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Exploring exosomes to provide evidence for the treatment and prediction of Alzheimer's disease

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Abstract: Exosomes are extracellular vesicles with a 30–150 nm diameter originating from endosomes. In recent years, scientists have regarded exosomes as an ideal small molecule carrier for the targeted treatment of Alzheimer's disease (AD) across the blood-brain barrier due to their nanoscale size and low immunogenicity. A large amount of evidence shows that exosomes are rich in biomarkers, and it has been found that the changes in biomarker content in blood, cerebrospinal fluid, and urine are often associated with the onset of AD patients. In this paper, some recent advances in the use of exosomes in the treatment of AD are reviewed, and various exosome markers and some latest detection methods are summarized to provide some evidence for the detection or treatment of AD by exosomes.

Introduction

Alzheimer's disease (AD) is the most common dementia among older individuals, accounting for 60%–80% of the total population with dementia (Gopalakrishna *et al.*, 2022). According to the data of Alzheimer's Disease International, every 3 s, a patient develops AD in the world, and it is expected that by 2050, the number of AD worldwide will increase to more than 150 million (Joe and Ringman, 2019). Typical pathological features of AD mainly include neurofibrillary tangles (NFT) and senile plaques (Ising and Heneka, 2018), which often lead to damage or loss of synapses and neurons (Gkanatsiou *et al.*, 2021). Accumulation of synaptic protein neurexin (axon protein) fragment in the brain also leads to specific memory loss (Fig. 1) (Sánchez-Hidalgo *et al.*, 2022). Typical clinical symptoms of AD patients include decreased episodic memory and executive function (Tarawneh and Holtzman, 2012), while atypical clinical symptoms generally occur in the non-memory domain and are manifested as agnosia, aphasia, and executive dysfunction (Lam *et al.*, 2013). Xia *et al.* (2022) found that human lifespan and memory storage are closely related to the C/EBPβ/AEP signaling pathway that drives AD, which closely links the pathogenesis of AD with the life cycle regulation. In this review, the association between exosomes and AD is explored and some evidence is provided for treating and predicting AD by exosomes. We hope to find a reasonable test and treatment to improve the quality of life of people affected with AD.

Exosomes are small as vesicles. In recent years, exosomes have attracted the attention of scientists as a targeted vector molecule for treating AD. Exosomes can be used as drug carriers to cross the blood-brain barrier (BBB) and improve intracranial drug concentration to achieve therapeutic effects. Exosomes can also participate in the cleaning process of pathogenic amyloid beta (Aβ) protein and Tau protein, proving that exosomes can potentially treat AD. Mesenchymal stem cells (MSCs) are pluripotent stem cells capable of self-renewal and multidirectional differentiation (Samsonraj *et al.*, 2017). MSC-exos is a subtype of extracellular microvesicles. As a type of exosome, its action process contributes to the improvement of immune regulation and neuroinflammation in pathological abnormal areas. It can significantly improve spatial learning ability and cognitive impairment of AD transgenic mice (Cui *et al.*, 2019). Exosomes have nanoscale size and low immunogenicity, so they can be carriers of small molecules across the BBB to treat AD. In medical tests, exosome-derived proteins, lncRNAs, or miRNAs can be stably detected in blood or cerebrospinal fluid (CSF). These molecules are considered new biomarkers for diagnosing neurodegenerative diseases and have significant promise in diagnosing AD.

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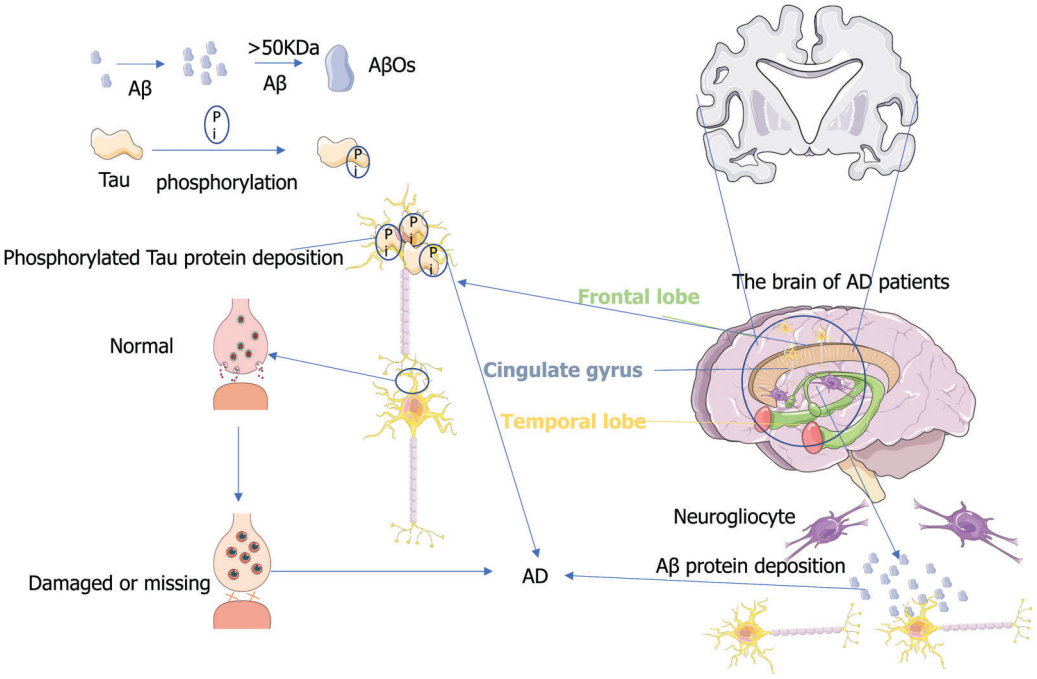

FIGURE 1. Pathogenesis of Alzheimer's disease (AD). The figure illustrates several theories of the pathogenesis of AD, including neuron neurofibrillary tangles (NFT), senile plaques, and damage or loss of synapses.

