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REVIEW ARTICLE

Extracellular vesicles in neurodegenerative diseases: Insights and new perspectives

Qian Yuan [a], Xiao-dong Li [b], Si-miao Zhang [a], Hong-wei Wang [c], Yun-liang Wang [a,d,*]

[a] *Department of Neurology, The Second Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan Province, 450014, PR China*
[b] *Department of Neurology, Zhengzhou Central Hospital, Zhengzhou, Henan Province, 450014, PR China*
[c] *Department of Medicine, The University of Chicago, IL, 60637, USA*
[d] *Department of Neurology, The 960th Hospital of Chinese PLA, Zibo, Shandong Province, 255300, PR China*

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Abstract Extracellular vesicles (EVs) are vesicle-like substances released by eukaryotic cells. Based on their origin and size, EVs are mainly divided into exosomes, microvesicles and apoptotic bodies, and they are secreted by eukaryotic cells under physiological and pathological conditions. EVs are enriched with nucleic acids, proteins and other factors. EVs can regulate the function of adjacent and distant cells, and they are even involved in the pathogenesis of diseases. They contain proteins associated with the pathogenesis of neurodegenerative diseases (NDs), such as the α-synuclein (α-syn) and tau proteins, which suggest potential roles for EVs as biomarkers and carriers of drugs and other therapeutic molecules that can cross the blood–brain barrier to treat NDs. In this review, we summarized the function of EVs in the pathogenesis of different NDs and related advances in EVs as diagnostic biomarkers and treatments for diseases.

Neurodegenerative diseases (NDs) mainly include Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD), which are very serious diseases. AD and PD are also very common in elderly people. However, because the early clinical manifestations of these diseases are not typical,

* Corresponding author.
E-mail address: Wangyunliang81@163.com (Y.-l. Wang).
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NDs may easily be confused with many other diseases. Because of the lack of effective biological markers, early diagnosis of NDs is quite difficult. Furthermore, although much research has been carried out in the past few decades, and the pathogenesis of NDs has been more recognized, there are still no drugs or measurements that can cure or delay the development of diseases in clinical practice, which brings profound psychological pressure and economic burden to patients and families. Many recent studies have reported that extracellular vesicles (EVs) may be involved in the pathogenesis of NDs[1,2] and have potential as diagnostic biomarkers[3] and therapeutic measures.[4,5] This paper reviewed the research progress into the relationship between EVs and NDs.

Introduction of EVs

EVs arise either from late endosomes, which are typically referred to as exosomes (50—150 nm in diameter), or they bud directly from the plasma membrane (PM), in which case they are referred to as microvesicles (100—1000 nm in diameter) (Fig. 1).[6,7] In addition to these two main members of the EV family, other extracellular structures have been described, such as apoptotic bodies (100—5000).[7] So far, the most studied type is the exosomes.

The secretion of exosomes is complicated because the vesicle structure secreted by cells directly enters the external environment. The International Society for Extracellular Vesicles (ISEV) clearly assist in EV definitions, isolation methods, but a lack of standards remains widespread.[8—10] EVs in the size range of exosomes are not able to effectively separated from due to overlapping physical properties and biometrics of the two[7,11]; although many articles use the term "exosomes" referring to EVs preparations isolated by physical

