

Cutting Edge Research on Drug Treatment Strategies and Emerging Therapies for Alzheimer's Disease

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Abstract. Alzheimer's disease (AD) is a devastating neurodegenerative disorder characterized by progressive cognitive decline and the memory loss. As the global population ages, the burden of AD continues to rise, highlighting the urgent need for effective treatment strategies. This abstract presents a comprehensive review of cutting edge research on drug treatment strategies and emerging therapies for AD. Recent advancements in understanding the underlying pathophysiology of AD have led to the identification of novel targets for drug development. Innovative therapeutic approaches, including disease-modifying drugs, have shown promise in preclinical and clinical studies. This thesis discusses the latest research on anti-amyloid β (A β) therapies, tau-based interventions, and inflammation-targeting drugs. Additionally, emerging therapies such as immunotherapies and gene therapies, and stem cell-based treatments are explored. The thesis emphasizes the potential of calcineurin and personalized approaches in AD treatment. The integration of biomarkers and neuroimaging techniques enables early detection and intervention, facilitating more targeted and effective therapies. Furthermore, the role of lifestyle modifications, such as diet, exercise, and cognitive training, is examined in the context of AD management. This article introduces the situation of the AD and the developing trend of the research on treatment of AD. By the power of innovative drug therapies, emerging technologies and holistic approaches, there is a flush of hope of slowing disease progression, improving cognitive function, and enhancing the quality of life for individuals affected by AD.

Keywords: Alzheimer's disease, drug treatment strategies, emerging therapies, calcineurin, challenges and future.

1. Introduction

AD is a neuronal degenerative disease commonly seen in the old over 65 years old which is also called the senile dementia [1]. Alzheimer's disease is named after the German neurologist Alois Alzheimer. In 1906, Dr. Alzheimer's first described the symptoms of the disease.

The reasons for the disease remain unclear right now. But most studies suggest it may be due to a combination of genetic, environmental and lifestyle factors such as age, cardiovascular health, family gene. A major pathological feature of AD is the production of a large number of beta-amyloid plaques and neurofibrillary tangles in the brain. Amyloid plaques are formed from beta-amyloid protein, while neurofibrillary tangles are formed from hyperphosphorylated tau protein. These plaques and tangles may interfere with communication between neurons, leading to damage and death of neurons. The mechanism of AD is about the beta-amyloid hypothesis and the tau protein hypothesis [2, 3]. In the Alzheimer's brain, protein aggregates called amyloid plaques form. These plaques are mainly composed of Amyloid-beta (A β) protein. A β is produced by beta-secretase and gamma-secretase cleavage of the larger Amyloid Precursor Protein (APP). In AD, the production and clearance of A β is unbalanced, causing A β to build up in the brain and form plaques. The second mechanism is the tau protein hypothesis. Another pathological feature of AD is neurofibrillary tangles. Neurofibrillary tangles are mainly composed of abnormally phosphorylated tau proteins. Normally, tau is involved in stabilizing the microtubule structure of neurons. But in AD, tau is abnormally phosphorylated, causing the microtubule structure to break down and form tangles. When the protein aggregates, the neuronal cells will be accelerated apoptosis. A large quantity of neuronal cells death triggers the brain shrink. Then the symptom of AD will begin to show the power.

2. The Clinical Symptoms of AD

The symptoms can be the memory loss which is one of the earliest and most recognizable. The patients will find it is hard to memorize the information obtained recently [4]. Cognitive decline is also a symptom of the patients. Some will suffer from the individual problem-solving. Find it hard to concentrate on one simple thing. Find it difficult to make decisions. Even worse, they may have difficulty in conversations.

The patients could also have disorientation and confusion. They may get confused and lose their way. They will be disoriented with time and space. Even the place which they are familiar with will trap them. Besides, the patients may change their personality and behavior, lose their initiatives, become more and more passive and numb [5].

Alzheimer's disease patients who have severe memory loss are unable to recall events from the day before or their loved ones. The body's health deteriorates over time, the trunk stiffens and loses its ability to bend, and the sphincter muscle function deteriorates. Prior to death, some individuals would experience a period of coma before passing away from systemic disorders such as bedsores, lung conditions, organ failure, infections, etc.