Exosome and Alzheimer's Disease

Introduction to exosomes

Extracellular vesicles (EVs) comprise a group of heterogeneous membrane-derived vesicles of different origins, sizes, and characteristics and play a crucial role in cellular exchange (Gould and Raposo, 2013). Exosomes are heterogeneous subgroups of EVs, with size in the range of 30–150 nm, and originating from endosomes (Baietti *et al.*, 2012; Bebelman *et al.*, 2018). Exosomes comprise proteins, DNA, mRNA, microRNA, long non-coding RNA, and circular RNA involved in intercellular communication (Dai *et al.*, 2020). Transmembrane ligands on the surface of the exosome can directly bind to surface receptors on recipient cells to generate downstream signaling cascades that activate target cells. Exosomes can also release molecules directly into the cytoplasm of target cells through fusion with the plasma membrane or internalization by the recipient cells for material exchange or information transfer (Gurung *et al.*, 2021), which is closely related to the occurrence, development, and treatment of diseases (Jella *et al.*, 2018).

Exosomes mediate the transcellular transduction of substances

Exosomes gradually lead to the pathogenesis of AD patients by delivering pathological forms of Aβ and Tau proteins (Song *et al.*, 2020). Oligo-Aβ-containing exosomes isolated from the brain of AD patients have been found to be absorbed by SH-SY5Y cells (Fig. 2), resulting in cytotoxicity and transmission to other receptor cells (Sardar Sinha *et al.*, 2018). Exosomes secreted by astrocytes can target Aβ to neuronal mitochondria, enhancing Aβ neurotoxicity by inducing apoptosis (Elsherbini *et al.*, 2020). Exosomes can mediate the clearance of pathological proteins to protect neurons. For example, exosomes derived from bone marrow mesenchymal stem cells (BM-MSCs) reduce Aβ deposition and promote cognitive function recovery in AD mice by activating the sphingosine kinase (SphK)/S1P signaling pathway (Wang and Yang, 2021). These studies suggest that exosomes can mediate the clearance of pathological proteins to protect neurons. Exosomes can also serve as cell-to-cell communication devices, mediating the effect of trans-cell transduction. For example, sirtuin 2 is transmitted from oligodendrocytes to axons through exosomes, which deacetylates mitochondrial protein and enhances ATP production, providing a target for promoting axon bioenergy metabolism in diseases of the nervous system (Fig. 2). It can increase the energy capacity of mitochondria in axons, and the results prove that exosome-mediated transcellular signaling is an effective and robust mechanism (Schiapparelli *et al.*, 2022).

Exosomes mediate some protein transport

Exosomes mediate the aggregation and clearance of Aβ

Aβ is produced by processing amyloid precursor protein (APP) as a physiological metabolite and its secretion into the extracellular environment. A balance between APP production and degradation/clearance controls the homeostasis level of extracellular Aβ. Studies have shown that in the case of endogenous injury, PC12-derived exosomes may promote the formation of Aβ protein fiber, which is closely related to the pathological dynamics of early AD (Yuyama *et al.*, 2012). During the development of AD, protein deposits are formed at the later stage of Aβ aggregation. Before visible deposits are formed, a small amount of pathogenic Aβ accumulates, like a "seed" of aggregation, triggering more pathological proteins to accumulate and snowball (Uhlmann *et al.*, 2020). When Aβ forms metastable oligomers heavier than 50 kDa, these are called Aβ oligomers (AβOs). AβOs can target to bind to dendritic spines, induce Tau mismatch, reduce neuronal activity (Schützmann *et al.*, 2021) and induce the onset of AD. Exosomal membranes are particularly rich in GM1

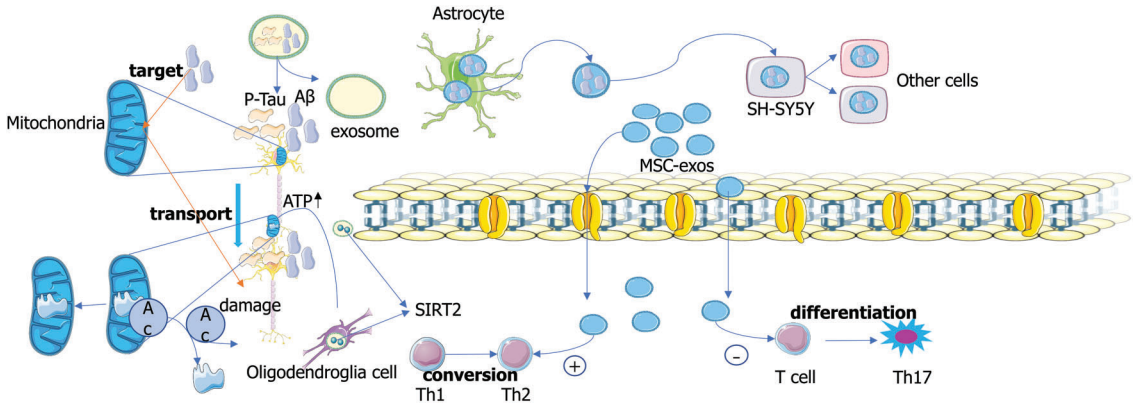

FIGURE 2. Exosomes mediate the transport of protein. Exosomes can deliver p-Tau and amyloid beta (Aβ) proteins. Sirtuin 2 (SIRT2) is transmitted from oligodendrocytes to axons through exosomes, which deacetylates mitochondrial proteins and enhances ATP production (Schiapparelli *et al.*, 2022). Oligo-Aβ-containing exosomes isolated from the brain of patients with AD are absorbed by SH-SY5Y cells, resulting in cytotoxicity and transmission to other receptor cells (Sardar Sinha *et al.*, 2018). Mesenchymal stem cells (MSC)-exos can induce Th1 cells to convert to Th2 cells and reduce the potential of T cells to differentiate into effector T cells (Th17) that produce interleukin 17 (Xie *et al.*, 2020).

ganglioside (He *et al.*, 2022), which drives conformational changes of Aβ to form non-toxic amyloid fibrils and promote absorption of Aβ (Fernandez-Perez *et al.*, 2017). Exosomes MExo-gem is a mannose-modified exosome containing the drug gemfibrozil. Macrophage-derived exosomes (Exos) drive conformational changes in Aβ to reduce amyloid fibrillary formation by binding Aβ and promoting microglial internalization of Aβ. Exosomes MExo-gem with Gem modification promotes microglia to clear Aβ by activating peroxisome proliferator-activated receptor-α, promoting nuclear translocation of transcription factor EB, and enhancing lysosomal activity (Hao *et al.*, 2022) (Fig. 3). These pieces of evidence suggest that different exosomes from brain cells promote the formation of Aβ protein fiber. In addition, some exosomes can promote the absorption of Aβ protein by changing the conformation of Aβ protein.

