding location.

Hyperphosphorylation, the cause of accumulation of neurofibrillary tangles, is linked to the NMDA receptors as well. CAMKII binds to the NMDAR subunit 2B (NR2B) on the catalytic site results in phosphorylation of peptides. CaMKII-induced phosphorylation of NR2B cause the dysfunction of NR2B and also further inhibit the dissociation of the binding complex. Uncontrolled phosphorylation causes accumulation of tau-proteins and leads to neurofibrillary tangles.

6. Risk factor protein of AD

A wide range of studies have identified multiple gene and their translation products as risk factors for AD [11]. Different risk factor proteins attend different stages of this disease. Some CaMBPs have also been demonstrated as risk factor proteins, containing cluster of differentiation 33 (CD33), complement receptor 1 (CR1), membrane-spanning 4-subfamily A proteins (MS4AE) and MS4A6S, bridging integrator 1 (BIN1), ephrin type A receptor 1 (EPHA1), apolipoprotein E (APOE, clusterin (CLU) and other unverified proteins that are possibly CaMBPs [11]. Among these CaMBPs, CR1, CLU, ABCA7 and MS4A has been proved to associate with the overactivated immune response in AD, when CLU, ABCA7 and SORL1 also attend to affect cholesterol metabolism [11]. These risk factor proteins proved new potential therapy target for AD.

7. Conclusion

Calcium hypothesis was first proposed for the pathophysiology of AD. However, researchers found that the hypothesis was limited and hard to fully explain the mechanism of the occurrence of AD. Furthermore, O'Day and his colleagues conjectured that CaM instead of calcium plays a central role in the development of this dementia. Within recent two decades, CaM's functions in regulating related proteins that involve in the progression of AD are highlighted. CaM serves as a kind of functional protein in the regulation of memory and neurotransmission in human brains. The formation of neurofibrillary tangles and A β plaques involves numerous CaM binding proteins and many other unverified proteins that potentially contain CaM binding domains. Uncontrollable calcium influx through critical receptors and ion channels are also potentially mediated by CaM. Eleven early onset proteins in AD have been identified to be CaM binding. Late onset genes encoded proteins, such as the APOE which is a determinant of late-onset AD, are potential to combine with calcium and be regulated. After examining the evidence, it is reasonable to be considered that the mechanism of CaM to CaM regulated proteins should be a central study on the effective treatments of preventing AD and slowing the progression of AD. Current research indicates that targeting CaM with CaM-peptides is successful treatment to patients with Huntington's disease, which is another form of dementia.

CaM are identified as the only critical substrate involved in almost all central signaling pathways in the dementia expect from tau and A β . Further studies on the CaM related treatments of AD also offer promising results. The critical step now is to provide pharmaceuticals to target CaM and/or specific CaMBPs to effectively against AD.

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