3. The Diagnosis of AD

There are many ways to diagnose the AD. Some traditional methods to diagnose are deserved to be introduced. The doctor diagnoses the patients' medical history and clinical evaluation. Doctors will ask patients about their symptoms, medical history, and the family history to understand the risk factors and development of AD. The doctor will use a series of cognitive tests which are called cognitive function assessments to assess the patient's memory, attention, language, thinking and executive function. These tests can help doctors determine if a patient has cognitive impairment. Besides, Brain imaging tests, such as MRI (magnetic resonance imaging) or CT (computed tomography), can help assess changes in the structure and function of the brain. These imaging tests can rule out other causes of cognitive impairment.

Then it comes to introduce some cutting-edge methods to diagnose the AD.

3.1. Immune Response

Immune response diagnosis of AD is a new diagnostic method that is based on the immune system's response to the disease. Alzheimer's disease is a progressive neurodegenerative disease that causes symptoms such as the memory loss, cognitive decline, and behavioral changes. There are two main ways to diagnose AD with an immune response: a blood test and a cerebrospinal fluid test. In a blood test, scientists look for specific proteins or antibodies like Tau proteins in the blood that have been linked to the onset and progression of Alzheimer's. Cerebrospinal fluid testing, on the other hand, detects specific markers like p-Tau protein or Tau protein in the cerebrospinal fluid to diagnose AD.

Chen developed p-tau181 for the single molecule array detection of plasma, serum, and CSF [6]. Use monoclonal antibodies capture p-tau81 and are detected with biotin-labeled tau antibodies. Can carry out ultra-low grade protein detection [7]. Besides, Budden has invented a reusable immune infrared sensor that can be used on surfaces to detect beta-amyloid and tau proteins. For the purpose of measuring the concentration of the tau-38 subtype in human plasma, Kim et al. used a surface plasmon resonance platform and a DNA aptamer antibody with specificity for the tau-38 subtype.

3.2. Probe-based Early AD Diagnosis

Probe-based diagnosis is an emerging Alzheimer's diagnosis technique that utilizes specific probes to label and detect AD-related biomarkers. These probes are typically fluorescent dyes or radioisotopes, which can bind to proteins or markers associated with AD and be visualized or quantitatively analyzed using imaging techniques. Probe-based diagnostic methods can provide non-invasive, highly sensitive and highly specific diagnostic methods for AD. The following are some commonly used probe-based diagnostics: Fluorescent dye probes for AD-associated proteins: These

probes can bind to AD-associated beta-amyloid, Tau proteins, etc., and use fluorescence imaging technology to detect and quantify the deposition of these proteins in the brain. There are mainly three types of probes to choose. The first is radioisotopically labeled probes of brain metabolism. These probes can be used by nuclear medicine imaging techniques, such as positron emission computed tomography (PET) or single photon emission computed tomography (SPECT), to detect areas of the brain that are metabolically abnormal in Alzheimer's patients. Then it comes to magnetic resonance imaging (fMRI) of brain function: fMRI can assess functional brain activity in Alzheimer's patients by detecting changes in blood flow and oxygenation levels in the brain, thereby providing information on diagnosis and disease progression.

Seo et al. created a near-infrared fluorescence probe that binds tau aggregates and beta amyloid aggregates with different isomorphous pictures, and only tau aggregates can light up fluorescent chemicals [8]. The third is early diagnosis of AD based on nano materials including nano biosensors and nano imaging. These sensors can be made to find beta-amyloid and tau proteins, which are biomarkers of AD. In biological samples like blood, cerebrospinal fluid, or urine, they might be able to spot slight variations in these indicators. Nano materials (such as quantum dots, gold nanoparticles, magnetic nanoparticles, and so on) can be utilized as imaging agents to improve the sensitivity and specificity of MRI, PET, optical imaging, and other imaging techniques.