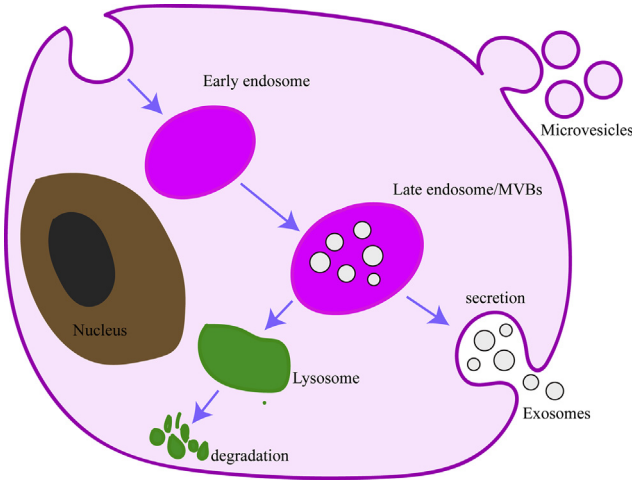

Figure 1 The release process of microvesicles and exosomes. Invagination of the plasma membrane form early endosome, followed by budding of payload into the endosomal membrane to form multivesicular endosomes (MVBs). MVBs can fuse with lysosome for degradation of their contents or fuse with the plasma membrane to release their intraluminal vesicles, then called exosomes. Microvesicles bud directly from the plasma membrane.

biological processes, they are likely referring to a mixture of the exosomes and non-exosome-like small EVs.[7]

EVs are rich in nucleic acids nucleic acids, proteins, and lipids.[11] To date, according to the EXOCARTA database (www.exocarta.org), 41,860 proteins, 3408 mRNAs, 2838 miRNAs, and 1116 lipids have been isolated from exosomes of different cell origins.

The important role for EVs in intercellular communication occurs via the transfer of proteins, lipids and nucleic acids, which has been confirmed in numerous studies.[12—15] EVs can also be isolated from various body fluids, such as the cerebrospinal fluid, blood, urine, and saliva.[8—10,16] Exosomes are very stable under different conditions and can protect "bio-goods (such as proteins or miRNAs)" from degradation and denaturation in the extracellular environment.[17] Increasing evidence has shown that they can function as biomarkers for NDs and that they are more reliable than biological fluids, such as pure cerebrospinal fluid, blood, or urine.[18] Finally, increasing evidence shows that EVs can also cross the blood—brain barrier,[19—21] and their characteristic low immunogenicity lays a theoretical foundation for them to function as biomarkers for diseases and "drug" delivery vehicles for the treatment of NDs.

Roles of EVs in the central nervous system

In the central nervous system (CNS), EVs are secreted by many cells, such as dendritic cells, neurons, oligodendrocytes, and astrocytes.[22] Emerging evidence indicates that EVs are key players in the intercellular communication that underlies physiological processes, such as synaptic plasticity and the maintenance of myelination.[23,24] Furthermore, upon injury to the CNS, EVs may propagate inflammation across the blood—brain barrier and beyond, and they also appear to mediate neuroprotection and modulate regenerative processes.[23] For example, Kramer-Albers and colleagues[25] show that functional EVs can be transmitted bidirectionally between oligodendrocytes and neurons; furthermore, the results indicate that oligodendrocyte-derived exosomes mediate neuroprotective functions. Moreover, the cargo delivered by exosomes includes a number of enzymes with metabolic functions, including catalase and superoxide dismutase-1, which can help neurons resist oxidative stress. Consistently, neurons exposed to oxidative stress or other harmful conditions survived better if they had been treated with oligodendroglial exosomes, suggesting that exosomes are protective and can increase the neuronal stress tolerance.[25,26] Xu et al[27] reported that exosomes transfer neuronal miR-132 from neurons to endothelial cells (ECs) to regulate the integrity of brain vasculature. Perez-Gonzalez et al[28] demonstrated that EVs containing CysC protect cultured cells from starvation-induced death. Unfortunately, EVs also have somewhat neurotoxic effects, and several studies have shown that EVs can promote the spread of proteins associated with the pathogenesis of NDs in the CNS.[29] Microglia-derived EVs carry bioactive lipids, including endocannabinoids, which acutely impact the neuronal firing rate.[30—32]

