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Extracellular vesicles as an emerging tool for the early detection of Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) is characterized by a series of interacting pathophysiological cascades, including the aggregation of β-amyloid plaques and the formation of neurofibrillary tangles derived from hyperphosphorylated tau proteins. AD is the cause of approximately 70 % of dementia, an irreversible and untreatable syndrome at its late stage. Hence, more efforts should be devoted to identifying at-risk or preclinical AD populations for early intervention and the improved design of drug trials. The exosome, a nanoscale subtype of extracellular vesicle that serves as a cell-to-cell communication messenger, is an emerging liquid biopsy tool for various diseases including AD. Recently, it has been discovered that brain-derived exosomes can flow through the blood-brain barrier to the peripheral blood, containing important protein and nucleic acid biomarkers that are associated with the pathogenesis and progression of AD. Other reports showed a strong involvement of exosomes in synaptic function, insulin resistance, and neuroinflammation, among others. Here, we summarize those studies and assess the value of exosomes as an emerging tool for the early detection of AD in conjunction with the current clinical diagnosis paradigm.

1. Introduction

Alzheimer's disease (AD), accounting for approximately two thirds of 50 million people with dementia, is a progressive neurodegenerative disorder characterized by a loss of memory and cognitive decline. The increasing morbidity has produced a heavy health and economic burden, with the current cost of AD being approximately one trillion US dollars a year, which is forecasted to double by 2030 (Patterson, 2018). However, the scanty five US FDA-approved drugs can only improve the late-stage symptoms, and the clinical trials targeting AD dementia or prodromal AD patients have all ended in failure (Jan et al., 2017; Pillai and Cummings, 2013).

The real pathophysiological process of AD is thought to begin many years before the clinical diagnosis of dementia. From the current perspective, the term AD refers to an aggregate of neuropathological changes that can be identified by biomarkers in living people, while dementia is just an ultimate outcome. The present scheme of biomarker profiles is labeled as A (aggregated β-amyloid (Aβ)), T (neurofibrillary tangles (NFTs)) and (N (neurodegeneration)) system, and an individual is thought to be in the AD continuum as long as the Aβ biomarker is positive (Jack et al., 2018). Actually, the pathogenesis of AD involves a series of interacting pathophysiological cascades, the core events of which manifest in the formation of NFTs derived from hyperphosphorylated tau proteins in addition to Aβ plaque aggregation, both of which are essential to defining AD (Jack et al., 2018; Ballard et al., 2011). Accumulating evidence underlines the importance of other molecular pathophysiological pathways, such as synaptic dysfunction and degeneration (DeKosky and Scheff, 1990; Goetzl et al., 2018a; Lista and Hampel, 2017; Reddy and Beal, 2008), abnormity of the ubiquitin-proteasome and autophagic-lysosomal systems (Ihara et al., 2012), vascular and blood-brain barrier (BBB) dysregulation (Iturria-Medina et al., 2016), innate immune responses and neuroinflammation (Heneka et al., 2015), mitochondrial dysfunction (Jan et al., 2017; Reddy and Beal, 2008), insulin resistance (Moloney et al., 2010; Talbot et al., 2012) and others (Ballard et al., 2011), across different stages of AD. Furthermore, other neurodegeneration-related proteins and pathologies can coexist with AD patients, including TDP-43, α-synuclein, vascular lesions and others (Kovacs et al., 2013; Rahimi and Kovacs, 2014). All

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these factors make the disease extremely complex.

The development of biomarkers in recent years has dramatically advanced AD diagnosis, making it possible in the preclinical stage (Jack et al., 2018), bringing dawn and hope to drug trials. However, the state-of-the-art clinical assessments of AD, for example, positron emission tomography (PET) amyloid imaging and cerebrospinal fluid (CSF) analysis, although with high sensitivity and specificity in classifying symptomatic patients versus normal controls (Curtis et al., 2015; Hansson et al., 2006), may be useful only for the confirmation of a clinical diagnosis rather than prediction, because they are expensive and invasive and their values in preclinical patients are not as strong (Curtis et al., 2015; Landau et al., 2013). In addition, the PET analysis is also influenced by the skills of the operators. In the real world, a clinical non-AD diagnosis does not exclude underlying AD pathology and the clinical diagnosis of AD does not predict underlying pathology (Salloway et al., 2014). Hence, there still exists an unmet need for convenient and inexpensive early diagnosis biomarkers for AD.

The discovery of extracellular vesicles (EVs) greatly changed our understanding of cell-to-cell communication. Exosomes, as an important subpopulation of EVs, are characterized by their round shape structure with a lipid bilayer and small size ranging from 30 to 100 nm (Urbanelli et al., 2016), and there are still no adequate tools to separate exosomes from EVs. The following features determine that exosomes may function as biomarkers. First, exosomes can be extracted from diverse body fluids (Raposo and Stoorvogel, 2013). Second, most cell types in the body release exosomes, including neurons and others (Raposo and Stoorvogel, 2013; Chiasserini et al., 2014). Third, exosomes can pass through the BBB because of their bilayer lipid structure (Jain, 2012). Fourth, various proteins, messenger RNAs (mRNA), microRNAs (miRNAs) and other components, collectively termed as “cargos”, were detected in exosomes, and the membrane can protect their substances from enzyme degradation (Chiasserini et al., 2014; Cheng et al., 2015). The cargos may vary depending on the cell of origin as well as the eliciting stimulus, eventually including biomarkers that are typical of the pathological state (Urbanelli et al., 2016; Fuhrmann et al., 2015). Notably, several studies successfully harvested and enriched “brain-derived exosomes” in blood by using immunochemical methods, thus eliminating the noises generated from a general pool of exosomes in the bloodstream (Mullins et al., 2017; Guix et al., 2018; Fiandaca et al., 2015). This near-noninvasive approach can more accurately reflect the pathological changes in the brain. It must be mentioned that the latest research changed the term “exosomes” to “EVs” due to the purity problem of this method (Kapogiannis et al., 2019), and we totally followed the original authors’ nomenclature in the following discussion.

It is encouraged to incorporate new biomarkers of the specific AD pathophysiological process (Jack et al., 2018). In this review, we summarized the current studies and reviewed the role of exosomes as AD biomarkers. The biomarkers are divided into predictive and diagnostic utility, reflecting the preclinical and clinical stages, respectively. The application of circulating exosome-based tests for AD patients can indicate the intrinsic pathological changes and improve the diagnostic accuracy and is important for targeting super-early secondary prevention clinical trials in AD (Fig. 1).

2. Relationship between Aβ, tau protein and exosomes

2.1. Roles of exosomes in Aβ propagation

The term Aβ usually refers mainly to a mixture of canonical 40–42 amino acid peptides excised by the endoproteolysis of β-amyloid precursor protein (β-APP) through the sequential proteolytic processing of β-APP cleaving enzyme (BACE) and γ-secretase (Rajendran et al., 2006). *Rajendran et al.* initially proved that Aβ can be packaged into multivesicular bodies and then a small part of the Aβ wrapped in exosomes is released into the extracellular environment (Rajendran et al., 2006). Subsequently, scientists found that β-APP and its processing products, C-terminal fragments and Aβ, as well as several key members of the secretase family of proteases, also existed in exosomes (Sharples et al., 2008; Vingtdeux et al., 2007). Another in vivo study proved that brain-derived exosomes from transgenic AD mouse models overexpressed β-APP and C-terminal fragments compared to wild-type mice (Perez-Gonzalez et al., 2012). These results proposed that exosomes may have great potential in deteriorating AD.

By injecting Aβ-enriched extracts directly into the brains of primates or mice, scientists found that this approach can initiate Aβ accumulation and the associated pathologies (Baker et al., 1994; Eisele et al., 2010). However, it was uncertain whether exosomes had effects. Another study confirmed that exosomes were gathered in the Aβ plaques of autopsied AD patient brain sections, but not in those of Parkinson’s disease (PD) patients or controls (Rajendran et al., 2006). Further studies verified that exosomes can promote the Aβ aggregation that contributes to plaque deposition in juvenile familial AD mice, and the plaque burden was significantly alleviated after the inhibition of exosome production (Dinkins et al., 2014, 2016). Importantly, a latest study proved that exosomes from AD patients’ brains contained increased levels of Aβ oligomers compared with controls, and these toxic exosomes were able to spread their cargos by direct transfer to other neurons and causing cytotoxicity, while the propagation can be blocked when inhibiting the formation, secretion or uptake of exosomes (Sardar et al., 2018). On the other hand, the Aβ-induced glial apoptosis is through an exosome-dependent manner (Wang et al., 2012), and exosomes also suppressed the glial clearance of Aβ (Dinkins et al., 2016). Together, these studies suggested that exosomes can be hijacked to aid in the aggregation, deposition and propagation of Aβ, characterized as a prion-like mechanism (Fig. 2).