Exosomes mediate Tau transport

Abnormal intracellular aggregation of misfolded Tau proteins into insoluble aggregates is called neurofibrillary tangles (NFT), a typical pathological marker of AD (Sinsky *et al.*, 2021). Tau spreads from the entorhinal cortex to the hippocampus early in the disease. The development of Tau pathology in AD is associated with progressive cognitive impairment and neuronal loss. Tau protein can be secreted from neurons through synaptic stimulation and transmitted along the nerve (Yamada *et al.*, 2014). Tau fibrin can be transferred from cells to cells *in vitro* and *in vivo* (Sanders *et al.*, 2014). Exosomes play an important role in cell communication and transport of pathogenic proteins associated with AD. Tau protein is recognized by specific cell vesicles called exosomes in the CSF of patients with AD (Saman *et al.*, 2012). Tau first appears in the entorhinal cortex (EC) before any symptoms and then develops in a graded pattern, distributing to the hippocampus and neocortex. Increasing evidence indicates that pathological Tau proteins can diffuse between cells and recruit native Tau proteins, leading to the transformation into fibrous aggregates of pathological Tau proteins (Iba *et al.*, 2013; Ahmed *et al.*, 2014; Boluda *et al.*, 2015). Intracellular accumulation of misfolded alpha-synuclein (αSyn) is a neuropathological marker of alpha-synuclein disease (Goedert *et al.*, 2013). For AD patients, Tau and aSyn exist

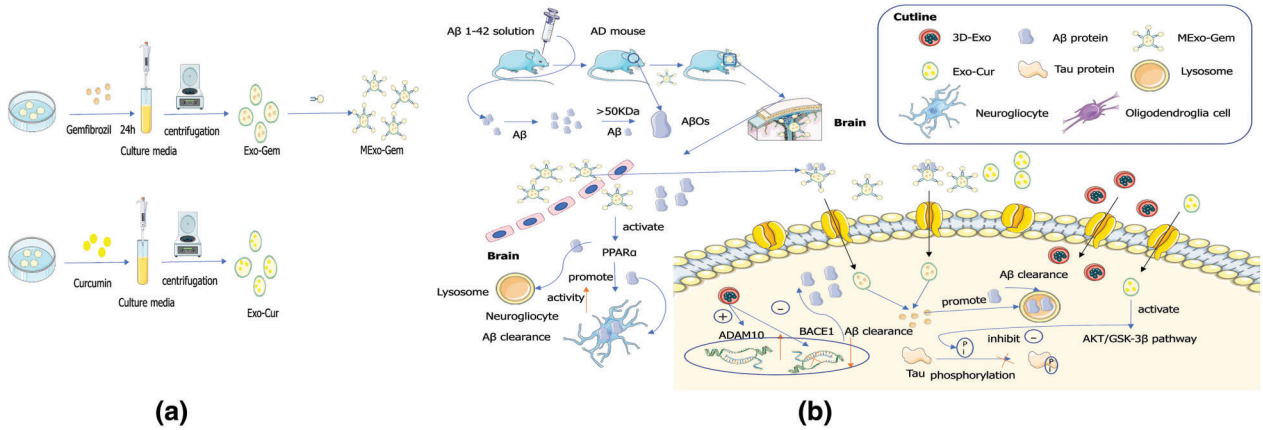

FIGURE 3. Engineered exosome targeting therapy mechanisms. (a) Preparation of mannose-modified exosome containing gemfibrozil (MExo-gem) and exosomes that envelope curcumin (Exo-Cur). (b) Mechanism analysis of MExo-gem, Exo-Cur, and 3D-exo in the treatment of Alzheimer's disease.

in the brain in the form of the copolymer, and the presence of mixed pathology of Tau and aSyn is associated with a higher risk of dementia (Sorrentino *et al.*, 2017). Studies have shown that inoculation of pre-formed fibrous Tau protein into Tau transgenic mice can produce AD-like NFT pathology at a fairly rapid rate in connected brain regions (Iba *et al.*, 2013; Ahmed *et al.*, 2014; Boluda *et al.*, 2015). Misfolded Tau proteins can diffuse through anatomically connected neurons, which indicates that Tau aggregates can spread beyond the trans-synaptic transmission, and pre-formed Tau aggregates can also diffuse beyond the synaptic connection (de Calignon *et al.*, 2012; Harris *et al.*, 2012; Liu *et al.*, 2012), indicating the existence of non-synaptic transmission pathways (de Calignon *et al.*, 2012). Microglia can effectively secrete exosomes. Studies have shown that microglia engulf Tau-containing cytopathic neurons or synapses and secrete Tau protein in exosomes, effectively delivering Tau protein to neurons (EL Andaloussi *et al.*, 2013). Neutral Sphingomyelinase 2 (nSMase2) is a phosphoprotein phosphorylated only at serine residues (Back *et al.*, 2018). Tau-containing exosomes secreted by microglia are sensitive to nSMase2 inhibition. This indicates that the synthesis of ceramides is crucial for exosome biogenesis (Asai *et al.*, 2015). Higher levels of myelocyte-derived exosomes in CSF were closely associated with higher levels of Tau protein in CSF (Agosta *et al.*, 2014). This evidence demonstrated that microglia depletion significantly inhibits Tau protein delivery to neuron exosomes and that microglia-derived exosomes deliver Tau protein to neurons in large quantities. In a recent study, the team used biotin molecular tags (Biotin) to label proteins, and electron microscopy showed that unbiased screening based on mass spectrometry identified about 200 transneuronal transported proteins (TNTP) isolated from the visual cortex. Most TNTP are present in exosomes, and viral TNTP, including Tau protein and β-synuclein, were detected in isolated exosomes and postsynaptic neurons. It indicates that the transport of TNTP by exosomes may be mediated by exosomes (Schiapparelli *et al.*, 2022), providing new evidence for previous studies on the transport of Aβ and Tau proteins by exosomes.

