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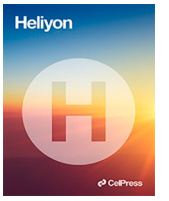

Review article

The role of exosomes in the diagnosis of Parkinson's disease

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ABSTRACT

Parkinson's disease is a common neurodegenerative disease characterized by intracellular aggregation of misfolded α-synuclein as a major pathological hallmark. Exosomes are cell-derived lipid bilayer membrane vesicles with various components, including proteins, RNA, and lipids, that mediate intercellular communication. Currently, exosomes are found to be responsible for transporting misfolded proteins from unhealthy neurons to nearby cells, spreading the disease from cell to cell. Such exosomes can also be found in the cerebrospinal fluid and blood. Thus, exosomes may serve as a potential tool to detect the pathology of Parkinson's disease for clinical diagnosis. In this article, the role and challenges of exosomes in the diagnosis of Parkinson's disease are outlined.

1. Introduction

Parkinson's disease (PD) is a common neurodegenerative disease. Global estimates in 2020 show that more than 9.4 million individuals living with PD, higher than the 6 million people previously reported in 2016, including China 2.7 million [1,2]. And since 1990, the national morbidity, mortality, and disability-adjusted life-years (DALYs) of PD have been increasing [3]. The rising prevalence of PD has increased the burden on families and society and has caused widespread concern. Environmental factors, such as exposure to pesticides and other environmental chemicals, high intake of dairy products, are associated with an increased risk of PD, while smoking, caffeine intake, physical exercise, and use of some drugs such as ibuprofen and statins were associated with a reduced risk [4]. Genetic factors also play a role in the pathogenesis of PD, *LRRK2*, *SNCA*, *Parkin*, *PINK1*, and *DJ1*, as well as *GBA* are the major causative genes [5].

The death of dopaminergic (DA) neurons in the substantia nigra compacta (SNc) and the aggregation of misfolded α-synuclein (α-syn) in the cytoplasm of residual neurons in the substantia nigra are two of the characteristic hallmarks of PD [6]. Braak proposed that pathology of PD could be divided into six stages. During stages 1 and 2, also called the presymptomatic stage, misfolded α-syn confined to the olfactory bulb, medulla oblongata, and pontine tegmentum. In stages 3 and 4, the substantia nigra and other nuclear grays of the midbrain and basal forebrain are affected and progressively aggravated. At this point the corresponding motor symptoms are already present. In stages 5 and 6, the pathological process invades the telencephalic cortex [7]. While symptoms of PD are divided into motor and non-motor symptoms. The main motor symptoms are bradykinesia, tremor, stiffness, and postural instability. Non-motor symptoms are olfactory impairment, constipation, rapid eye movement sleep behavior disorder (RBD), dementia,

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depression, and anxiety [8]. Based on the Braak hypothesis, these non-motor symptoms can precede the motor symptoms in PD, because affected brain regions in the early stages, such as dorsal vagal nucleus, locus coeruleus, and olfactory bulb, are associated with these non-motor symptoms, which may predict future risk of PD [9].

Early symptoms of PD are uncharacteristic, and the overlapping of early symptoms of atypical Parkinsonian syndromes (APS), including multiple systems atrophy (MSA), dementia with Lewy bodies (DLB), progressive supranuclear palsy (PSP) corticobasal degeneration (CBD) frontotemporal dementia (FTD) and some rare disorders, making a definitive diagnosis difficult. According to the diagnostic criteria established by the International Parkinson's and Movement Disorders Association, motor symptoms are the core feature of the PD, defined as bradykinesia combined with rest tremor or rigidity, and at least two supportive criteria, such as significant beneficial response to dopaminergic treatment and presence of levodopa-induced dyskinesia, and without red flags, such as severe autonomic failure in the first 5 years of disease [10]. But when classic motor symptoms are present, the mortality of dopaminergic neurons in the substantia nigra is already more than 50 % and DA concentrations in the striatum are already below 80 % [11]. In addition, imaging is also utilized to make a clinical aided diagnosis of PD. A systematic review analysis showed that single-photon emission computed tomography (SPECT) and positron emission tomography (PET) can confirm the presence of dopaminergic neurodegeneration and be used as a clinical support diagnosis, but they cannot reveal the preceding disease process [12]. Another systematic review analysis revealed that fMRI and ^{18}F-FDG PET may be able to predict brain alterations in prodromal PD, but the results are not very compelling due to inconsistencies between studies [13]. Thus, there is no imaging biomarker suitable for a definitive early diagnosis of PD [14]. Braak suggested that the pathogenesis of PD may be first initiated in the olfactory structures and the gut enteric nerves, spreading to the substantia nigra after years or even decades, causing dopaminergic neuron death and PD motor dysfunction [15]. This implies that pathological changes precede the appearance of motor symptoms and that by the time PD is definitively diagnosed, disease progression is already severe. Therefore, it is important to develop a new biomarker capable of early detection of PD. We need biomarkers that can reflect early pathological changes in PD to help us perform early diagnosis. Based on this view, exosomes released by specific neuronal cells become promising potential biomarkers. Recently, exosomes were found to be involved in interneuronal and neuron–glia communication, and carry misfolded α-syn from the parent cells, spreading the disease from cell to cell [16,17]. Several studies have shown that Central Nervous System (CNS)-derived exosomes are free to cross the blood-brain barrier (BBB) [18] and can be isolated from serum or plasma by specific neural markers, thus providing a "window" for detecting brain pathology, which holds promise for non-invasive analysis of brain biomarkers [19]. This offered a new option for the early diagnosis of PD.