In contrast, other results have made the role of exosomes puzzling. *Yuyama et al.* published a series of articles proved that exosomes drive the conformational changes of Aβ to form nontoxic amyloid fibrils and promote its uptake by microglia; in addition, the injection of exosomes into the brains of APP transgenic mice can markedly reduce Aβ deposition (Yuyama et al., 2012, 2014; 2015). Exosomes also assist in degrading extracellular Aβ by releasing insulin-degrading enzymes (Bulloj et al., 2010; Tamboli et al., 2010). The role of exosomes in Aβ remains challenging and further research is needed.

2.2. Exosomes function as vesicles for tau seeding

The intraneuronal deposition of NFTs, which are composed of the aggregated microtubule associated protein tau, is another feature of AD (Ballard et al., 2011). Curiously, tau pathology always developed through the brain in a rigid and stationary manner (Braak and Braak, 1995), and this form of spreading led researchers to speculate that tau transmission is also through a prion-like mechanism; in addition to affecting the host neuron, tau “seeds” also cause damage and the aggregation of tau proteins in recipient cells. Traditionally, it was thought that the release of intracellular tau is only by cell death and then through the infection of neighboring cells (Gómez-Ramos et al., 2006); however, a series of studies have proved that extracellular tau could also arise by exosomes (Guix et al., 2018; Asai et al., 2015; Saman et al., 2012; Wang et al., 2017; Simón et al., 2012), and exosomes also participated in the tau efflux from the ventricular system to the blood (Shi et al., 2016). This release approach may be a forced protective mechanism of tau overload in cells (Saman et al., 2012; Wang et al., 2017; Simón et al., 2012; Shi et al., 2016), or a routine mechanism (Guix et al., 2018; Wang et al., 2017). The tau that is released by exosomes is full length and is therefore capable of self-aggregation and potentially seed aggregation upon entry into recipient cells (Guix et al., 2018). More recently, several studies demonstrated that exosomes are involved in the trans-synaptic transmission of tau between neurons; thus, they may contribute to the spread of tau pathology (Wang et al., 2017; Polanco et al., 2016, 2018). In addition, microglia can also propagate

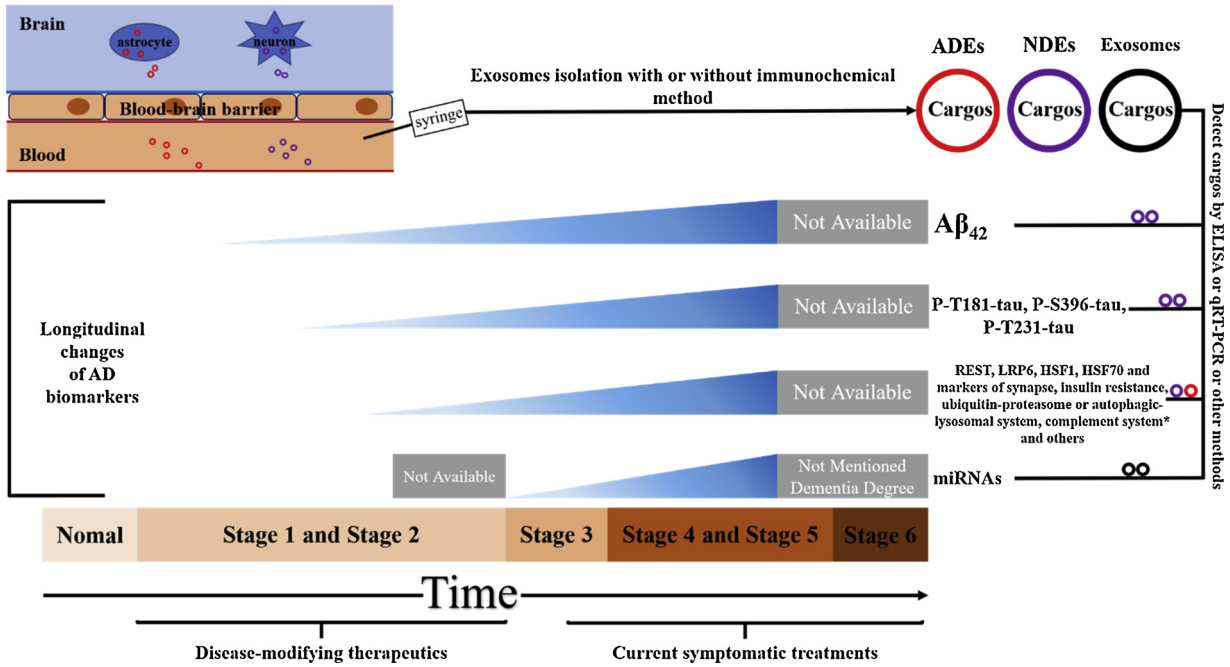

Fig. 1. Longitudinal changes in AD exosomal biomarkers during disease progression.
As time goes by, the cognitive function of individuals in the Alzheimer's continuum deteriorates gradually. The numeric clinical stages are in accordance with the 2018 NIA-AA Research Framework: stage 1 and stage 2 both describe a preclinical state; stage 3 is similar to mild cognitive impairment; and stages 4–6 are identical to mild, moderate, and severe dementia. Studies have proven that the levels of blood-derived exosomal Aβ, phosphorylated tau, and markers of other pathological changes are all dysregulated as early as 1–10 years before the cognitive decline of patients in the preclinical stage, and we speculated that this pathway is still in the order of A-T-(N). Not Available means a lack of research on patients in this stage. Studies on miRNAs lack a classification of dementia severity. *, cargos derived from ADEs. AD, Alzheimer's disease; ADEs, astrocyte-derived exosomes; NDEs, neuron-derived exosomes; ELISA, enzyme-linked immunosorbent assay; qRT-PCR, quantitative reverse transcription PCR; Aβ, β-amyloid; REST, repressor element 1-silencing transcription factor; LRP6, low-density lipoprotein receptor-related protein 6; HSF, heat shock factor.

tauopathy by internalizing and secreting exosomes, and the spreading was seriously suppressed when inhibiting exosome synthesis (Asai et al., 2015; Wang et al., 2017). To gain more insights, scientists injected neuron- or microglia-derived tau-containing exosomes directly into the brains of rodents and observed enlarged regions of tau pathogenic manifestations (Asai et al., 2015; Winston et al., 2016). Exosomes are actually just one of the pathways in the spreading of tau, and its weight deserves further research (Fig. 3).

2.3. Exosomes as biomarkers reflect core pathological events of AD

The identification of the mechanisms of the cell-to-cell transmission of pathology-associated proteins would provide an exosome-dependent molecular pathway that could be detected by novel convenient approaches with the aim of reflecting the intrinsic pathological changes of AD, ultimately making exosomes vehicles for biomarkers to amplify the existing AT(N) profiles (Jack et al., 2018). Increasing evidence based on primates and rodents suggests the possibility of the function of exosomes as biomarkers (Yuyama et al., 2015; Shi et al., 2016); moreover,