EVs and occurrence of NDs

EVs and occurrence of AD

Senile plaques and neurofibrillary tangles (NFTs) are two specific neuropathological features of AD. Senile plaques are mainly composed of deposited amyloid β-protein (Aβ), while NFTs are mainly composed of hyperphosphorylated tau protein. According to current views, the accumulation of Aβ and tau proteins is toxic to neurons, but exosomes can mediate the diffusion of these toxic proteins among AD neurons.[17] Yakama et al[33] confirmed that exosomes can release AD-related proteins and peptides, namely, amyloid precursor protein (APP), APP C-terminal fragment, or APP intracellular domain, to the outside of cells. In addition, other research groups have found exosomal marker proteins in amyloid plaques deposited in mouse brain tissue and the brains of postmortem AD patients, indicating that exosomes have transported Aβ to plaques during the pathogenesis of AD.[34] Furthermore, Zheng[35] observed in an AD model that plasma exosomes aggregate around Aβ, and these findings provide a possible interpretation for the extracellular amyloid deposition in the brain of AD patients. It is reported that the neurotoxicity of exosomes may be due to exosome lipids promoting the formation of soluble Aβ out of extracellular insoluble Aβ. Aβ is internalized by microglia and then sorted into exosomes, in which it exists as the toxic form of Aβ.[35] In addition to the release and aggregation of Aβ, exosomes are also associated with tau in the pathogenesis of AD. Studies have found that microglia can diffuse tau by releasing tau in exosomes, and microglia can significantly reduce the synthesis of exosomes, resulting in significantly reduced abnormal aggregation of tau proteins *in vitro* and *in vivo*. These studies indicate that exosomes contribute to the spread of tau protein.[36] The neurotoxic effects of exosomes on AD patients are related not only to pathological proteins but also probably to the fact that exosomes can impair the neuronal function during pathological progression of AD by other means; for example, AD patient exosome contents induce neuronal apoptosis in AD models. In 2012, Wang[37] performed a series of experiments and demonstrated that Aβ induces the secretion of exosomes containing ceramide and proapoptotic response factor-4 (PAR-4) through the neutral sphingomyelinase 2 (nSMase2)-dependent pathway, leading to the apoptosis of astrocytes; Eitan[38] found that EVs may also cause dysregulation of neuronal Ca^{2+} and impair mitochondrial function, leading to neurons' vulnerability to excitotoxicity and further damage. All of the above data illustrate the neurotoxicity of exosomes. On the other hand, EVs also have neuroprotective effects in AD. Yuyama[39] added a mixture of exosomes and Aβ to primary cortical cells, which then significantly inhibited oligomerization and the resultant toxicity, revealing the ability of exosomes to capture Aβ and promote its clearance from microglia. Correspondingly, there is also *in vivo* evidence that exosomes derived from mouse neuroblastoma N2a cells or human cerebrospinal fluid can eliminate the neurotoxicity caused by Aβ derived from the brains of patients with AD,[33] but they believe that this protective effect was due to the exosome proteins; the proteins can interfere with the assembly of toxic Aβ rather hydrolyze the Aβ protein.[33] Finally, cystatin C, a protein thought to be neuroprotective against AD, has been shown to secrete mouse primary neurons out of the cell through the exosomes[40]; immunoproteomics analysis has revealed the presence of at least 9 different cystatin C glycosylation forms in exosomes. In addition, the overexpression of familial AD-associated presenilin gene mutations can result in decreased levels of all cystatin C forms (natural and glycosylated) and exosomal APP metabolites.[40] In conclusion, despite all of this evidence, the pathogenic or protective effects of exosomes have not been totally confirmed in AD patients, and whether the dual role of exosomes is ubiquitous in all exosomes and to what extent can they promote or prevent the clearance of Aβ peptides remains controversial. To date, there is no marker information on exosomes derived from different neuronal cell types; further research is needed to solve these problems step by step.