Exploration of Exosome and Neuronal Regulation

Microglia mediate neuronal damage

In AD, astrocytes and microglia individually or jointly stimulate neuroinflammation through cell crosstalk, promoting pro-inflammatory cytokine release and neuronal loss (Kaur *et al.*, 2019). Microglia can mediate synaptic elimination through phagocytosis (Lee and Chung, 2019), and activated microglia can release NO and pro-inflammatory cytokines, increase ROS levels, and induce oxidative stress damage of dopaminergic neurons (Jiang *et al.*, 2019). Neuropathological analysis of AD showed that Aβ protein pathologic plaques promote the development of Tau protein pathology. The aggregation form of microtubule-associated protein Tau consists of paired helical filaments (PHF) (Li *et al.*, 2016). The human natural PHF (AD-PHF) extracted from the brain of sporadic AD could induce Tau aggregation in the brain of wild-type (WT) mice. This PHF contains six human wild-types Tau isomers and all post-translational modification features of the PHF from the AD brain (Audouard *et al.*, 2016). In AD-PHF injected 5*FAD mice, insoluble Tau protein levels were higher, Tau cortical diffusion was more important than WT mice, and the CDK5 kinase p25 activator was increased. Data showed that *in vivo*, Aβ enhanced the seeding of experimentally induced pathological fiber Tau protein (Vergara *et al.*, 2019). Primary microglia can internalize Aβ fibrils and release vesicular bodies containing Aβ peptides (Gouwens *et al.*, 2018). Microglia can directly absorb Aβ and degrade it in the lysosomal compartment (Govindpani *et al.*, 2019).

Exosomes are involved in the bidirectional regulation of neurons

Neuron-derived exosomes can prevent the pro-inflammatory response of microglia cells by removing the Aβ protein (Yuyama *et al.*, 2012). Moreover, adding exosomes inhibits the formation of toxic oligomers, thus effectively avoiding neuron injury. On the other hand, the transport of Tau protein by exosomes may cause neurotoxicity. The release and uptake of Tau protein between adjacent cells may play an important role in the pathological spread of Tau protein (Mohamed *et al.*, 2013). Tau-containing exosomes from neurons can be specifically absorbed by microglia. When Tau protein cannot be degraded by microglia, it can be released through microglia exosomes and absorbed by neurons (Asai *et al.*, 2015). Tau protein can be transported forward or backward through the endolysosomal pathway, ultimately enhancing the Tau protein-associated pathologic condition in recipient neurons (Wu *et al.*, 2013).

There is increasing evidence that exosome-mediated communication plays an important physiological role in neuronal development, synaptic function, nerve regeneration, and neuron-glial interactions at the neural network level (Rajendran *et al.*, 2014). According to the above existing studies, exosomes may have different regulatory effects on AD. Exosomes can inhibit the formation of toxic oligomers, remove Aβ protein, reduce the pro-inflammatory response of microglia cells, and effectively avoid neuronal damage in this way. On the other hand, microglia depletion significantly inhibited the transfer of Tau protein to neuronal exosomes, and many microglia-derived exosomes transferred Tau protein to neurons. Exosomes also promote the aggregation of glial Aβ protein. Abnormal aggregation of Tau and Aβ proteins will increase the probability of AD inflammatory induction.

The mechanism of mesenchymal stem cells—exos inhibiting neuroinflammation

Exosomes have great potential in medical detection and drug delivery due to their characteristics as transport vectors. Exosomes, as natural biological agents, can act as active molecules for cell-cell transport with good biocompatibility and instantaneously regulate the function of targeted cells (Iranifar *et al.*, 2019). According to relevant studies, exosomes derived from mesenchymal stem cells (MSC-exos)

can interact with target cells through different mechanisms. For example, MSC-exos can bind directly to membrane receptors, internalizing their contents into target cells. MSC-exos can also deliver bioactive substances to target cells by fusion with the plasma membrane. Composed of microvascular endothelial cells that line the brain's capillaries, BBB acts as a highly selective membrane barrier that facilitates transport between systemic circulation and the central nervous system (Kadry *et al.*, 2020). Exosomes can easily improve intracranial drug concentration through BBB (Qu *et al.*, 2018). Compared with traditional administration methods, exosome administration avoids intracranial complications such as infection, non-specific absorption, and drug toxicity (Wang *et al.*, 2019). The lipid bilayer structure of exosomes contributes to improving hydrophobic or hydrophilic drug transport efficiency (Kim *et al.*, 2018). MSC-exos can regulate immunity, promote Aβ degradation and regulate Aβ degradation. MSC-exos regulates immune cells. MSC-exos can help inhibit the proliferation and differentiation of lymphocytes (Blazquez *et al.*, 2014). In addition, MSC-exos is involved in inducing lymphocyte differentiation into anti-inflammatory type. This small molecule can induce Th1 cells to convert to Th2 cells and reduce the potential of T cells to differentiate into effector T cells (Th17) that produce interleukin 17 (Xie *et al.*, 2020) (Fig. 2). In addition, some studies have suggested that inflammatory cytokines and proteins contained in MSC-exos have immunomodulatory effects. The excessive accumulation of Aβ in the brain triggers a neuroinflammatory process. MSC-exos contributes to improving immune regulation and neuroinflammation in pathologically abnormal areas. MSC-exos can reduce the expression of pro-inflammatory factors and up-regulate the expression of the anti-inflammatory factors in immune cells, exerting an immunosuppressive effect (Matthay and Abman, 2018).

Prospects for Exosomes in Therapy and Detection/Prediction of Alzheimer's Disease