3.3. Fluorescence

Fluorescence is a fluorescent dye-based Alzheimer's diagnosis method that utilizes specific fluorescent dyes to bind to proteins or markers associated with AD through imaging techniques to visualize or quantify the deposition of these markers. Here are some commonly used fluorescent dyes and their use in Alzheimer's diagnosis. Thioflavin dyes bind to deposits of beta-amyloid and emit a fluorescent signal. Using fluorescence microscopy or fluorescence imaging technology, it is possible to visualize the deposition of beta-amyloid protein in the brain. Then it comes to Congo Red dye. Congo Red dye can also bind to beta-amyloid and produce a fluorescent signal. It is commonly used in fluorescence microscopic analysis of tissue slices to assess the extent of beta-amyloid deposition in the brains of Alzheimer's patients. The last one is methylthioninium chloride dye. Methylthioninium chloride is a soluble fluorescent dye that binds to abnormal aggregation of Tau proteins and emits a fluorescent signal. The dye can be used to detect deposits of Tau protein in the brains of people with AD. Fluorescence diagnosis can provide a non-invasive, highly sensitive and highly specific method for diagnosing AD.

In order to diagnose AD, Zhang et al. used methylene blue as a fluorescent indication and bcl-2 and ROS as double targets for the first time [9]. The principle is through enzymatic hydrolysis and REDOX reaction, so that methylene blue fluorescence reaction. Zhang et al. developed a cascade catalytic amplification reaction of hairpin structure mediated by quadrivalent cross DNA nanostructures. Zhang et al. discovered a hairpin structure cascade catalytic amplification reaction mediated by quadrivalent cross DNA nanostructure.

3.4. Electrochemistry

Electrochemical diagnosis is a method of detecting and diagnosing Alzheimer's disease through electrochemical sensors. It is based on electrochemical principles, using electrochemical sensors to detect and analyze the content or activity of AD-related biomarkers in body fluids. Here are some commonly used electrochemical sensors and their applications in Alzheimer's diagnosis. The first is carbon nanotube sensors: Carbon nanotube sensors can detect and quantitatively analyze proteins or markers associated with AD by measuring current or potential changes. For example, carbon nanotubes can be modified to specific biomolecular recognition elements, such as antibodies or DNA probes, to detect, for example, beta-amyloid or Tau proteins. Then it comes to metal nanoparticle sensors: Metal nanoparticle sensors detect markers associated with AD by measuring changes in electrochemical signals. For example, electrodes modified with metal nanoparticles can be used to detect, for example, beta-amyloid or Tau proteins. The last one is biosensors. Biosensors are devices

that combine biomolecules with electrochemical sensors to detect and analyze biomarkers associated with AD. For example, an antibody or nucleic acid probe can be fixed on the electrode surface, interact with a specific protein or nucleic acid, and be detected and quantitatively analyzed by electrochemical signals. Electrochemical diagnostic technology has the advantages of high sensitivity, strong specificity and simple operation.

Duan et al. created an electrochemical biosensor by overlaying a double-layer phospholipid membrane with a porous -11-thiocarbodecarbonate electrode [10].

3.5. miRNA

MiRNA (microRNA) is a class of short non-coding RNA molecules, which plays an important role in gene expression regulation. In recent years, researchers have discovered that miRNAs play an important role in the onset and progression of AD. Therefore, the detection of miRNA is considered as a potential diagnostic method for AD. MiRNA diagnostic methods for AD mainly include the following aspects. The first is miRNA expression profile: The researchers established a miRNA expression profile for AD by measuring miRNA expression levels in blood, cerebrospinal fluid, or other body fluids. These miRNA expression profiles can be used to diagnose AD as well as to assess disease progression and prognosis. The second is the specific miRNA markers. Researchers have identified a number of miRNA markers associated with AD. By detecting the expression levels of these miRNA markers, it is possible to provide information on the diagnosis of Alzheimer's and disease progression. The third is miRNA vectors. Several studies are developing vectors that utilize miRNA as a diagnostic tool. By labeling the miRNAs with fluorescent dyes or radioisotopes, the distribution and activity of mirnas in Alzheimer's patients can be detected by imaging techniques.

Dong et al. initially used high-throughput solea sequencing to screen the entire gene expression profile of AD serum miRNA, and then used multiple RT-qPCR to determine the AD-specific miRNA profile at the individual level [11]. To discover RNA linked with ad, Chandrashekar et al. used an approach called miRacles. To discover RNA linked with ad, Chandrashekar et al. used an approach called miRacles [12]. When the target miRNA is discovered, the dna nanostructure shifts from linear to ring, resulting in a change in the electrophoresis rate. As a result, the switching state of miRNA can be reported using electrophoretic reading, and miRNA may be determined.