EVs and occurrence of PD

Due to the central role of α-synuclein (α-syn) in sporadic PD, the potential association of α-syn with exosomes has begun to attract attention. However, the pathogenesis of PD is related not only to α-syn but also to the DJ-1 protein and leucine-rich repeat kinase 2 (LRRK2) protein; researchers have also identified and studied them. A number of studies have shown that neuron-derived exosomes transfer α-syn between neurons and nonneuronal cells (such as astrocytes and microglia), thereby contributing to the diffusion of PD. The deposition of α-syn in glial cells induces inflammation, which can be further transmitted to other glial cells and neurons.[41,42] Tofaris[43] suggested that when the lysosomal function is inhibited, the amount of exosome-released α-syn increases, and the exosomes promote the uptake of α-syn by recipient cells; then, these exosome-associated α-syn oligomers are more likely to be absorbed by recipient cells than free α-syn, which results in toxicity. The above evidence indicate that the exosomes promote the transmission and aggregation of α-syn, but the exact trigger mechanism for the incorporation of α-syn into exosomes or for its release remain unclear. Further, Chang et al[44] once reported that α-syn was capable of inducing secretion of exosomes from microglia, which are rich in major histocompatibility complex (MHC II), tumor necrosis factor (TNF), and proinflammatory cytokines, and the exosomes induced neuronal apoptosis in a TNF-dependent manner. Whether the exosomes have neuroprotective effects has not yet been confirmed. Because the role of exosomes in PD-related brain inflammation has not been well known, further research in this area should be performed.

EVs and occurrence of HD

HD is a neurodegenerative disease that mainly affects motor function. It is an autosomal dominant hereditary disease caused by expanded repeats of the CAG base sequence in the *IT15* gene. The repeated CAG sequence occurs in a gene that encodes a polyglutamine sequence (PolyQ); the number of repeats is less than 35 in healthy individuals and more than 37 in patients. Its pathogenic gene (*IT15*) encodes a protein with a relative molecular weight of 350,000Da, consisting of 3144 amino acids, and is

named mutant Huntingtin (mHtt). The main pathological change observed in HD is the appearance of inclusion bodies and aggregates formed by mHtt in the neurons of the central nervous system. Zhang[45] used human embryonic kidney 293T cells as donor cells for EVs in an experiment and found that the released EVs contained polyglutamine (polyQ) and RNA with CAG repeats, indicating that EVs have the potential to transmit the repeated RNA of a toxically amplified trinucleotide from one cell to another. These EVs may be used as biomarkers for disease states and responses to treatment, but more research is needed to confirm this.

EVs and occurrence of ALS

ALS is a fatal ND characterized by progressive muscle paralysis that is caused by motor neuron degeneration. ALS is accompanied by the accumulation of pathological protein superoxide dismutase-1 (SOD1), TAR DNA binding protein-43 (TDP- 43), and sarcoma fusion transporter (FUS), which interferes with neuronal function and ultimately leads to cell death. The pathogenesis of ALS mainly includes abnormal aggregation of proteins, mitochondrial dysfunction, glutamate excitotoxicity, oxidative stress, and neuroinflammation.[46] Recent studies have shown that peripheral blood mononuclear cells also play an important role in the pathogenesis of ALS.[47] Therefore, some researchers[48] stimulated $CD14^{++}$ monocytes with exosomes isolated from the serum of ALS patients and interestingly found that the mononuclear cells showed an obvious inflammatory reaction; further, this change was not observed in the control group. This indicates that exosomes may play a role in promoting the ALS inflammatory response; many studies have also confirmed that exosomes can transport proteins associated with the pathogenesis of ALS,[49] but fluid tissues have been researched more than solid tissues. Therefore, Silverman[2] once isolated the exosomes from the frozen brain tissue of ALS mice and found that CNS-derived EVs contained pathogenic ALS proteins; further, the astrocytes and neurons, instead of the microglia, were the main source of EVs, which confirmed that exosomes are involved in the transmission of toxic proteins during ALS progression. It is possible that the exosomes from different sources have different effects on the pathogenesis of ALS, but further development of techniques for identifying exosomes from different sources are needed to address this issue.