The prospect of exosomes for the treatment of Alzheimer's disease

In some recent studies, the team used a 3-dimensional graphene scaffold and 2-dimensional graphene sheet as human umbilical cord mesenchymal stem cells (hUMSCs) culture substrate, extracted supernatant from hUMSCs and isolated exosomes, and found that 3D-Exo up-regulated A Disintegrin And Metalloproteinase 10 (ADAM10), down-regulated beta-secretase 1 (BACE1) expression, and decreased Aβ deposition *in vitro* and *in vivo*. It also reduces inflammation and oxidative stress in the brain by inhibiting microglia. 3D-exo significantly improved cognitive and memory abilities of AD model rats (Yang *et al.*, 2020). This study provides a novel therapeutic intervention and the potential clinical application of exosomes extracted from hUMSCs grown on three-dimensional scaffolds for treating AD and other diseases. It demonstrates its efficacy and safety. Targeting amyloid dynamic balance is an important therapeutic strategy for AD. Through exosome modification, MExo-gem containing mannose-modified exosomes can bind to Aβ, and also possibly specifically target microglia by the interaction between mannose delivered by exosomes and mannose receptors expressed in microglia, thereby promoting Aβ entry into microglia. MExo-gem activates lysosome activity and accelerates Aβ clearance in microglia. MExo-gem improved AD model mice's learning and memory ability (Hao *et al.*, 2022). Exosomes are also used as drug carriers for AD therapy in nanomedicine delivery systems, where receptor-mediated endocytosis increases the solubility and permeability of curcumin through BBB. Engineered exosomes loaded with curcumin can prevent neuronal death by activating the AKT/GSK-3β pathway to inhibit Tau phosphorylation (Wang *et al.*, 2019) (Fig. 3). Similarly, quercetin loaded in plasma exosomes can prevent Tau pathology better than its free form, thus achieving better AD treatment in quercetin exosystem agents (Qi *et al.*, 2020). An exosecreting agent was created by combining genetic engineering with the co-transfection of parental cells. Rabies virus glycoprotein (RVG) is a cell-penetrating peptide with 29 amino acid residues. It can cross the BBB (Yang *et al.*, 2023). The exosecreting agent exhibited RVG peptide on its surface, targeted at α7-nAChR, and enriched with neutral lysozyme variants with higher specificity and Aβ degradation. Exoinstitutional agents are preferentially internalized into cell lines in a level-dependent manner with α7-nAChR expression. When incubated with Aβ-producing N2a cells, it significantly reduced cellular endocrine levels of Aβ40. Exosystemic agents preferentially target the brain's hippocampus, significantly reducing the expression of pro-inflammatory genes interleukin (IL)1α, tumor necrosis factor-α, and nuclear factor (NF)-κB, while increasing the expression of anti-inflammatory gene IL10 (Yu *et al.*, 2021). Silibinin (SIB)-loaded macrophage-derived exosomes reverse Aβ-induced neuronal injury and reduce cognitive impairment in AD mice by regulating the NF-κB pathway (Huo *et al.*, 2021). The above studies suggest that exosomes acting as bioengineered vectors or targeted drug delivery molecules can cross the BBB, improving transport rates and preventing complications of infection. Moreover, exosome-targeted vector therapy may improve learning and cognitive ability of mice with AD. These studies provide a basis for the clinical development of exosome drug therapy for AD.

Application of exosome-derived biomarkers in medical detection

Exosome derivatives predict the onset of Alzheimer's disease

Exosomes can be used as markers for diagnosing and predicting diseases, including diabetes, obesity, cancer, and cardiovascular and neurodegenerative diseases (Lauritzen *et al.*, 2020), and exosome biomarkers were found to be heterogeneous (Johnson *et al.*, 2022). Reay *et al.* (2022) through linkage disequilibrium regression analysis technique and generalized statistical analysis of Genome-Wide Association Studies, found through gene association, that there is clear evidence of genetic overlap between blood biomarkers and psychiatric characteristics, including a series of biochemical indicators that tend to be associated with similar profiles of psychiatric disorders. Extensive changes in

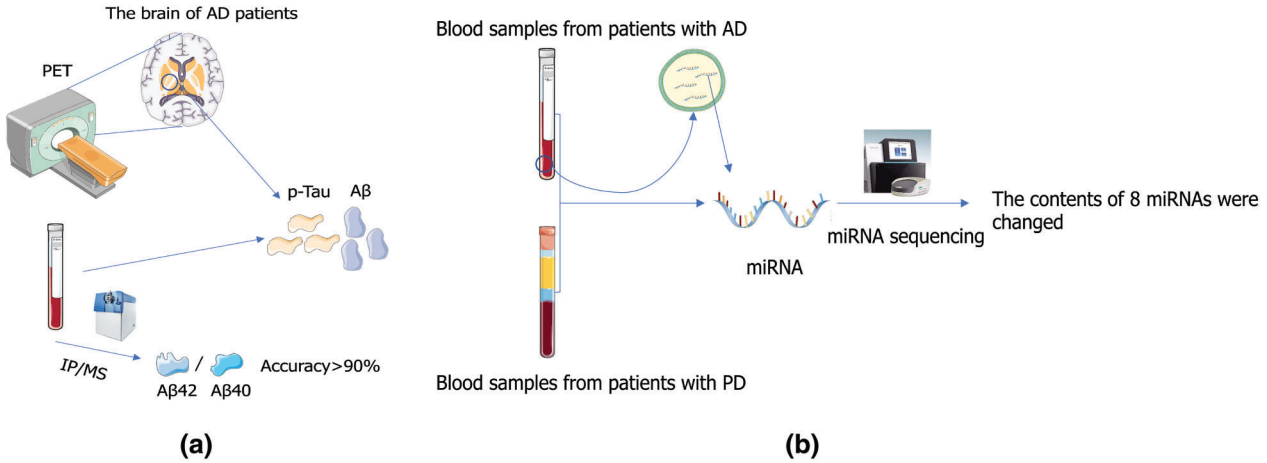

FIGURE 4. Application of exosome in medical detection. (a) Alzheimer's disease (AD) can be diagnosed by positron emission tomography (PET) or by measuring concentrations of Aβ and P-Tau in cerebrospinal fluid (Sonuç Karaboga and Sezgintürk, 2020). (b) Extensive changes in exo-miRNA expression levels were detected in patients with AD and PD patients by small RNA sequencing, and eight miRNAs were significantly elevated/decreased in AD and PD samples compared to those in controls (Nie *et al.*, 2020).

exo-miRNA expression levels were detected in AD and Parkinson's disease (PD) patients by small RNA sequencing, and eight miRNAs were significantly elevated/decreased in AD and PD samples compared to controls (Nie *et al.*, 2020) (Fig. 4), suggesting that the expression of exo-miRNA is somehow related to the pathogenesis of AD and PD.

Exosome derivative biomarkers in cerebrospinal fluid can accurately predict Alzheimer's disease

CSF exosome derivative biomarkers can accurately predict AD. The concentration of p-Tau in CSF is closely correlated with AD (Hanes *et al.*, 2020), and the phosphorylation spectrum of soluble Tau in the brain of AD patients is highly correlated with that of CSF in AD patients (Horie *et al.*, 2020). Aβ protein is also one of the biomarkers of AD patients, and Aβ deposition is a relatively late result of Aβ aggregation in AD. Increased Aβ42/Aβ40 ratio can directly lead to accumulation and aggregation of Tau protein (Kwak *et al.*, 2020), which can be detected by using positron emission tomography imaging or by measuring Aβ and p-Tau concentrations in CSF as biomarkers for diagnosis and tracking of AD in patients (Fig. 4) (Blennow, 2021). At present, plasma Aβ42/Aβ40 ratio measurement by immunoprecipitation mass spectrometry (IP-MS) can achieve more than 90% accuracy in identifying brain amyloid Aβ protein degeneration (Nakamura *et al.*, 2018). As one of these biomarkers, Aβ has great potential in predicting AD. Therefore, the researchers established a method to detect the ratio of Aβ monomer (AD biomarker) in CSF using the competitive synergistic effect of Cu^{2+} between CDs and Aβ monomer using the adaptive characteristics of Eu/GMP network (Liu *et al.*, 2020). This method provides an idea for early diagnosis of AD and a better understanding of the chemical nature of AD. The combination of exosome growth-associated protein 43 (GAP43), neuro granule protein, synaptosome-associated protein 25, and synaptotagmin 1 was found to detect preclinical AD 5 to 7 years before cognitive impairment by comparing AD patients with healthy individuals (Jia *et al.*, 2021).