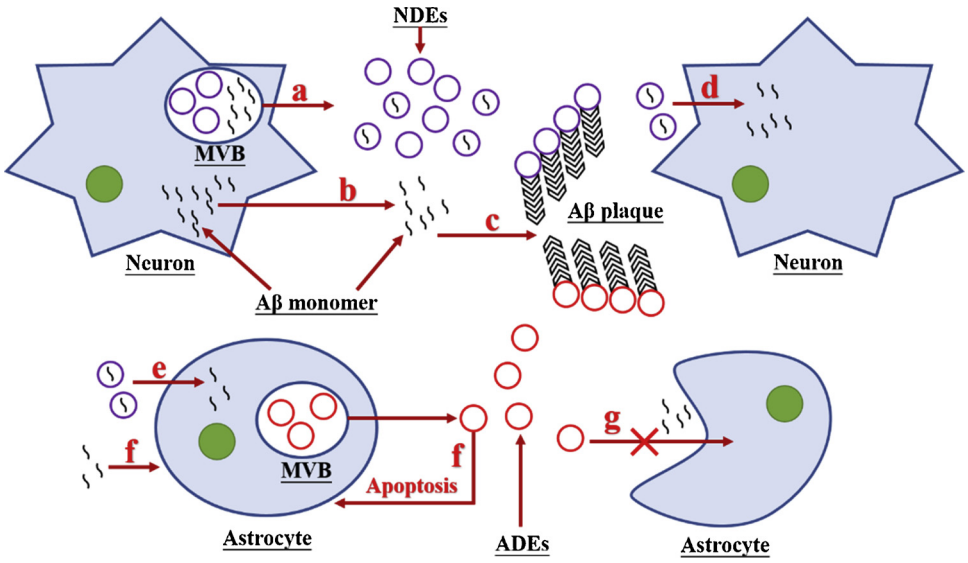

Fig. 2. Roles of exosomes in Aβ.
a, Aβ monomers are packaged into MVBs, and then parts are released into the extracellular environment through exosomes; b, Aβ monomers enter the extracellular environment through other pathways; c, in familial AD models, both NDEs and ADEs can promote Aβ aggregation, leading to plaque deposition; d, e, Aβ-containing NDEs infect other neurons or astrocytes; f, Aβ-induced astrocyte apoptosis is dependent on ADEs; g, ADEs extracted from familial AD models can block the glial clearance of Aβ. MVB, multivesicular body; AD, Alzheimer's disease; ADEs, astrocyte-derived exosomes; NDEs, neuron-derived exosomes; Aβ, β amyloid.

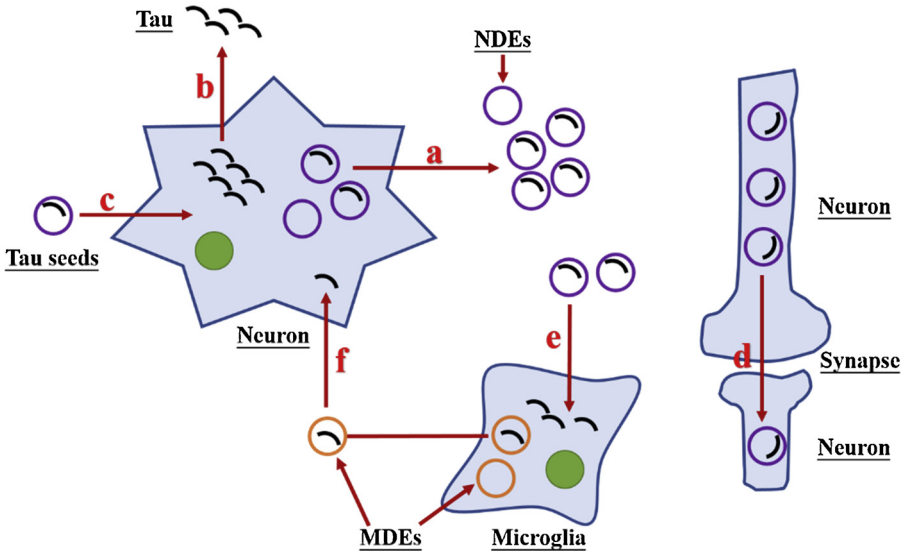

Fig. 3. Exosomes function as vesicles for tau seeding. a, Tau-containing exosomes are released into the extracellular space; b, tau seeds enter the extracellular environment through other pathways; c, e, tau-containing NDEs infect other neurons or microglia; d, the internalized exosomes undergo axonal transport and finally are released at presynaptic terminals, then they are taken up by the synaptically connected neurons, resulting in the distribution of tau in these recipient cells; f, microglia release tau seeds by exosomes, and these MDEs can infect other neurons. NDEs, neuron-derived exosomes; MDEs, microglia-derived exosomes.

exosomal tau extracted from the CSF of controls or AD patients was differently phosphorylated (Saman et al., 2012). Consequently, much work so far has focused on exosomal Aβ and tau as biomarkers in AD patients (Table 1).

Fiandaca et al. performed an important case-control study that played a leading role in the follow-up studies. They proved that the total tau, P-T181-tau, P-S396-tau and $A\beta_{42}$ levels of plasma neuron-derived exosomes (NDEs) from amnestic mild cognitive impairment (aMCI) due to AD (AD-aMCI) or AD dementia (ADD) patients were all significantly higher than those of controls, and the last three indicators have high discrimination. In addition, exosomal P-S396-tau can also distinguish AD from frontotemporal dementia (FTD) perfectly. Although there were no differences between AD-aMCI and ADD patients in the four indicators, the levels of P-T181-tau, P-S396-tau and $A\beta_{42}$ have already elevated for up to 10 years at the preclinical stage, and the amount of $A\beta_{42}$ increased gradually with the progression of AD (Fiandaca et al., 2015). Identical conclusions of increased levels of exosomal P-T181-tau, P-S396-tau and $A\beta_{42}$ from AD patients were obtained in another two studies (Winston et al., 2016; Goetzl et al., 2016a). *Goetzl et al.* also considered astrocyte-derived exosomes (ADEs) and other Aβ-related proteins (Goetzl et al., 2016a), with only $A\beta_{42}$ in plasma ADEs showing reduced differential amount between patients and controls; there was no difference in P-T181-tau or P-S396-tau. In addition, the levels of BACE-1 and soluble β-APP were increased in ADEs from AD patients, and soluble β-APP can distinguish AD from FTD. The levels of γ-secretase and soluble α-APP in ADEs were not changed. In NDEs, the levels of soluble α-APP and β-APP were significantly higher for AD patients than for controls, with no such difference in BACE-1 or γ-secretase. Septin-8, which has been implicated in the regulation of the amyloidogenic processing of APP, was found to be downregulated in the ADEs of AD patients compared with controls or FTD patients but was not changed in NDEs (Goetzl et al., 2016a). *Winston* et al. focused on aMCI patients specifically and divided them into stable and unstable groups depending on whether they developed dementia within 36 months. They found that the NDE levels of $A\beta_{42}$ and P-S396-tau in the unstable MCI group were similar to those in the ADD group, and both were significantly higher than those in the control or stable group. Importantly, there was no difference between the levels in the stable MCI and control groups. Therefore, the two indicators can predict whether the cognition of MCI patients continues to deteriorate or not. By contrast, the exosomal P-T181-tau amount did not show such ability (Winston et al., 2016). During analysis, it should be noted that the first two studies considered AD-aMCI and ADD patients as a single AD group (Fiandaca et al., 2015; Goetzl et al., 2016a), and the last one considered them separately (Winston et al., 2016). Notably, based on pure clinical diagnosis, a latest multicenter study verified that the NDE concentrations of $A\beta_{42}$, T-tau, and P-T181-tau showed a ladder-like change pattern among ADD, aMCI and control groups, demonstrating their possible diagnostic biomarker roles again (Jia et al., 2019).

Amyloid and other pathological features in AD have also been observed in Down syndrome (DS) (Webb, 2012); therefore, biomarkers that reflect DS pathology are of considerable interest. Researchers extracted plasma NDEs from DS patients and controls and found that the NDE levels of $A\beta_{42}$, P-T181-tau and P-S396-tau in the young (non-cognitively impaired, 8–35 years age) or the adult DS group (non-cognitively impaired or dementia, more than 35 years) were all increased compared with the age-matched control group. Furthermore, $A\beta_{42}$ levels were significantly decreased, whereas P-S396-tau levels were increased, in adult DS patients with dementia relative to those with normal cognition (Hamlett et al., 2017). These results indicated that exosomal $A\beta_{42}$ levels have already increased in cognitively healthy DS patients and will downregulate in the late dementia stage, while the amount of exosomal P-S396-tau will increase gradually with DS progression. The levels of P-T181-tau were not changed with the deterioration of cognition.