3.6. Nanometers Materials

Nano materials have potential applications in AD diagnosis. Here are some examples of applications of nano materials in Alzheimer's diagnosis: The first is the magnetic nanoparticles. Magnetic nanoparticles can be used as carriers for fluorescent probes to provide high-contrast imaging of AD-related markers in magnetic resonance imaging (MRI). These nanoparticles can bind to proteins or markers associated with AD and be detected and quantified by MRI technology. The second is Gold nanoparticles. Gold nanoparticles have excellent optical properties and can be used as surface-enhanced Raman scattering (SERS) probes to detect low concentrations of AD-related markers. These nanoparticles can improve sensitivity and selectivity to proteins or nucleic acids by binding to specific antibodies or DNA probes. The third is the nano fluorescent probes. Nano fluorescent probes are nanoparticles that utilize fluorescent materials that can bind to proteins or markers associated with AD and emit a specific fluorescent signal. These nano probes can be used to visualize and quantify deposits in the brain using fluorescence imaging technology.

Zhang et al. 2 developed a multiplex surge-enhanced Raman scattering biosensor to detect A81-42 oligomers and tau proteins [13]. They coupled an aptamer for target recognition with a polyA modified on the surface of gold nanoparticles (AuNPs), and when the target protein specifically bound to the aptamer AuNPs, the polyA oligonucleotide deviated, destroying the stability of the nanocouplings in solution and triggering a plasma resonance effect of adjacent AuNPs, enabling SERS detection of protein biomarkers. The biosensor can detect (au protein and A81-42 oligomer) in 15 minutes and can detect both at the same time.

4. The Treatment Strategies for Alzheimer's Disease

At present, there is no cure for Alzheimer's disease, and the treatment is mainly through drugs and psychological intervention to control symptoms and delay the development of the disease. Researchers are constantly exploring new treatments, including preventing or removing amyloid plaques and neurofibrillary tangles in the brain [14].

4.1. Medication

At present, the drugs used in clinical treatment of Alzheimer's disease mainly include cholinesterase inhibitors and NMDA receptor antagonists. These drugs have different mechanisms of action and can improve symptoms and cognitive function in Alzheimer's patients to some extent.

4.1.1 Regular medication therapies

Cholinesterase inhibitors, includes Donepezil, Galantamine, and Rivastigmine, improve cognitive function and behavioral symptoms in Alzheimer's patients by inhibiting the activity of cholinesterase and increasing the concentration of acetylcholine [15]. NMDA receptor antagonists: includes Memantine, improves cognitive function and behavioral symptoms in Alzheimer's patients by modulating glutamate signaling and reducing neuronal firing [16]. In addition to the above-mentioned medications, there are a number of other medications and treatments that are also used in the adjuvant treatment of AD, including antidepressants, anti-anxiety drugs, antipsychotics and behavioral therapies.

4.1.2 Calcineurin

The calcineurin is one of the most potential treating therapies of the AD. Calcineurin has various features that help it operate and play a role in cellular activities. It has abundant of properties. Some are crucial to the treatment of AD. The first is the Serine/threonine protein phosphatase activity. Calcineurin dephosphorylates target proteins by removing phosphate groups from particular serine or threonine residues. The second is calcium-dependent activation. Calcium ions (Ca^{2+}) binding to the regulatory protein calmodulin activates calcineurin. Increased intracellular calcium levels cause calcium ions to bind to calmodulin, which then activates calcineurin. The third is substrate specificity. Calcineurin selectively dephosphorylates specific target proteins, including transcription factors like NFAT (nuclear factor of activated T-cells). It recognizes and binds to specific protein motifs or regulatory regions on its target proteins, allowing for substrate specificity.