Prediction of blood exosome biomarkers and their derivatives on Alzheimer's disease

Because of the trauma of the biopsy, a cheap, safe, and accurate prediction method is needed to detect AD. As an ideal method, the detection of exosome biomarkers has come into people's field of vision. In one experiment, the concentrations of Aβ42, T-Tau, and p-Tau-T181 secreted in exosomes of AD were higher than those in control groups, confirming the consistency between CSF and exosome biomarkers. Exosome Aβ42, T-Tau, and p-Tau-T181 were confirmed to have the same ability to diagnose AD as CSF (Jia *et al.*, 2019). The continuous increase in p-Tau protein levels in the blood and its high specificity with AD make blood-derived p-Tau a potential marker for the pathophysiological detection of AD (Benussi *et al.*, 2020). Plasma p-Tau231—a p-Tau protein with high diagnostic accuracy in distinguishing between AD and non-AD dementia (AUC = 0.93), demonstrating the potential clinical utility of plasma p-Tau (Ashton *et al.*, 2021). A disposable neurobiosensor probe for the determination of Tau-441 proteins has been developed using a nanocomposite composed of reduced graphene oxide (rGO) and gold nanoparticles (AuNP) using electrochemical impedance spectroscopy and cyclic voltammetry (CV). The nanocomposite surface (rGO-AuNP) modified with 11-mercaptoundecanoic acid (11-MUA) covalent anchor showed higher sensitivity. The neurobiosensor probe could capture the Tau-441 target protein in serum and CSF samples with recovery rates ranging from 96% to 108% (Sonuç Karaboga and Sezgintürk, 2020), suggesting that AD can be detected. The researchers used an interdigitated microelectrode (IME) as an impedance biosensor and detected blood-based Aβ using the gold nanoparticles (AuNPs) sandwich method. The IMEs sensor can detect Aβ with high sensitivity and selectivity according to its level. Mouse plasma samples were prepared from the blood of double-mutant APP/PS1 transgenic (TG) and wild-type (WT) mice, and the diagnostic ability of AD was tested by Aβ assay in the plasma samples. They found that the

AuNPs sandwich method assisted Aβ detection and successfully distinguished TG and WT mice groups. Therefore, this sensing system can detect Aβ with high sensitivity and selectivity (Yoo *et al.*, 2020). Rossi *et al.* (2020) efficiently and completely extracted the HAS-Aβ peptide complex from plasma by the Pierce albumin removal method, which could be identified using albumin biomarkers. Nano-probes can deliver clear signals to Aβ targets, which is a good choice for early diagnosis of AD (Hamd-Ghadareh *et al.*, 2022). Due to the transient heterogeneity of Aβ aggregates, it is not easy to dynamically monitor Aβ and its aggregation intermediates by constructing a two-dimensional manganese dioxide nano-enzyme sensor array. The nano-enzyme biosensor system can accurately detect Aβ species and related aggregation processes in clinical blood samples (Hu *et al.*, 2022).

According to the definition of AD by the National Institute of Aging (NIA) and the Alzheimer's Association (AA), the clinical symptoms of AD can be divided into six stages. The existing research on the prediction of AD mainly focuses on the second stage: subjective cognitive decline (SCD), and the third stage: mild cognitive impairment (MCI). In one trial, two biomarkers, Aβ42 and a sniffer stick (SS-16), were used separately and in combination in patients with MCI and AD dementia. Lower SS-16 scores and higher Aβ42 levels in NDE were found in MCI and AD dementia. For the longitudinal group, 8 individuals with MCI developed AD dementia within 2 years, and 16 individuals with MCI developed AD dementia within 3 years. The combination of the SS-16 score and Aβ42 level in NDE showed better prediction of the transition from MCI to AD dementia at 2–3 years than did a single indicator (Zhao *et al.*, 2020). Exosome-derived microRNAs are also good markers for predicting AD. ATP-binding cassette transporter A1 (ABCA1) is used as a marker to capture specific exosomes. Evaluation of the levels of ABCA1-labeled exosomes microRNA-135a (miR-135a) revealed that ABCA1 exosomes harvested from HT-22 cells and neuronal media were significantly higher than erythrocytes and leukocytes. The level of ABCA1-labeled exosome miR-135a in patients with MCI and AD (DAT) was higher than that in the control group and slightly increased in the serum of patients with SCD, suggesting that the level of exosome marker miR-135a can be used to diagnose AD (Liu *et al.*, 2021). Another study reported that miR-30b-5p, miR-22-3p, and miR-378a-3p were significantly dysregulated in AD patients (Dong *et al.*, 2021). A predictive model for AD was established by logistic regression analysis of serum exosome secretion markers. Combining these three miRs yielded better diagnostic ability (AUC = 0.88).

Urine exosome biomarker detection—a potential predictive method for Alzheimer's disease

Exosomes in the blood can predict the progression of AD, and biomarkers can also be extracted to predict the progression of AD in urine. In one study, enzyme-linked immunoadsorption assay (ELISA) was used to detect levels of Aβ42 and p-Tau-S396 (standardized by CD63) in urinary exosomes from patients with AD and matched healthy subjects. The exosome concentration, particle size, and phenotype were measured and the nanoparticles were observed by transmission electron microscopy. The levels of Aβ42 and p-Tau-S396 in the urine exosomes of patients with AD were higher than those of matched healthy controls, and more exosomes were extracted from patients with AD. This experiment showed that early AD diagnosis can be achieved by detecting Aβ42, p-Tau-S396 levels, and urinary exosome content (Sun *et al.*, 2019). These results suggest that marker detection is more accurate in CSF than in blood and urine marker. However, some studies have shown that combining some biomarkers in blood can achieve a similar detection effect as CSF biomarkers and confirmed that blood biomarkers such as Aβ42, T-Tau, and p-Tau-T181 have the same diagnostic ability as CSF markers. Urine exosome secretion markers also have certain predictive potential. Exosome-derived biomarkers in blood and urine are expected to provide a suitable method for accurately detecting AD in the future.