EVs and diagnosis of NDs (Table .1)

EVs and diagnosis of AD

Exosomes have the potential to be diagnostic markers for AD. Fiandacaf[50] measured the levels of total tau protein, two versions of phosphorylated tau protein (P-T181-tau and P—S396-tau), and Aβ1-42 in neuron-derived exosomes isolated from the blood of AD patients; the research showed that the expression levels of P—S396-tau, P-T181-tau, and Aβ1-42 were significantly higher 1—10 years before the diagnosis of AD, and the blood exosomal level of Aβ1-42 continued to increase from the preclinical stage to the diagnosis of AD. These results suggest that the above three proteins have the potential to be AD biomarkers; it is also speculated that exosome-derived Aβ 1—42 may be the biomarkers for monitoring disease progression. Later, Winston[51] performed a similar study and confirmed that during patient progress from mild cognitive impairment (MCI) to dementia, the levels of the above three markers increased; they also found that Neurogranin (NRGN) and neuron-restricted silencing factor (REST) were elevated, so these five indicators can be used to accurately predict the

Table 1 The biomarkers in NDs.

Diseases	Source	Biomarker	Reference
AD	plasma exosomes	P-T181-tau, P—S396-tau, Aβ1-42	50,51
	plasma exosomes	NRGN, REST	51
	plasma exosomes	IRS-1, P-IRS-1	52
	plasma exosomes	cathepsin D, LAMP-1, ubiquitinylated proteins, heat-shock protein 70	53
	plasma exosomes	neurexin 2α, GluA4-containing glutamate receptor, neuroligin 1, neuronal pentraxin 2	54
	CSF exosomes	miR-193b	55
	plasma exosomes	miR-23b-3p, miR-24-3p, miR-29b-3p, miR-125b-5p, miR-138-5p, miR-139-5p, miR-141-3p, miR-150-5p, miR-152-3p, miR-185-5p, miR-338-3p, miR-342-3p, miR-342-5p, miR-548at-5p, miR-659-5p, miR-3065-5p, miR-3613-3p, miR-3916, miR-4772-3p, miR-5001-3p	56
PD	CSF exosomes	α-synuclein	58
	plasma exosomes	α-synuclein	62
	plasma exosomes	tau	62
	salivary EVs	α-synuclein	64
	urine exosomes	DJ-1	65
	urine exosomes	Ser(P)-1292 LRRK2	66
	urine exosomes	ratio of phosphorylated Ser-1292 LRRK2 to total LRRK2	67
	CSF exosomes	miR-153, miR-409-3p, miR-10a-5p, let-7g-3p	68
	serum microvesicles.	miR-19b, miR-24 miR-195	69
ALS	serum exosomes	miR-27a-3p	74

transformation of MCI to AD dementia; other studies have suggested that neuronal-derived AD pathogenesis-associated exosomes may carry proteins that have the potential to become AD biomarkers, such as phosphorylated insulin receptor 1 protein,[52] cysteine D (cathepsin D),[53] and synaptic protein (Synaptic protein).[54]

In addition to proteins, miRNAs have been shown to be biomarkers in several studies. In 2014, Liu et al[55] showed that miR-193b may bind to the 3′-untranslated region of APP and inhibit the expression of APP-related mRNA and protein, suggesting that miR-193b may be involved in the progression of NDs. Compared with MCI and the control group, in AD patients, the expression levels of miR-193b in the exosomes of cerebrospinal fluid and blood were lower, demonstrating that exosome miR-193b is a potentially unique biomarker of AD; thereafter, Lugli et al[56] used Illumina deep sequencing and differential centrifugation to separate the plasma exosomes and identify the miRNAs expressed by the exosomes. There were 20 miRNAs that were significantly changed in the AD group (miR-23b-3p, miR-24-3p, miR-29b-3p, miR-125b-5p, miR-138-5p, miR-139-5p, miR-141-3p, miR-150-5p, miR-152-3p, miR-185-5p, miR-338- 3p, miR-342-3p, miR-342-5p, miR-548at-5p, miR-659-5p, miR-3065-5p, miR-3613-3p, miR-3916, miR-4772-3p, and miR-5001-3p); these miRNAs are expected to be diagnostic markers for AD. However, there are conflicting reports about these miRNAs, which is probably due to different separation and identification techniques. Research on different cell sources and different uses may require tissue-specific exosome markers, and further studies are needed in the future.