To validate the role of NDEs as biomarker candidates in the preclinical stage of AD, *Kapogiannis et al.* recently performed a longitudinal study with the largest number of case-control samples so far (Kapogiannis et al., 2019). Though using similar methods (Fiandaca et al., 2015; Winston et al., 2016; Goetzl et al., 2016a; Jia et al., 2019; Hamlett et al., 2017), the authors used the term neuronal-enriched EVs (nEVs) instead of NDEs. They collected 887 longitudinal plasma samples from 350 cognitively healthy participants, including 128 individuals who developed AD ultimately and 222 matched controls who remained cognitively healthy at the end of the follow-up. Participants with future AD showed a higher levels of P-T181-tau and P-T231-tau in nEVs than controls; however, the levels of $A\beta_{42}$ did not change in the preclinical stage as previously reported (Fiandaca et al., 2015; Hamlett et al., 2017), nor did total tau. Longitudinal trajectories of these nEVs biomarkers indicated that the difference in P-T181-tau was more obvious in the elderly subjects, while longitudinal changes for P-T231-tau tended to diverge in younger age groups. The authors did not measure the amount of P-S396-tau, and it remains challenging to explain the different results of $A\beta_{42}$.

A recent study extracted NDEs with an optimized method and found that the levels of exosomal P-T181-tau from MCI or ADD patients both showed no difference from controls (Guix et al., 2018), which is in contrast to the above five studies (Fiandaca et al., 2015; Winston et al.,

Table 1
Exosomes (or neuronal-enriched EVs) as biomarkers reflect core (and other) pathological events of AD.

Experimental Design	Diagnosis	Exosomes	Detecting Cargoes	Significances	Outcome	References
Cross-sectional study: AD, 57 (aMCI, 29, CDR 0.5; dementia, 28, CDR 1.0); AC, 57.	Petersen 2004 criteria, NIA-AA criteria 2011, revised NINCDS-ADRDA criteria 2007; all patients have detected CSF $A\beta_{42}$ (emailed to the corresponding author).	Plasma NDEs; 2 μg mouse anti-human NCAM or 1 μg mouse anti-human L1CAM.	Total tau, P-T181-tau, P-S396-tau and $A\beta_{42}$. ELISA.	Increased.	Diagnostic biomarkers.	Fiandaca et al. (2015)
Longitudinal study: aMCI, 13; dementia, 11. First blood collection: 1-10 years before diagnosis (AP); Second: initial diagnosis (AD). AC, 24.				Increased in AP/AD group: P-T181-tau, P-S396-tau, Aβ42.	Predictive biomarkers.	
AD, 12 (aMCI, CDR 0.5; mild dementia, CDR 1.0); AC, 10.	Petersen 2004 criteria, NIA-AA criteria 2011, revised NINCDS-ADRDA criteria 2007; most patients have detected CSF $A\beta_{42}$ and tau.	Plasma ADEs; 1.5 μg mouse anti-human GLAST. Plasma NDEs; 1 or 2 μg mouse anti-human L1CAM.	P-T181-tau, P-S396-tau, $A\beta_{42}$, BACE-1, γ-secretase, Sα-APP, Sβ-APP, septin-8, GDNF. ELISA.	Increased: BACE-1, Sβ-APP; Decreased: GDNF, septin-8, $A\beta_{42}$. Increased: P-T181-tau, P-S396-tau, $A\beta_{42}$, Sα-APP, Sβ-APP.	Diagnostic biomarkers.	Goetzl et al. (2016a)
Mild-to-moderate AD-dementia, 10; Stable MCI, 20; Unstable MCI, 20; AC, 10.	all participants were diagnosed according to MMSE, ADL and levels of CSF $A\beta_{42}$.	Plasma NDEs; 2 μg mouse anti-human L1CAM.	P-S396-tau, $A\beta_{42}$, NRGN, REST. ELISA.	Increased in dementia and unstable MCI groups: P-S396-tau, $A\beta_{42}$; Decreased: NRGN, REST.	Diagnostic and predictive biomarkers.	Winston et al. (2016)
			P-T181-tau. ELISA.	Increased in dementia and MCI groups.	Diagnostic biomarkers.	
Discovery stage: AD-dementia, 28; aMCI, 25; Controls, 29. Validation stage: AD-dementia, 73; aMCI, 71; Controls, 72.	NIA-AA criteria 2011 for AD-dementia; Gauthier et al. criteria 2006 for aMCI; clinical diagnosis.	Plasma NDEs; according to Fiandaca et al., 2015.	T-tau, P-T181-tau and $A\beta_{42}$. ELISA.	Stepwise increased among the three groups.	Diagnostic biomarkers.	Jia et al. (2019)
DS, 14 (8-35 years, normal); DS, 33 (> 35 years, normal, 16; dementia, 17); Controls (8-35 years, 9; > 35 years, 28).	Definite diagnosis.	Plasma NDEs; same as Fiandaca et al., 2015.	P-T181-tau, P-S396-tau, $A\beta_{42}$. ELISA.	Increased in DS.	Diagnostic biomarkers.	Hamlett et al. (2017)
			P-S396-tau, $A\beta_{42}$. ELISA.	Decreased in DS-dementia than DS-normal: $A\beta_{42}$; Increased: P-S396-tau.	Predictive biomarkers.	
AD-dementia, 128; AC, 222. Several blood collections before cognitive impairment or deadline of follow-up. Finally, 887 longitudinal plasma samples were analyzed.	Clinical diagnosis based on all available clinical and neuropsychological data; not detected biomarkers.	Plasma neuronal-enriched EVs; slightly different to Fiandaca et al., 2015; 4 μg mouse anti-human L1CAM.	Total tau, P-T181-tau, P-T231-tau, P-panY-IRS-1, P-S312-IRS-1, TSG101.Mesoscale discovery electrochemiluminescence assays. $A\beta_{42}$. SIMOA assay. Neuronal-enriched EV concentration and diameter.	Average EV diameter, P-T181-tau, P-T231-tau, P-panY-IRS-1 and P-S312-IRS-1 were increased in preclinial stage of AD. The EV concentration, total tau, TSG101 and $A\beta_{42}$ were not changed.	Predictive biomarkers.	Kapogiannis et al. (2019)
AD-dementia, 20 (mild, 10, CDR 0.5-1; moderate, 10, CDR 2); MCI, 10, CDR 0.5; AC, 10.	pure clinical diagnosis; others not shown.	Plasma NDEs; optimized method; 4 μg mouse anti-human L1CAM.	full-length tau, mid-region tau, P-T181-tau. ELISA.	Not Changed.	Not Avaliable.	Guix et al. (2018)
AD-dementia, 27; MCI, 21; Controls (< 40 years, 15; 40-65 years, 21; > 65 years, 23).	NIA-AA criteria 2011; All participants have detected CSF $A\beta_{42}$ and tau.	CSF derived EVs particles; flow cytometric assay method.	Apo-AI, annexin V. Flow cytometric assay method.	Decreased.	Diagnostic biomarkers.	Yang et al. (2015)
			ApoE, $A\beta_{42}$. Flow cytometric assay method.	Not Changed.	Not Avaliable.	
AD-dementia, 28; AC, 27.	NINCDS-ADRDA criteria 1984; all participants have detected CSF tau.	CSF derived; differential centrifugation.	APP, α-syn, DJ-1/PARK7, Fractalkine, NF-L, neurofilament, Tau, RP11-462G22.1, PCA3. qRT-PCR.	Increased: NF-L, neurofilament, RP11-462G22.1, PCA3; Decreased: APP, α-syn, DJ-1/PARK7, Fractalkine, tau.	Diagnostic biomarkers.	Gui et al. (2015)

Note: AD, Alzheimer's disease; aMCI, amnestic mild cognitive impairment; CDR, Clinical Dementia Rating; AC, AD case-controls; AP, preclinical; NIA-AA, National Institute of Aging-Alzheimer's Association; Aβ, β-amyloid protein; CSF, cerebrospinal fluid; NDEs, neuron-derived exosomes; NCAM, neural cell adhesion molecule; L1CAM, L1 cell adhesion molecule; ELISA, enzyme-linked immunosorbent assay; ADEs, astrocyte-derived exosomes; GLAST, glutamine aspartate transporter; BACE-1, β-site amyloid precursor protein-cleaving enzyme 1; APP, amyloid precursor protein; Sα(β)-APP, soluble α(β)-APP; GDNF, glial cell-derived neurotrophic factor; NRGN, neurogranin; REST, repressor element 1-silencing transcription factor; MMSE, mini-mental state examination; ADL, activities of daily living; DS, Down syndrome; EVs, extracellular vesicles; IRS-1, insulin receptor substrate-1; P-S312-IRS-1, Phospho-serine 312-IRS-1; P-panY-IRS-1, Phospho-pan-tyrosine-IRS-1; TSG101, tumor susceptibility gene 101; Apo, apolipoprotein; α-syn, α-synuclein; NF-L, light gene; qRT-PCR, quantitative reverse transcription PCR.