Multiple omics analysis can be used to predict multiple markers of Alzheimer's disease

Johnson *et al.* (2022) analyzed the proteomics of more than 1000 AD brain tissues using tandem mass tag mass spectrometry (TMT-MS). RNA expression network modules constructed by transcription data and proteins obtained from TMT-MS overlap with the TMT AD protein network in 168 RNA networks. It was also found that protein network modules were more strongly correlated with cognitive function than RNA network modules. Using laser capture microscopy (LCM) and laber-free unlabeled quantitative method proteomic analysis, several TMT AD protein network modules were enriched in NFTs. Such as silk crack the originally activated protein kinase (MARK)/ metabolic modules, and there is space between the iconic AD symptoms of consistency, the team by using the method of multiple omics analysis, they found a close relationship between the protein and the AD network module, and signaling by different protein markers and RNA network analysis can reveal the pathogenesis of AD. Exosome derivative markers and corresponding detection methods can also be used to predict AD development (Table 1) accurately. Combined analysis of blood or urine samples using a combination of exosome derivative markers can greatly improve the accuracy of prediction and replace the method of AD prediction by CSF, which is safer to implement. ELISA and similar methods have a price advantage over omics analysis. It has great potential value in medical detection applications. In the future, a clinical detection method is expected to be established based on the characteristics of biomarkers derived from different exosomes, that can use the combined analysis of multiple markers to accurately predict AD incidence.

The clinical situation of exosome application

Clinical progress of exosome biomarker detection methods

Through *ClinicalTrials.Gov* database (https://clinicaltrials.gov/) to collect AD, PD, and other neurological problems related to clinical data (Tables 2 and 3). Most of the clinical trials using biomarkers to detect AD in recent years stayed in the pre-phase III or phase NA. Very few trials enter the

TABLE 1

Exosome derivative markers and corresponding detection methods

Source	Biomarkers	Test method	Ref.
Blood	miR-30b-5p/miR-22-3p/miR-378a-3p	Logistic regression analysis techniques	Dong *et al.* (2021)
Blood	miR-135a	ABCA1-labeled exosomes	Liu *et al.* (2021)
Blood	Aβ42/Aβ40	IP-MS	Nakamura *et al.* (2018)
Blood	HAS-Aβ peptide complex	Pierce albumin removal method	Rossi *et al.* (2020)
Blood	Aβ	MnO_2 nano-enzyme sensor	Hu *et al.* (2022)
		Nano-probes	
Blood	Aβ42	Combined detection of Aβ42 and SS-16	Zhao *et al.* (2020)
Blood	Aβ	IMEs	Yoo *et al.* (2020)
Blood	Tau-441	rGO-AuNP	Sonuç Karaboga and Sezgintürk (2020)
Blood	p-Tau(p-Tau231)/Aβ/Aβ42/T-tau/p-Tau-T181	Similar to the marker assay in CSF	Jia *et al.* (2019)
CSF	GAP43/neurogranin/SNAP25	TMT-MS	Jia *et al.* (2021), Johnson *et al.* (2022)
		LCM	
		LFQ	
CSF	Aβ	Competitive Synergy of Cu^{2+} between CDs and Aβ monomer	Liu *et al.* (2020)
CSF	Tau-441	rGO-AuNP	Sonuç Karaboga and Sezgintürk (2020)
CSF	Proteome	TMT-MS	Ashton *et al.* (2021)
		LCM	
		LFQ	
Urine	Aβ1-42 and p-Tau-S396	ELISA	Sun *et al.* (2019)
		Transmission electron microscopy and nanoparticle tracking analysis	

Note: Aβ: amyloid beta; GAP43: growth-associated protein 43; IMEs: interdigitated microelectrode; rGO-AuNP: reduced graphene oxide-gold nanoparticles; TMT-MS: tandem mass tag mass spectrometry; LCM: laser capture microscopy; IP-MS: immunoprecipitation mass spectrometry; LFQ: label-free quantitation; ELISA: enzyme-linked immunoadsorption assay; SNAP25: synaptosome-associated protein 25.

TABLE 2

Clinical status of exosomes in detecting or treating Alzheimer's disease

ND	Phase	Initial year	Application	Origin	Therapeutic cargo	Outcome	CTN
AD	I/II	2020	Theranostic	Blood	Naive	Unknown	NCT04388982
	I	2014	Diagnostic	Body fluid	Naive	Unknown	NCT03275363
	I/II	2019	Diagnostic	Blood/CSF	Naive	Unknown	NCT03944603
	II/III	2014	Detective	Blood/CSF	Aβ42	Terminated	NCT02245737
	IV	2010	Detective	CSF	Aβ42/Total Tau/p-Tau181	Aβ42: Down	NCT01142336
						Total Tau: Up	
						p-Tau181: Up	
	NA	2019	Prognostic	Blood	miRNAs	Unknown	NCT04137926
	NA	2014	Prognostic	ONE	Aβ	Unknown	NCT02129452
					Tau		
					miRNA		
	NA	2017	Diagnostic	CSF	Tau/p-Tau181/Aβ42/Ng/SNAP-25	Unknown	NCT03300726
	NA	2013	Diagnostic	Blood/Saliva/Urine	Tau/p-Tau/Aβ42	Unknown	NCT01773915

(Continued)

Table 2 (continued)

ND	Phase	Initial year	Application	Origin	Therapeutic cargo	Outcome	CTN
	II	2021	Detective	Blood/CSF	p-Tau181 p-Tau217/Aβ40/Aβ42/NFL/T-tau/sTREM2/YKL-40/Neurogranin	Ongoing	NCT04693520
	NA	2018	Detective	Blood/CSF	Aβ42/Tau/NFL	Aβ42 in Plasma: Up Aβ42 in CSF: Down Tau in Plasma/CSF: Down NFL in Plasma/CSF: Down	NCT03405662

TABLE 3

Clinical status of exosomes in the detection of PD and other neurological diseases