EVs and diagnosis of PD

Wang[57] suggested that the level of leucine-rich repeat kinase 2 (LRRK2) protein in CSF exosomes is not related to the diagnosis of PD; Stuendl[58] measured the α-syn content in the CSF exosomes of PD patients. The results showed that the α-syn content in exosomes from PD patients was lower than that in the control group, suggesting that α-syn be used as a biomarker for PD patients. Blood can be easily obtained than CSF, so blood is also being studied by many people. Because peripheral cells, especially red blood cells and platelets, can produce abundant a-syn,[59] they cannot be directly used as diagnostic markers.[60] Therefore, some people have studied α-syn in the exosomes from plasma patients with PD.[61] The comparison of dozens of PD patients without GABI gene mutations with a control group revealed that the level of α-syn in plasma exosomes was significantly higher in the PD group than in the control group, and the ratio of plasma exosome α-syn to plasma total α-syn was found to be inversely related to the severity of the disease. Later, Shi collected a few hundred samples and found that the α-syn content in plasma exosomes from PD patients was significantly higher than that in the control group,[62] which confirms that the plasma exosome α-syn is likely to be a useful marker for later PD diagnosis; then, Shi applied a similar method to detect the exogenous tau content of CNS-derived plasma exosomes. Surprisingly, tau in plasma exosomes of PD patients was also found to have the potential to be a biomarker.[63] In general, CSF, plasma exosome α-syn, and plasma exogenous tau protein may become clinically significant biomarkers.

Because saliva collection is simple and noninvasive, in 2018, Cao[64] measured the content of α-syn in EVs from the saliva collected from dozens of patients with PD and a control group. The results showed that the content of oligomeric α-syn and the ratio of oligomeric α-syn to total α-syn can be used as diagnostic markers for PD. However, this study has some limitations, such as a small sample size. There are few studies on salivary exosomes as diagnostic markers for PD, and the diagnosis of PD by Parkinson's disease-associated salivary exosomes remains to be further studied.

Urine is another example of an accessible biological fluid. One study[65] reported the detection of PD-related proteins, DJ-1 and LRRK2, in urine exosomes, of which DJ-1 showed significant sex differences. The level of DJ-I in urine exosomes was significantly higher in male PD patients than in the control group. This study showed that DJ-1 could be used as a marker for the diagnosis of male PD patients, but LRRK2 does not show a difference between the two groups. However, in another study, Fraser[66] reported sex differences in phosphorylated LRRK2 (Ser(P)-1292 LRRK2) levels in urinary exosomes, which shows that in patients with idiopathic PD, the level of Ser(P)-1292 LRRK2 in male exosomes is higher than that in female exosomes, and the level of Ser(P)-1292 LRRK2 was positively correlated with the severity of the disease. In the same year, these authors focused on familial PD and found that in LRRK2 mutation carriers, the ratio of Ser(P)-1292 LRRK2 to total LRRK2 was related to the risk of PD. The higher the ratio was, the higher the risk of PD. In the future, more research is expected to focus on whether lowering the ratio can reduce the incidence risk of PD.[67] In 2017, Wang[57] showed that Ser(P)-1292 LRRK2 in urine exosomes may have a diagnostic effect on male PD. In general, the levels of DJ-1 and Ser(P)-1292 LRRK2 in urine exosomes have the potential to diagnose PD, and Ser(P)-1292 LRRK2 may also have the ability to monitor the progression and prognosis of the disease.