2016; Goetzl et al., 2016a; Jia et al., 2019; Hamlett et al., 2017). This research made the original clear conclusions questionable. The authors attributed this difference to the small sample size and inaccurate pure clinical diagnosis (Guix et al., 2018). However, we should also realize that the above five studies performed similar NDE extraction methods, and the source of patients included in three of the four AD studies is relatively monotonous and may create overlaps among enrolled patients (Fiandaca et al., 2015; Winston et al., 2016; Goetzl et al., 2016a). *Yang* et al. developed a novel CSF flow cytometry method detecting EV particles (they used the term EV rather than exosome) and found that the $A\beta_{42}$-positive particle concentrations were not changed in MCI or ADD patients (Yang et al., 2015). Together, it currently remains challenging to view exosomal Aβ or tau as diagnostic or predictive biomarkers in AD, and a more precise method for extracting exosomes and multicenter collaboration is needed.

3. Exosomal miRNAs as biomarkers for AD

During the past several years, miRNAs have emerged as important posttranscriptional regulators of gene expression, providing a completely new level of controlling gene expression, and they have shown extraordinary diagnostic and therapeutic potential, especially in the field of cancer and neurodegenerative diseases. It has been revealed that the brain is the organ with the highest expression of tissue-specific miRNAs, and these miRNAs are involved in the development of AD pathogenesis and may be candidates as diagnostic biomarkers (Kumar and Reddy, 2016; Dehghani et al., 2018). However, the dysregulated extracellular miRNAs released by cells in the pathogenic state must pass through the BBB to reach the circulatory system and face the risk of being degraded, and blood contains many interfering substances that increase the signal-to-noise ratio. Inspiringly, exosomes mediate miRNA secretion (Kumar and Reddy, 2016). In addition, exosomes function as miRNA carriers that cross the endothelial cellular layers of the BBB, and this could promote communications between the brain and distant organs via biological fluids (Haqqani et al., 2013; Montecalvo et al., 2013). The membrane-derived vesicles also protect miRNAs from RNases (Cheng et al., 2015). Consequently, the exosomal miRNAs possess an advantage over miRNAs in fluids and may serve as a sort of biomarker in AD (Table 2).

Cheng et al. screened the deregulated miRNAs of serum-derived exosomes from ADD patients, compared them to those of controls by deep sequencing, and then validated them by quantitative reverse transcription PCR (qRT-PCR) (Cheng et al., 2015). Finally, 16 miRNAs (miR-1306-5p, miR-342-3p and others) were thought to be diagnostic biomarkers; the sensitivity and specificity of using these 16 miRNA signatures to identify ADD patients and controls reached 87 % and 77 %, respectively, and the mistaken controls showed either high Aβ burdens in the brain upon PET neuroimaging or low burden but carried with APOE ε4, suggesting a high risk of the preclinical stage of AD (Cheng et al., 2015). However, the qRT-PCR data of several miRNAs showed great overlaps between patients and controls, but the result is still inspiring that the biomarker profile has the potential to predict AD at the preclinical stage. Instead of using commercial kits, researchers isolated exosomes by differential centrifugation and found that these plasma-derived exosomes have 20 dysregulated miRNAs (miR-342-3p, miR-342-5p and others) between ADD patients and controls by Illumina deep sequencing (Lugli et al., 2015). The diagnostic accuracy for an individual person can reach 83 %–89 % according to the levels of 7 miRNA signatures. However, the deep sequencing results of the two studies were completely different except for miR-342-3p (Cheng et al., 2015; Lugli et al., 2015), implying that there are serious deficiencies. Previous studies have identified important features of miR-29c, miR-135a, miR-193b and miR-384 in regulating the expression of APP or BACE-1; accordingly, researchers have confirmed that the levels of blood-derived exosomal miR-29c, miR-135a and miR-384 were upregulated while those of miR-193b were downregulated in AD-MCI or

Table 2
Exosomal miRNAs as biomarkers of AD.

Experimental Design	Diagnosis	Exosomes	Detecting Cargoes	Significances	Outcome	References
HC, 23; MCI, 3; ADD, 23.	NINCDS-ADRDA criteria 1984; 83 participants have detected Aβ-PET.	Plasma/Serum Exosomal RNA Isolation Kit.	miRNAs; Deep sequencing. Screened miRNAs; qRT-PCR validation.	Upregulated: miR-361-5p, miR-30e-5p and others; Downregulated: miR-1306-5p, miR-342-3p, miR-15b-3p.	Diagnostic and possible predictive biomarkers.	Cheng et al. (2015)
HC, 36; MCI, 8; ADD, 16.						
HC, 35; ADD, 35.	Screened and verdified by clinicians; all patients have detected CSF, PET.	Plasma-derived; differential centrifugation.	miRNAs; Illumina deep sequencing.	Upregulated: miR-548at-5p, miR-138-5p, miR-5001-3p, miR-659-5p; Downregulated: miR-23b-3p, miR-342-3p, miR-342-5p and others.	Diagnostic biomarkers.	Lugli et al. (2015)
HC, not shown; MCI, 43; ADD, 51.	Not shown.	Serum/Plasma-derived; kit.	miR-193b; qRT-PCR.	Downregulated.	Diagnostic biomarkers.	Liu et al. (2014a), (2014b)
HC, not shown; ADD, 7.		CSF-derived; kit.				
HC, not shown; AD-MCI, 101; ADD, 107.	NIA-AA criteria 2011; not mentioned biomarkers.	Serum-derived; kit.	miR-135a, miR-193b, miR-384; qRT-PCR.	Upregulated: miR-135a, miR-384; Downregulated: miR-193b.	Diagnostic biomarkers.	Yang et al. (2018)
HC, 60 (for MCI, 30; for ADD, 30); AD-MCI, 78; ADD, 124.	NIA-AA criteria 2011; not mentioned biomarkers.	Plasma-derived; kit.	miR-29c; qRT-PCR.	Upregulated.	Diagnostic biomarkers.	Liu and Yang (2018)
HC, 16; dementia, 32 (AD, 22; VaD, 10).	pure clinical diagnosis; NINCDS-ADRDA criteria 1984 and other clinical data.	Serum-derived; kit.	miR-223; qRT-PCR.	Downregulated.	Possible Diagnostic biomarkers.	Wei et al. (2018)
HC, 27; ADD, 28.	NINCDS-ADRDA criteria 1984; all participants have detected CSF tau.	CSF-derived; differential centrifugation.	miRNAs; TaqMan miRNA arrays.	Upregulated: miR-132-5p, miR-151, miR-485-5p; Downregulated: miR-29c, miR-136-3p, miR-16-2, miR-331-5p.	Diagnostic biomarkers.	Gui et al. (2015)

Note: HC, Healthy controls; MCI, mild cognitive impairment; AD, Alzheimer's disease; ADD, AD induced dementia; NIA-AA, National Institute of Aging-Alzheimer's Association; CSF, cerebrospinal fluid; Aβ, β-amyloid protein; PET, positron emission tomography; miRNAs, microRNAs; qRT-PCR, quantitative reverse transcription PCR; VaD, vascular dementia.

Table 3
Exosomal biomarkers reflect brain synaptic function, insulin resistance and other events of AD.