ND	Phase	Year of initiation	Application	Origin	Therapeutic cargo	Outcome	CTN
PD	I/II	2013	Detective	Blood/Urine	LRRK2	Unknown	NCT01860118
	NA	2023	Detective	Blood	Naive	Ongoing	NCT05871359
	I	2020	Detective	CNS	Naive	Unknown	NCT04350177
	NA	2023	Prognostic	Blood	Naive	Ongoing	NCT05815524
MG	NA	2023	Diagnostic	Serum	miRNAs	Ongoing	NCT05888558
CTE	NA	2021	Diagnostic	Blood	S100B/GFAP/UCH-L1/NFL/T-Tau/p-Tau181/	Ongoing	NCT04928534
MSA	NA	2020	Detective	Blood	IRS-1pS312	Ongoing	NCT04250493
MS	NA	2017	Detective	Blood/CSF	NFL/Aβ/Tau/Inflammatory cytokines/miRNAs	The microRNA let-7b-5p is negatively associated with inflammation and disease severity in multiple sclerosis	NCT03217396

Note: According to the definition set out by the U.S. Food and Drug Administration (FDA), the clinical trial phase when a drug is being researched is divided into five phases: Early Phase 1 (formerly known as Phase 0), Phase 1, Phase 2, Phase 3, and Phase 4. NA is used to describe trials that do not have a phase defined by the FDA, including trials of devices or behavioral interventions. CTN: Clinical trial number.
Naive refers to a natural biomarker or a specific marker not mentioned in the clinic when exosomes are not used for drug delivery or when natural exosomes are used for testing.
In the table, Up means the content or ratio increases. Down means the content or ratio decreases.
Cerebrospinal fluid: CSF; myasthenia gravis: MG; chronic traumatic encephalopathy: CTE; multiple system atrophy: MSA; multiple sclerosis: MS; central nervous system: CNS; Ng: neurogranin; PD: Parkinson's disease; soluble TREM2: sTREM2; S100 calcium-binding protein B: S100B; glial fibrillary acidic protein: GFAP; neurofilament light chain protein: NFL; insulin receptor substrate-1 phosphorylated at serine 312: IRS-1pS312; leucine-rich repeat kinase 2: LRRK2; synaptosomal-associated protein 25: SNAP-25; recombinant ubiquitin carboxyl terminal hydrolase L1: UCH-L1;YKL-40: a secreted heparin-binding glycoprotein; olfactory neuroepithelium: ONE.

clinical phase IV. According to the current clinical results and clinical data, most exosome-derived biomarkers to predict related neurological diseases such as AD and PD are exosome-derived biomarkers. Biomarkers in patients with AD were investigated in two clinical trials using simvastatin administration (CTN: NCT01142336) and photo bioregulatory therapy (CTN: NCT03405662). These two clinical trials observed changes in the levels of typical AD-related biomarkers, such as Aβ42 and Tau, in the blood or CSF of these subjects. However, after analysis, the data were not statistically significant. The clinical data on these psychiatric disorders and the related reports mentioned above give us some experience in predicting AD. Using the correlation level changes of biomarkers, combined with gene association analysis, small RNA sequencing, SS-16, and other combined detection methods to predict whether patients are in the SCD and MCI stages, seems to prevent the conversion of SCD and MCI to AD to a certain extent. In a recent report, to further explore the potential of biomarkers to predict SCD, the Aß42/Aß40 ratio and the levels of T-tau and p-Tau in the CSF were used to explore differences between patients with SCD and controls (CO) of normal patients. The domain value of the Aß42/Aß40 ratio is calculated by means of data modeling for the definition of

amyloid-positive protein. Comprehensive analysis of positive rates of different proteins defined in this clinical trial proved that the positive predictive value (PPV) of AD was 0.9 (Jessen *et al*., 2023), which provides a new scheme for the future clinical use of exosome-derived biomarkers to predict AD.

Advances in the clinical treatment of exosomes

The clinical evidence on the use of exosomes in the treatment of AD is still in a relatively nascent stage. Although researchers have carried out relevant clinical trials to explore the safety evaluation of exosomes in patients with AD (CTN: NCT04388982), clear clinical data is needed to prove the reliability and safety of exosomes in treating AD. However, new progress has been made in using exosomes to treat ischemic brain pawns. Neural stem cells (NSC) combined with NSC-derived exosomes have a significant therapeutic effect on ischemic stroke. Exosomes regulate downstream target genes through the miRNA they carry, thereby downregulating oxidative stress and inflammation in brain tissue, inhibiting cell apoptosis, and ultimately promoting the survival and differentiation of transplanted NSC (Zhang *et al*., 2023). This study showed that exosome drugs have great potential for treating neuropathic diseases. Currently, exosome-related drugs have been developed for the treatment of tumors. Exosome candidate therapy exoSTING can activate the local dose-dependent STING signaling pathway in tumors, activating the immune response, and promoting the uptake of tumor-resident antigen-presenting cells (Jang *et al*., 2021). This innovative therapy is the first clinical proof of concept. Although no exosome drugs have entered the clinical validation stage for AD treatment, the advantages of exosomes themselves is expected to be used to achieve targeted therapy for AD in the future.

Conclusions

Exosomes have the ability to transmit Aβ and Tau proteins; therefore, they are associated with the onset of AD. In addition, exosomes have the function of clearing Aβ protein to reduce neuroinflammation. Exosomes are considered potential drug vectors for the treatment of AD due to their BBB-crossing and low immunity properties. The synthetic exosomes MExo-gem promotes microglia to clear Aβ by activating PPARα. 3D-EXO up-regulates ADAM10, down-regulates BACE1 expression, and reduces Aβ deposition *in vivo* and *in vitro*. It reduces inflammation and oxidative stress in the brain by inhibiting microglia. SIB-Exo reverses Aβ-induced neuronal damage and reduces cognitive impairment in AD mice by regulating the NF-κB pathway. These experiments provide evidence for the future clinical treatment of AD with exosomes.

As research continues to evolve, partial biomarkers in the blood/urine/CSF show the potential to detect AD. According to our collection of clinical evidence and existing studies, some exosome-derived protein biomarkers have the ability to diagnose AD early, such as Aβ42, T-tau, and p-Tau. MicroRNAs derived from exosomes in blood/urine/CSF have also been found to have the ability to diagnose AD, such as miR-135a, miR-30b-5p, and miR-22-3p. Although to date, no exosome drug has been approved for clinical use in AD patients. It is expected that the detection of AD by exosome-derived biomarkers and the treatment of AD by exosome drugs can be realized in the future due to the potential excellent properties of exosomes.

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