Besides, Calcineurin possesses several important properties that contribute to its function and role in cellular processes. Here are some key properties of calcineurin. The first is the regulatory subunits. Calcineurin consists of a catalytic subunit (calcineurin A) and a regulatory subunit (calcineurin B) [17]. The regulatory subunit, typically composed of multiple EF-hand calcium-binding domains, interacts with calcium-bound calmodulin to activate the catalytic subunit. It is composed off 542 amino acids and has a weight of 61502 Da [18]. Then the second is about the immunophilin binding. Calcineurin requires immunophilins, specifically cyclophilin or FKBP (FK506-binding protein), as co-factors for its full enzymatic activity. These immunophilins bind to calcineurin and facilitate its interaction with immunosuppressive drugs like cyclosporine A or tacrolimus (FK506). The third is about the cellular localization. Calcineurin is found in various subcellular compartments, including the cytoplasm, nucleus, and cell membrane, depending on its targets and functions within different cell types. Its localization allows for spatial regulation of its activity and interaction with specific substrates. Furthermore, it comes to introduce the role in signal transduction. Calcineurin acts as a key mediator in calcium-dependent signaling pathways. It plays important roles in cellular processes such as neuronal development, synaptic plasticity, immune response regulation and gene expression. Finally, it is about the regulation by inhibitors. Calcineurin activity can be inhibited by specific drugs, such as cyclosporine A and tacrolimus, which bind to immunophilins and interfere with the calcineurin-immunophilin complex. These inhibitors are used therapeutically as immunosuppressants to prevent organ transplant rejection.

Understanding the properties of calcineurin is crucial for comprehending its functional roles and its potential as a therapeutic target in various diseases and conditions. Ongoing research continues to shed light on the intricacies of calcineurin biology and its implications for human health.

Protein phosphorylation: Protein kinases are important enzymes in the phosphorylation process, which phosphorylates proteins by transferring gamma-site phosphate groups from ATP (adenosine triphosphate) to certain amino acid residues in proteins (often serine, threonine, or tyrosine). Protein kinases are important enzymes in the phosphorylation process, which phosphorylate proteins by transferring gamma-site phosphate groups from ATP (adenosine triphosphate) to certain amino acid residues in proteins (often serine, threonine, or tyrosine) [19].

Protein dephosphorylation: Protein phosphatase uses a hydrolysis reaction to remove the phosphoric acid group from the protein, a process known as protein dephosphorylation. This procedure can modify the function of the phosphorylated protein by returning it to its initial state.

Protein phosphorylation and dephosphorylation are usually rapid, reversible processes that fine-regulate cell signaling. The imbalance of these two processes can lead to cell dysfunction, which can lead to various diseases. Therefore, understanding the regulatory mechanism of protein phosphorylation and dephosphorylation is of great significance for the diagnosis and treatment of diseases.

The imbalance of intracellular calcium ion levels is a prevalent event in the degenerative phase of AD. Excess calcium ions can activate calcineurin, causing aberrant cell activities such as cell survival, neurotransmitter release, learning, and memory [20].

The pathogenesis of AD is mainly divided into two types [21]. Intracellular neurogenic tangles nft and intercellular senile plaques. The main component of nft is the hyperphosphorylated microtubule-associated protein tau. Tau proteins are made up of clustered pairs of spiral filaments. Calcineurin can dephosphorylate tau protein. Neurotransmitters, hormones, and other signals that stimulate the cell membrane will cause the calcium channel on the cell membrane to open, enabling calcium ions from outside the cell to enter, increasing the concentration of calcium ions inside the cell. Calcium/calmodulin complexes are created when calcium ions bind to calmodulin. Each of the four calcium ion binding sites in the calcium/calmodulin complex causes a local conformational change in calmodulin. The regulatory subunit B (CAN-B) of CaN binds to the calcium/calmodulin complex, which causes CaN's overall conformation to alter. There are more points of interaction between CAN-B and catalytic subunit A (CaN-A) thanks to CaN-B's four calcium ion binding sites. CaN-A can interact with the substrate protein and dephosphorylate it since its active site is exposed. A divalent zinc ion is present in the CaN-A active site and is engaged in the hydrolysis of substrate phosphate ester linkages. Protein dephosphorylation is accomplished by CaN by removing the phosphate group from the serine or threonine residue of a particular protein. Dephosphorylation changes the way a protein functions. For instance, nuclear factors to activate T cells (NFAT) can be dephosphorylated by CaN, allowing them to enter the nucleus from the cytoplasm and further control the expression of particular genes.