Experimental Design	Diagnosis	Exosomes	Detecting Cargoes	Significances	Outcome	References
Cross-sectional study: AD, 12 (aMCI, CDR 0.5; mild dementia, CDR 1.0); AC, 12.	Petersen 2004 criteria, NIA-AA criteria 2011, revised NINCDS-ADRDA criteria 2007; all patients have detected CSF $A\beta_{42}$ and tau.	Plasma NDEs; 1.5 μg mouse anti-human L1CAM.	synaptotagmin, synaptopodin, synaptophysin, neurogranin, GAP43, synapsin1, P-S9-synapsin1. ELISA.	Decreased.	Diagnostic biomarkers.	Goetzl et al. (2016b)
Longitudinal study: probable mild-to-moderate dementia, 9, CDR > = 1.0. First blood collection: 1-10 years before diagnosis (AP); Second: diagnosis (AD). AC, 9.				Decreased in AP/AD group: synaptophysin, synaptotagmin, synaptopodin, neurogranin and GAP43.	Predictive biomarkers.	
Cross-sectional study: AD, 28 (aMCI, CDR 0.5; mild dementia, CDR 1.0); AC, 28.	Petersen 2004 criteria, NIA-AA criteria 2011; all patients have detected CSF $A\beta_{42}$ and tau.	Plasma NDEs; same as Goetzl et al., 2016b.	NPTX2, AMPA4, NLGN1, NRXN2α. ELISA.	Decreased.	Diagnostic biomarkers.	Goetzl, et al. (2018a)
Longitudinal study: probable mild-to-moderate dementia, 18, CDR 1.0. First blood collection: 6-11 years before diagnosis (AP); Second: diagnosis (AD). AC, 18.	revised NINCDS-ADRDA criteria 2007; not mentioned biomarkers.			Decreased in AP/AD group: AMPA4, NLGN1 and NRXN2α.	Predictive biomarkers.	
Cross-sectional study: AD, 26 (aMCI, 16, CDR 0.5; mild-to-moderate dementia, 10, CDR 1.0); AC, 26.	Petersen 2004 criteria, NIA-AA criteria 2011, revised NINCDS-ADRDA criteria 2007; all patients have detected CSF $A\beta_{42}$.	Plasma NDEs; 1 μg mouse anti-human L1CAM.	IRS-1, P-S312-IRS-1, P-panY-IRS-1. ELISA.	Increased: P-S312-IRS-1, R; Decreased: P-panY-IRS-1.	Diagnostic biomarkers.	Kapogiannis et al. (2015)
Longitudinal study: aMCI, 11; dementia, 11. First blood collection: 1-10 years before diagnosis (AP); Second: initial diagnosis (AD). AC, 22.				Increased in AP/AD group: P-S312-IRS-1 and R; Decreased: P-panY-IRS-1.	Predictive biomarkers.	
Cross-sectional study: AD, 26 (aMCI, 18, CDR 0.5; mild-to-moderate dementia, 8, CDR 1.0); AC, 26.	Petersen 2004 criteria, NIA-AA criteria 2011, revised NINCDS-ADRDA criteria 2007; some patients have detected CSF $A\beta_{42}$ and tau.	Plasma NDEs; 2 μg mouse anti-human L1CAM.	Ubiquitin, LAMP-1, HSF70, cathepsin D. ELISA.	Increased: Ubiquitin, LAMP-1, cathepsin D; Decreased: HSF70.	Diagnostic biomarkers.	Goetzl et al. (2015a)
Longitudinal study: dementia, 20. First blood collection: 1-10 years before diagnosis (AP); Second: initial diagnosis (AD). AC, 20.				Increased in AP/AD group: Ubiquitin, LAMP-1 and cathepsin D; Decreased: HSF70.	Predictive biomarkers.	
Cross-sectional study: AD, 28 (aMCI, CDR 0.5; mild dementia, CDR 1.0); AC, 28.	Petersen 2004 criteria, NIA-AA criteria 2011; all patients have detected CSF $A\beta_{42}$ and tau.	Plasma ADEs; 1.5 μg mouse anti-human GLAST.	IL-6, TNF-α, IL-1β, C1q, C4b, factor B, factor D, fragment Bb, C3b, C3d, C5b-C9 TCC, MBL, CR1, CD46, CD59, DAF, factor H, factor I. ELISA.	Increased: IL-6, TNF-α, IL-1β, C1q, C4b, factor B, factor D, fragment Bb, C3b, C3d, C5b-C9 TCC; Decreased: CR1, CD46, CD59, DAF.	Diagnostic biomarkers.	Goetzl et al. (2018b)
Longitudinal study: probable mild-to-moderate dementia, 16. First blood collection: 5-12 years before diagnosis (AP); Second: diagnosis (AD). AC, 16.	revised NINCDS-ADRDA criteria 2007; not mentioned biomarkers.			Decreased in AP/AD group: CD59 and DAF.	Predictive biomarkers.	
Mild-to-moderate AD-dementia, 20; Stable MCI, 20; Unstable MCI, 20; AC, 20.	NIA-AA criteria 2011; all participants have detected CSF $A\beta_{42}$.	Plasma ADEs; 1.5 μg mouse anti-human GLAST.	C1q, C4b, factor D, fragment Bb, C3b, C5b, C5b-C9 TCC, MBL, CR1, CD46, CD59, DAF. ELISA.	Increased in dementia and unstable MCI groups: C1q, C4b, factor D, fragment Bb, C3b, C5b and C5b-C9 TCC; Decreased: CR1, CD46, CD59, DAF.	Diagnostic and predictive biomarkers.	Winston et al. (2019)
Cross-sectional study: AD, 24 (aMCI, 16; dementia, 8); AC, 24.	Petersen 2004 criteria, NIA-AA criteria 2011, revised NINCDS-ADRDA criteria 2007; all patients have detected CSF $A\beta_{42}$ (emailed to the corresponding author).	Plasma NDEs; ? μg mouse anti-human L1CAM.	REST, LRP6, HSF1. ELISA.	All decreased.	Diagnostic biomarkers.	Goetzl et al. (2015b)
Longitudinal study: aMCI, 7; dementia, 9. First blood collection: 2-10 years before diagnosis (AP); Second: initial diagnosis (AD). AC, 16.				All decreased in AP/AD group.	Predictive biomarkers.	

Note: AD, Alzheimer's disease; aMCI, amnestic mild cognitive impairment; CDR, Clinical Dementia Rating; AC, AD case-controls; AP, preclinical; NIA-AA, National Institute of Aging-Alzheimer's Association; Aβ, β-amyloid protein; CSF, cerebrospinal fluid; NDEs, neuron-derived exosomes; L1CAM, L1 cell adhesion molecule; ELISA, enzyme-linked immunosorbent assay; ADEs, astrocyte-derived exosomes; GLAST, glutamine aspartate transporter; GAP43, growth-associated protein43; P-S9-synapsin1, phosphorylation of serine9 in synapsin1; NPTX2, neuronal pentraxin 2; AMPA4, GluA4-containing glutamate; NLGN1, neuroligin1; NRXN2α, Neurexins2α; IRS-1, insulin receptor substrate-1; P-S312-IRS-1, Phospho-serine 312-IRS-1; P-panY-IRS-1, Phospho-pan-tyrosine-IRS-1; R, P-S312-IRS-1/P-panY-IRS-1; LAMP-1, Lysosomal associated membrane protein 1; HSF, Heat shock factor; IL, interleukin; TNF, tumor necrosis factor; TCC, terminal complement complex; MBL, mannose-binding lectin; CR1, complement receptor type 1; DAF, decay-accelerating factor; REST, Repressor element 1-silencing transcription factor; LRP6, low-density lipoprotein receptor-related protein 6.

ADD patients compared with controls (Liu et al., 2014a; Liu and Yang, 2018; Yang et al., 2018). The expression of miR-29c, miR-193b and miR-384 can also distinguish MCI from dementia; in addition, miR-29c and miR-384 both were suitable biomarkers to discriminate AD, vascular dementia and PD with dementia. miR-223 was regarded as an inflammation-related miRNA, its expression in serum-derived exosomes performed higher abundances and concordances with that of serum, and the exosomal miR-223 levels in dementia (AD and vascular dementia) patients were downregulated compared to controls, correlating with serum concentrations of typical inflammatory factors and cognitive function scales and magnetic resonance spectroscopy spectral ratios (Wei et al., 2018). However, the authors did not specialize in AD patients, therefore the results need further verification.