In studies of miRNAs in exosomes from PD patients, CSF exosomal miRNAs have been identified, and the results reveal that miR-1 and miR-19b-3p are significantly downregulated, while miR-153, miR-409-3p, and miR-10a-5p are upregulated significantly. These 6 miRNAs are expected to serve as diagnostic markers for PD.[68] Another study collected plasma from 109 PD patients and 40 normal controls and identified 24 miRNAs (miR-24, mi R-30a-3p, mi R-30e-3p, miR-195, miR-223, miR -324-3p, miR-331-5p, miR-338-3p, miR-505, miR-626, miR-15b, miR-162-3p, miR-19a, miR-19b, miR-29a, miR-29c, miR-30c, miR-148b, miR-181a, miR-185, miR-221, miR-339-5p, miR-450b-3p, and miR-1294); the results show that miR-24 and miR-195 are expressed at higher levels than the control group, while miR-19b expression is low. It is thought that miR-24, miR-195, and miR-19b have potential as diagnostic markers for PD.[69] This evidence demonstrates the potential value of cerebrospinal fluid and plasma exosome miRNAs in the diagnosis and assessment of PD. Among the miRNAs, miR-153 has been shown to bind to the 3′-untranslated region of α-syn and downregulate its mRNA and protein levels, thus participating in the pathogenesis of PD[70]; in addition,

it is also confirmed that the pathogenic role of miR-7 in PD has a similar effect as that caused by miR-153,[71] but the above potential markers do not overlap, and large-sample control studies are needed in the future to verify the roles and diagnostic utilities of the above miRNAs.

EVs and diagnosis of HD

Studies have shown that in all types of blood cells, the platelets contain the highest concentration of mHtt,[72] so Denis[73] collected samples from 59 HD patients and 54 control groups; however, no HTT was detected in platelet-derived EVs, and the correlation analysis also failed to reveal any association between the number of platelet-derived EVs and patients' age, CAG repeats, total exercise score of unified Huntington's disease rating scale, total function score, or disease burden score. Therefore, platelet-derived EVs from HD patients are not valuable biomarkers for HD. However, there are few studies on exosomes as HD biomarkers, so more research is needed to find better circulating biomarkers in the blood.

EVs and diagnosis of ALS

There are studies using exosomes as biomarkers. Xu[74] measured the expression of miR-27a-3p in serum exosomes from 10 ALS patients and healthy subjects and found that miR-27a-3p is downregulated in patients with ALS; therefore, miR-27a-3p is thought to be a biomarker for ALS. In addition to miRNAs, Otake[75] recently suggested that exosome mRNAs and genes may also be biomarkers of diseases. In general, there are few studies on ALS biomarkers. This may be due to the small number of ALS cases in general. In the future, large-sample multiple-center studies are needed to verify the above results and transition into clinical application as soon as possible.

EVs and treatment of NDs

EVs and treatment of AD

Exosomes are also attractive candidates as therapeutic delivery vehicles for NDs. In 2011, Alvarez[20] applied exogenous small interfering RNA (siRNA) to Lamp2b protein (an exosome membrane protein fused to a neuron-specific rabies virus glycoprotein)-expressible dendritic cell-derived exosomes by electroporation. Intravenously injected exosomes targeted the specific transmission of siRNA to neurons, microglia, and oligodendrocytes in the brain, and BACE1 gene knockout was observed; moreover, both BACE1 mRNA (60%) and protein (62%) were downregulated. Recently, Li[76] revealed a new method of loading therapeutic substances into exosomes, which was mediated by fusing the exosomal membrane protein CD9 with an RNA-binding protein with high affinity for miR-155. The fused CD9-RNA enriched miR-155 in the exosomes, and the encapsulated miR-155 was efficiently delivered to recipient cells, where it recognized endogenous targets. This method can also be designed to enable exosome enrichment with CRISPR/dCas9. These studies have laid the foundation for the use of exosomes to treat NDs in the clinic.