Overall, calcineurin may be a significant factor in the onset and progression of AD and may one day serve as a target for therapeutic intervention. The question of how to precisely control calcineurin activities and how to prevent adverse consequences still has to be answered.

To express and purify calcineurin, the gene encoding this enzyme was cloned into the pET-28a(+) vector and transformed into *E. coli* DH5 α competent cells. Transformants were grown overnight on LB solid medium containing 50 μ g/ml kanamycin. A single colony was picked to inoculate LB liquid medium with the same kanamycin concentration and grown at 37°C, pH 7.4. When OD₆₀₀ reached 0.6, IPTG was added to a final concentration of 0.5 mM to induce protein expression. After induction for 4 hours, cells were harvested by centrifugation. The tagged protein was purified by Ni-NTA affinity chromatography based on the affinity of His-tags to Ni-NTA resin. Finally, the purified product was identified and characterized by SDS-PAGE and enzyme activity assay.

A brief description of the production steps: Insert the target gene into the plasmid. The recombinant plasmid was transformed into *Escherichia coli* DH5 α . Successful conversion was tested using

conamycin. The expression of target protein was induced by IPTG. Prolonging culture and inducing target protein expression. The cell is ruptured and the target protein is then isolated and purified.

4.2. The Cognitive Stimulation

Cognitive stimulation is a non-drug treatment for mild to severe AD and other types of dementia. The cognitive stimulation can also be a treatment for AD. Engaging in the stimulation activates may help with the patients, such as puzzles, memory exercises, reading or learning new skills. These can slow down the cognitive decline and improve cognitive functions. Cognitive stimulation is frequently used in conjunction with non-pharmacological treatments such as cognitive rehabilitation and occupational therapy, and it may also be used in conjunction with medicine to improve therapeutic effects. For lasting effects, cognitive stimulation needs to be done regularly. Although cognitive stimulation is beneficial to many people with AD, not everyone is suitable for this treatment. A permission of doctors and the professional is required.

4.3. The Lifestyle Modifications

The lifestyle modification also plays a crucial role in managing AD. Recent research has indicated that some actions can aid in delaying the onset of the illness and promoting the restoration of cognitive function. Regular aerobic activity, such as walking, swimming, cycling, and so on, can improve heart health and blood circulation, lowering the risk of AD. Eating a nutritious diet, particularly one rich in fruits, vegetables, whole grains, low-fat dairy products, fish, poultry, and legumes, can supply a multitude of nutrients to support brain function. Utechniques, such as meditation, relaxation training, deep breathing, etc., can help reduce stress. Getting enough sleep is essential for a healthy brain. Supportive care like supportive environment is also key to recover.

5. Conclusion

AD is a neurological disease with no cure that poses significant challenges for individuals and their families. In the future, research and development in this field will encounter the following significant problems and challenges:

The current medical treatment for AD is primarily symptomatic; there is no cure. Researchers are exploring for more effective treatment options, such as preventing or decreasing the spread of lesions, although more in-depth research is required.

Because the early symptoms of AD are difficult to identify, early detection is critical for delaying the disease's progression. As a result, future research should focus on how to effectively diagnose AD at an early stage. While some lifestyle changes can lessen the risk of developing the disease, the specific etiology of AD is still unknown. More study could help us better understand the disease's causes and develop effective prevention strategies. As the world population ages, the frequency of AD is predicted to rise, putting a significant strain on society and the economy. As a result, allocating resources efficiently to address this issue is a significant social concern. People with AD require long-term care and attention, putting enormous strain on caretakers. Another critical issue is how to provide adequate support to help people cope with stress.

Researchers will continue to uncover more effective ways to treat and prevent AD in the future, while efforts from all sectors of society will be required to assist patients and their families in coping with the problems of this disease.

References

- [1] Alzheimer disease. *Nat Rev Dis Primers*. 2021 May 13; 7(1): 34.
- [2] Ma C, Hong F, Yang S. Amyloidosis in Alzheimer's Disease: Pathogeny, Etiology, and Related Therapeutic Directions. *Molecules*. 2022 Feb 11; 27(4): 1210.