CSF is another important source of miRNAs, yet few studies exist that are focused on CSF-derived exosomal miRNAs. In addition to serum-derived exosomal miR-193b, the CSF-derived exosomal miR-193b also had the ability to distinguish ADD patients from controls. Furthermore, its levels were negatively correlated with CSF $A\beta_{42}$ levels, indicating that it may predict clinical severity (Liu et al., 2014a). *Gui* et al. detected exosomal miRNAs in CSF by TaqMan miRNA arrays and found that levels of miR-29c, miR-136-3p, miR-16-2 and miR-331-5p were reduced, while those of miR-132-5p, miR-151 and miR-485-5p were increased in ADD patients compared to controls (Gui et al., 2015). Notably, the variation trends in miR-16-2, miR-331-5p, miR-151 and miR-136-3p in ADD were opposite of those in PD, suggesting the possibility of differentiating other types of dementia. It should be mentioned that invasive manipulation makes exosomes less convenient when used as biomarkers, but the future is still promising because CSF may be more representative than blood for the pathological changes in AD.

It is less likely to diagnose AD by using just a single exosomal miRNA; a set would be a better choice. Unfortunately, there is no direct evidence that exosomal miRNAs function as biomarkers in the preclinical stage of AD up to now.

4. Exosomal biomarkers reflect other pathological events of AD

The clinical syndrome of AD lags behind its pathological changes, and current evidence most strongly supports the amyloid cascade reaction hypothesis pathway (Jack et al., 2018); hence, we believe the subsequent pathological events all belong to the downstream manifestations of neurodegeneration or neuronal injury after Aβ deposition. Theoretically, these exosome-based biomarkers are not specific and are just indicators of outcome events attributed to AD or other conditions; however, they performed well in distinguishing FTD or PD and even changed their levels as early as the preclinical stage of AD, which is consistent with exosomal Aβ or phosphorylated tau. However, uncertainty still exists. For example, can exosome-based neurodegeneration evidence differentiate other neurodegenerative diseases or trauma? Does the degree of the alteration of these indicators correlate with the severity of cognition deterioration? The quest for more clinical evidence continues to be a great impetus for incorporating new biomarkers. Furthermore, the huge failure in developing sole-mechanism anti-AD drugs has forced us to focus on multitarget drugs, and exosomes may be novel candidates because they are involved in a variety of intricate and interrelated pathophysiological mechanisms of AD.

4.1. Exosomes and brain synaptic function

Synaptic dysfunction and degeneration are other pathological earmarks of AD (DeKosky and Scheff, 1990; Goetzl et al., 2018a; Lista and Hampel, 2017; Reddy and Beal, 2008). The severity of synaptic damage in the postmortem brain tissues of AD patients negatively correlates with the function of premortem cognition (DeKosky and Scheff, 1990). Synaptophysin, synaptotagmin, growth-associated protein 43 (GAP43), synapsin 1 and its variant with phosphorylation of serine 9 all are presynaptic proteins that regulate vesicle fusion, release or recycling (Sudhof, 2004; Südhof et al., 1989). Synaptopodin and neurogranin as postsynaptic proteins are important in monitoring intracellular calcium concentration (Reddy et al., 2005). In addition, neuronal pentraxin 2 (NPTX2), GluA4-containing glutamate (AMPA4), neurexins 2α (NRXN2α) and neuroligin 1 (NLGN1) also play vital roles in maintaining synaptic function (Chang et al., 2010; Südhof, 2008). Current evidence indicates that synapse markers may serve as mechanism-of-action biomarkers aiding in the in vivo investigation of AD-related pathological pathways (Lista and Hampel, 2017).

Few research efforts have been devoted to the exosome-based brain synaptic functions to date. Importantly, the levels of above 11 synapse-related markers in plasma NDEs were all reduced in AD-aMCI or ADD patients compared to controls (Goetzl et al., 2018a, b). Further analysis indicated that AD patients had lower levels of synaptophysin, synaptotagmin, GAP43, synaptopodin, neurogranin and synapsin 1 than FTD patients. In addition, it is remarkable that the levels of synaptophysin, NRXN2α and other markers were already downregulated as early as 1–10 years before dementia in cognitively intact patients (Table 3). Moreover, levels of exosomal synaptophysin, synaptotagmin, synaptopodin, AMPA4 and NLGN1, but not $A\beta_{42}$ or P-T181-tau, were inversely correlated with cognitive function scales. In addition, the decline in the amount of exosomal neurogranin in MCI patients suggested that they will progress to dementia within 36 months (Winston et al., 2016) (Table 1).

4.2. Exosomes and brain insulin resistance

The signaling pathways components of insulin, such as growth factor-1 receptor and insulin receptor, control important brain functions such as glucose uptake and energy metabolism. Many endeavors have been devoted to their signaling resistance in AD, which appears to be an early and common feature (Moloney et al., 2010; Talbot et al., 2012). Insulin receptor substrate-1 (IRS-1) serves as the effector molecule of the insulin receptor; phosphorylation of its several tyrosine residues will activate insulin signaling functions, whereas dephosphorylation and/or phosphorylation of some serine and threonine residues will lead to restrained signaling (Kapogiannis et al., 2015). Specific IRS-1 phosphorylation forms may serve as biomarkers of brain insulin resistance, having been found to be changed in the brain tissues of AD patients (Moloney et al., 2010; Talbot et al., 2012).

Little information is available related to exosomes and insulin resistance in AD. A study detected the plasma NDE levels of IRS-1, phospho-serine 312-IRS-1 (P-S312-IRS-1) and phospho-pan-tyrosine-IRS-1 (P-panY-IRS-1) of AD-aMCI or ADD patients and found that exosomal P-panY-IRS-1 levels were reduced, whereas the levels of P-S312-IRS-1 and insulin resistance index (R, P-S312-IRS-1/P-panY-IRS-1) were increased significantly, and IRS-1 was not changed in AD patients. Only R can distinguish AD patients from controls, FTD or diabetes patients simultaneously and effectively. There was no difference between AD-aMCI and ADD patients in any forms of IRS-1, but changes in exosomal P-S312-IRS-1, P-panY-IRS-1 and R have already happened 1–10 years before AD-induced cognitive decline (Kapogiannis et al., 2015) (Table 3). Notably, the increase in exosomal P-S312-IRS-1 or the decrease in P-panY-IRS-1 was associated with brain atrophy, indicating their detrimental and protective roles in AD pathogenesis, respectively (Mullins et al., 2017). However, a recent large longitudinal study performed by the same researchers found an opposite result for P-panY-IRS-1; they found that the P-panY-IRS-1 levels in nEVs were also increased in the preclinical stage, similar to those of P-S312-IRS-1 (Kapogiannis et al., 2019) (Table 1), and the authors attributed this surprising result to a difference in the tyrosine epitopes recognized by the detection antibody in different methods. The details of the study were introduced in the above Section 2.3. By using the same detection method, researchers found P-S312-IRS-1 and P-panY-IRS-1 responded to intranasal insulin therapy in the same direction in aMCI or ADD

patients (Mustapic et al., 2019).

4.3. Exosomes and the ubiquitin-proteasome or autophagic-lysosomal systems

AD and other proteinopathies are accompanied by an impaired function of impurity-clearing systems, resulting in the accumulation of insoluble protein species (Ihara et al., 2012). The plasma NDE levels of ubiquitin, lysosomal-associated membrane protein 1 and lysosomal proteolytic enzyme cathepsin D from AD-aMCI or ADD patients were all higher than those of controls or FTD patients. In the longitudinal study, the three indicators all exhibited vital differences between age-matched controls and preclinical-stage AD patients (Goetzl et al., 2015a) (Table 3). The capability of eliminating a growing burden of toxins and damaged organelles is indispensable to neuron survival, and these results suggested a compensatory mechanism indicating the early appearance of neuronal ubiquitin and lysosomal dysfunction in living AD patients.

4.4. Exosomes and neuroinflammation

In recent years, evidence from clinical and experimental studies has indicated that brain inflammation is an intrinsic feature of AD. The exposure of microglia or astrocytes to aggregated Aβ results in a continuous release of pro-inflammatory and complement factors; conversely, complement system activation continues to progress under the chronic low-grade inflammatory conditions of AD, driving a vicious cycle and leading to synaptic dysfunction and the aggravation of Aβ plaque deposition (Heneka et al., 2015). In vivo, the transfer of these astrocyte-released pro-inflammatory and complementary toxic mediators to neurons may involve exosomes. By extracting ADEs from plasma, researchers found that the levels of exosomal inflammatory cytokines (interleukin-6, tumor necrosis factor-α, interleukin-1β) and complement effector proteins (C1q, C4b, factor B, factor D, fragment Bb, C3b, C3d, C5b-C9 terminal complement complex) from AD-aMCI or ADD patients were both significantly higher than those of healthy controls, whereas membrane-associated complement regulatory proteins (complement receptor type 1, CD46, CD59, decay-accelerating factor) were lower (Goetzl et al., 2018b; Winston et al., 2019). In the longitudinal study, these complement proteins may be predictive biomarkers of MCI conversion to dementia within 36 months (Winston et al., 2019); furthermore, CD59 and decay-accelerating factor showed differential expression levels between preclinical-stage AD patients and age-matched controls, and they had already decreased 5–12 years before the appearance of cognitive impairment (Goetzl et al., 2018b) (Table 3). Although the possibility that astrocytes spread toxins through ADEs remains to be explored, the authors extended the neuroinflammation knowledge to a new level.