Wang et al found that exosomes carrying curcumin inhibited tau phosphorylation, holding great potential for improving targeted drug delivery and the recovery of neuronal function in AD therapy.[21]

EVs and treatment of PD

In terms of treatment, Hall et al[77] integrated a therapeutic PD protein catalase into exosomes. When activated macrophages were treated with these exosomes, the levels of reactive oxygen species in treated macrophages decreased, which was confirmed later in C57BL/6 mice. Other research groups also loaded glial cell-derived neurotrophic factor (GDNF) into exosomes and introduced them to a 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)-induced monkey PD model; the results confirm that exosome-mediated GDNF delivery has a strong neuroprotective effect.[78] All of these findings indicate that exosomes are good carriers for delivering drugs to the CNS, but more research is needed in the future on the delivery of therapeutic drugs such as siRNAs and miRNAs and on the development of instruments or methods for engineering exosomes.

EVs and treatment of HD

Lee[79] injected exosomes from adipose-derived stem cells (ASCs) into R6/2 mice and observed reduced mHtt aggregates in R6/2 mouse neurons. These exosomes also appear to improve abnormal apoptotic protein levels and reduce mitochondrial dysfunction and apoptosis in an *in vitro* HD model, but this study did not clarify which substances in the exosomes provided these protective effects. Didiot[80] coincubated Cy3-labeled hydrophobically modified small interfering RNA (Cy3-h siRNA) with exosomes to produce Cy3-h siRNA-carrying exosomes and then injected the exosomes into the unilateral corpus striatum of mice. It is exciting to observe the silencing of bilateral target mRNA. The widespread distribution and efficacy of siRNA-carrying exosomes is expected to advance the development of therapeutic approaches for the treatment of HD and other NDs. Subsequently, human exosomal miRNAs have been explored in research. Lee[81] transfected a miR-124 expression vector into HEK293 cells to generate a cell line stably expressing miR-124 and confirmed the miR-124 expression by qPCR. Then, the HEK293 cells overexpressing miR-124 were cultured in exosome-free Dulbecco's modified Eagle's medium, and the exosomes inside were isolated from the culture medium. The EXO-124 exosomes were injected into the bilateral corpus striatum of 6-week-old R6/2-type transgenic HD mice. The results showed that the expression of target gene was reduced. Although this treatment did not reduce the motor symptoms of mice, it showed the feasibility of using exosomes to deliver miRNA to the brain for the treatment of degenerative diseases. Current studies have provided evidence for the delivery of exosome-based siRNAs and miRNAs to the brain. To further validate the possibility of exosomes as a neurodegenerative drug carrier, more research is needed.

EVs and treatment of ALS

Studies have also applied exosomes to the treatment of ALS. Lee[82] found that exosomes released from ADSCs could reduce the accumulation of mutant SOD1 in G93A neurons. This is further validated by Bonafede,[83] who found that exosomes secreted by mouse ADSCs (0.2 μg/ml) could protect NSC-34 cells from oxidative damage and increase cell viability, thereby protecting the cells. These findings indicate that exosomes released by ADSCs have therapeutic potential against ALS, but further research is needed on whether these methods can be used for clinical treatment.

Summary and outlook

The field of EVs research is an emerging and rapidly developing field, and in recent years, it has received increasing attention from researchers. Over the past 10 years, EVs have evolved from the initial characterization as the "trash can" of cells into a key player in many biological processes in the physiological and pathological environment of the body. EVs play an important role in the pathogenesis, diagnosis, and treatment of NDs, but research on EVs still requires a great deal of technical development. First, the extraction process of EVs must be simple, fast, and inexpensive. Preanalytical procedures should be used to maintain the structural and molecular integrity of EVs; second, gold standards for instrumentation and analysis of exosome-associated biomarkers are urgently needed. Moreover, proteomics combined with other histological screenings, such as lipidomics, metabolomics, or transcriptomics, may quickly identify useful candidate biomarkers for validation in a wide range of clinical studies. Standard operating procedures for the collection, storage, processing, and analysis of EVs are still urgently needed. Although EVs have been shown to have the ability to spread diseases, it is believed that techniques related to EVs can still be used to benefit patients.

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Conflict of Interests

The authors declare no conflict of interests.

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