- [3] Wang Jianzhi, Tian Qing. Mechanism of Tau hyperphosphorylation and its role in neuronal degeneration in Alzheimer's disease [J]. *Advances in Biochemistry and Biophysics*, 2012, 39 (8): 771-777.
- [4] Alzheimer disease. *Nat Rev Dis Primers* 7, 34 (2021).
- [5] Segerstrom SC. Personality and Incident Alzheimer's Disease: Theory, Evidence, and Future Directions. *J Gerontol B Psychol Sci Soc Sci.* 2020 Feb 14; 75(3): 513-521.
- [6] Chen Y, Chang L, Blennow K, Zetterberg H, Ball A J. *Alzheimer's Dement.* 2020. 16(S4): e041238.
- [7] Preische O, Schultz SA, Apel A, Kuhle J, Kaeser SA, Barro C, Gräber S, Kuder-Bulletta E, LaFougere C, Laske C, Vöglein J, Levin J, Masters CL, Martins R, Schofield PR, Rossor MN, Graff-Radford NR, Salloway S, Ghetti B, Ringman JM, Noble JM, Chhatwal J, Goate AM, Benzinger TLS, Morris JC, Bateman RJ, Wang G, Fagan AM, McDade EM, Gordon BA, Jucker M; Dominantly Inherited Alzheimer Network. Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer's disease. *Nat Med.* 2019 Feb; 25(2): 277-283.
- [8] Seo Y, Park KS, HaT, Kim MK, Hwang Y J, Lee J, Ryu H, Choo H, Chong Y. *ACS Chem. Neurosci.*, 2016, 7: 1474 - 1481.
- [9] Zhang P, FuC X, Liu H H, Guo X J, Zhang Q. Gao J. Chen W J. Yuan W, Ding C F. *Anal. Chem.*, 2021, 93: 11337 - 11345.
- [10] Duan Y M, Chen J, Jin Y, Tu QY, Wang SH, Xiang J. *Anal. Chem.*, 2021, 93(7): 3611 -3617.
- [11] Dong H, LiJL, Huang L, Chen X, LiD H, Wang T, HuG Y, Xu J, Zhang C N, Zen K, Xiao S F, Yan Q, Wang C, Zhang C Y. *Dis. Markers*, 2015, 2015(9): 625659.
- [12] Chandrasekaran A R. Halvorsen K. *ACS Sens.*, 2021, 6(9): 3176 - 3181.
- [13] Zhang X, LiuS, Song X D, Wang HW, Wang J F, Wang Y, Huang JD, Yu J H. *ACS Sens.*, 2019, 4(9): 2140-2149.
- [14] Atri A. Current and Future Treatments in Alzheimer's Disease. *Semin Neurol.* 2019 Apr; 39(2): 227-240.
- [15] Marucci G, Buccioni M, Ben DD, Lambertucci C, Volpini R, Amenta F. Efficacy of acetylcholinesterase inhibitors in Alzheimer's disease. *Neuropharmacology.* 2021 Jun 1; 190: 108352.
- [16] Koola MM. Galantamine-Memantine combination in the treatment of Alzheimer's disease and beyond. *Psychiatry Res.* 2020 Nov; 293: 113409.
- [17] Yang SJ, Zhao W, Wei Q. Activities and properties of calcineurin catalytic domain [J]. *Chin Sci Bull*, 2000, 45(4): 381-386.
- [18] Calvo-Rodriguez M, Bacsikai BJ. Mitochondria and Calcium in Alzheimer's Disease: From Cell Signaling to Neuronal Cell Death. *Trends Neurosci.* 2021 Feb; 44(2): 136-151.
- [19] BioRun, Protein post-translational modification - phosphorylation, 2022.01.12, 2023.7.20.
- [20] ianqian. Effects of calcineurin on learning and memory in rats with Alzheimer's disease induced by A β 1-42 and its related mechanisms. Guangxi Medical University, 2015.
- [21] Jing Qun. A new target of calcineurin for drug therapy of Alzheimer's disease. *Chin J Pharmacol Toxicol* 18(1): 71-74.