4.5. Exosomes and other pathological events

Repressor element 1-silencing transcription factor (REST) is a repressor of neuronal genes that is downregulated once terminal neuronal differentiation has occurred; it potently protects neurons from oxidative stress and Aβ toxicity (Lu et al., 2014). The low-density lipoprotein receptor-related protein 6 (LRP6) is a coreceptor for Wnt/β-catenin signaling, and its deficiency has been proven to lead to an exacerbated burden of Aβ and poor cognition (Liu et al., 2014b). The heat shock factor 1 (HSF1) and 70 (HSF70) proteins both maintain neuronal defenses and protect neurons from neuropathic protein aggregation and toxicity (Guo et al., 2016). Scientists have demonstrated that the levels of REST, LRP6, and HSF1 in the plasma NDEs of AD-aMCI or ADD patients were reduced compared to that of controls or FTD patients, and HSF70 was reduced compared to controls and was increased compared to FTD patients (Goetzl et al., 2015a, b). In addition, their expression levels have already declined by the preclinical stage of AD, making the diagnosis up to 10 years ahead of schedule (Table 3). For MCI patients, the decreased amount of REST means that the cognitive impairment will progress to dementia within 36 months (Winston et al., 2016) (Table 1). Glial cell-derived neurotrophic factor is an effective neurotrophic factor with therapeutic potential against a series of neurodegenerative conditions including AD; its amount in plasma ADEs from AD-aMCI or ADD patients has been found to be decreased compared to that in controls, but not in NDEs (Goetzl et al., 2016a) (Table 1). In addition, researchers have found that cognitively healthy subjects who eventually developed AD had higher nEV diameter than healthy controls, indicating the predictive biomarker possibility, while the two groups had a similar amount of nEVs containing tumor susceptibility gene 101 and nEV concentration (Kapogiannis et al., 2019) (Table 1).

YaXing et al. isolated CSF-derived exosomes and verified that their mRNA expression levels of APP, α-synuclein, DJ-1/PARK7, Fractalkine, light gene, neurofilament and long noncoding RNAs (RP11-462G22.1, PCA3) were all deregulated between ADD or PD patients and controls; however, there was no difference between the two types of patients. The exosomal tau mRNA levels were not changed in PD but were downregulated in ADD patients when compared to controls, suggesting the possibility of use as a biomarker (Gui et al., 2015) (Table 1). In addition to the previously mentioned CSF-derived $A\beta_{42}$-positive EV particles isolated by a flow cytometry method (Yang et al., 2015), the levels of apolipoprotein AI-positive and annexin V-positive particles were both reduced in MCI and ADD patients versus age-matched controls, while the apolipoprotein E-positive particle concentrations were not changed (Yang et al., 2015) (Table 1).

5. Conclusion and perspective

An urgent need still exists, but it is fundamentally a significant challenge to treat AD patients. The identification of individuals in the Alzheimer's continuum is becoming essential for targeting super-early secondary prevention clinical trials in AD. The exosome, as a new star in AD, has been receiving increasing attention from researchers. To some extent, exosomes function as Trojan horses, facilitating the accumulation and spread of AD-associated toxic cargos in the brain while enhancing intercellular communication. During the process, scientists hope to grasp some clues that can reflect the pathological changes of AD; thus, in this review, we summarized the recent literature regarding the possible biomarker roles of exosomes.

Though limited by invasiveness or high costs, we will still need to rely on CSF and PET markers of Aβ and tau proteins to diagnose AD for a long time to come. Under the circumstances, exosomes may best be used as a supplementary tool for screening AD patients. General neurology clinics or memory clinics in many countries usually receive a broad range of referrals covering various diseases; reasonable referral could have a substantial impact on health care utilization and costs. Then, the conveniently acquired exosome-based biomarkers can help primary care practitioners in deciding which patients should receive a referral. To date, most biomarkers have focused on diagnostic utility for specialty clinic settings, but patients there often miss the best treatment opportunity. Therefore, it is inspiring that the blood-based exosome test can advance the diagnosis of AD by 10 years; furthermore, a diagnostic model based on demographic information and nEVs (can also be called NDEs) biomarkers is highly sensitive and specific for identifying AD patients in the preclinical stage (Kapogiannis et al., 2019), and the levels of exosomal $A\beta_{42}$, T-tau, and P-T181-tau were highly correlated with that in CSF of symptomatic patients, which is worthy of verification in the preclinical stage (Jia et al., 2019). We suppose that the exosome-based diagnostic paradigm can make more at-risk or preclinical AD individuals acquire early intervention, reducing the incidence of dementia. However, there are still huge gaps in clinical application. The predictive utility and partial diagnostic utility were founded on the successful extraction of brain-derived exosomes from blood, though it has been proven that NDEs isolated by previously

reported immunochemical methods were enriched for numerous neuronal markers (Patterson et al., 2018; Pulliam et al., 2019; Sun et al., 2017), but the target antigens in the methods are not specifically unique to the brain as they are also expressed throughout the renal system (Pulliam et al., 2019; Allory et al., 2008). In addition, it should be noted that the immunocaptured exosomes may not be bona fide, as the ExoQuick is a polymer-based reagent that can coprecipitate nonexosomal contaminants including microvesicles and lipoproteins (Van Deun et al., 2014). In the future, other possible exosomal biomarkers should be screened, such as receptor for advanced glycation end products, high-mobility group box 1 and neurofilament light (Patterson et al., 2018; Sun et al., 2017), and we need an optimized and unified method to obtain exosomes and more clinical trials to verify the current results, determine the biomarker level boundaries, and, importantly, ensure whether there are intersections with other conditions and whether the biomarkers can truly function as liquid biopsy tools reflecting the pathological changes of AD by comparison with the current gold standard.

Some limitations of this review exist. First, *Goetzl* was involved in most of the researches (Goetzl et al., 2018a; Fiandaca et al., 2015; Kapogiannis et al., 2019; Winston et al., 2016; Goetzl et al., 2016a; Hamlett et al., 2017; Goetzl et al., 2016b; Kapogiannis et al., 2015; Goetzl et al., 2015a, b; Winston et al., 2019; Goetzl et al., 2015b), he and his collaborators, such as *Kapogiannis*, were highly commendable for their outstanding contributions in this field; in spite of their efforts to expand the source of participants, this still inevitably led to a relatively less extensive source and the repeated utilization of patients. In addition, the results were not validated in other more laboratories, and even the same research group arrived at different conclusions ($A\beta_{42}$ (Fiandaca et al., 2015; Kapogiannis et al., 2019), and P-panY-IRS-1 (Kapogiannis et al., 2019, 2015), in the preclinical stage) by using different experimental methods in patients from different sources. Second, almost all studies were small in scale except the latest one (Kapogiannis et al., 2019). Third, the diagnosis of AD patients or "healthy" controls in some studies was based solely on clinical criteria and was not supported by autopsy or accepted biomarkers. Fourth, a recent study found that memantine can influence the expression of exosomal miRNAs (Wei et al., 2018); therefore, it may not appropriate to put all patients in one group. Fifth, the exosomal miRNA-based biomarkers are poorly reproducible, and there was no study targeting the preclinical stage. Sixth, studies seldom considered the effect of comorbidity. However, with further understanding of the function of exosomes in specific cellular events, the continuous technological advances in accurate and cost-effective extraction and cargo detection show a very promising role for exosomes as novel biomarkers of AD.

Declaration of Competing Interest

On behalf of all authors, the corresponding author confirms no conflict of interest.

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