2. Exosome

Exosomes are small intracellular membrane-based vesicles, which have a size ranging from 40 nm to 100 nm and serve as vehicles to carry different types of cellular cargo such as proteins, lipids and nucleic acids to the recipient cells [20]. Exosomes are generated from multivesicular bodies (MVBs). The invagination of the cell membrane forms a cup-like structure that encapsulates cell surface proteins and soluble proteins associated with the extracellular environment. These vesicles fuse to form early sorting endosomes, which gradually become late sorting endosomes. The invagination of late sorting endosomal membranes produces many small vesicles, which are called intraluminal vesicles (ILVs). At this time, these late sorting endosomes are known as MVBs. Then the MVBs can fuse with the plasma membrane to release the ILVs as exosomes or fuse with lysosomes for degradation [21]. Exosomes are secreted by all cell types, such as neurons, mesenchymal stem cells (MSC) and immune cells, and can be found in most body fluids, including blood, Cerebrospinal fluid (CSF), saliva, and urine [22].

Exosomes contain various cargoes including specific lipids, proteins and genetic material, such as DNA and RNA [23]. Lipids are the important components of exosomes, not only for their structural role in the exosome membrane, but also as crucial players in the formation and release of exosomes into the extracellular environment. Lipids in exosomes have a differential distribution between the inner and outer membranes. It is reported that sphingolipids and phosphatidylcholine are mainly found in the outer membrane, while other lipids are mainly present in the inner membrane [24]. The protein components of exosome are primarily divided into two categories, one is the common components, the other is specific components. The common components mainly include heat shock protein 70 (HSP70), heat shock protein 90 (HSP90), CD63, CD81, tumor susceptibility gene 101 (Tsg101), ALG2-interacting protein X (Alix), membrane transport and fusion-related proteins (Rab, GTPases), which are involved in the process of exosome formation and secretion [25]. The specific components are tightly linked to their parent cells. For example, mast cell-derived exosomes expressing MHC-II, CD86, lymphocyte function-associated antigen 1 (LFA-1) and intercellular adhesion molecule 1 (ICAM-1) induce the proliferation of B and T cells [26]. PD-L1 on melanoma-derived exosomes inhibits $CD8^+$ T cell anti-tumor function in vivo and promotes tumor growth [27]. In addition, exosomes also carry genetic material, such as DNA and RNA, that can be transferred between cells and regulate gene expression in recipient cells [28,29].

Exosomes play an important role in the transmission of molecules between neighboring cells as a tool for intercellular communication [30]. Thus, exosomescan contribute to the development and progression of different diseases, which is also the basis for their role in the pathogenesis of PD. The accumulation of α-syn in neurons is a hallmark of PD. Exosomes have been reported to convey misfolded α-syn from damaged neurons to normal neurons, leading to protein accumulation and cell death [31]. This process is thought to be associated with autophagic damage. Evidence from post-mortem patient brain tissue and rodent models of PD clearly support the idea that autophagy-lysosome pathway (ALP) damage is present [32]. Georgia Minakaki et al. further investigated that damage to ALP increases the amount of α-syn in neural-derived exosomes and that exosomes are able to transfer α-syn from cell to cell in vivo [33]. In addition, compared with free α-synuclein-oligomers, α-synuclein-oligomers encapsulated in exosomes are more easily internalized into the recipient cells [17,34]. Huang et al. found that injection of neural-derived exosomes from PD patients into the

striatum of mice could induce dopaminergic neurons degeneration and motor deficits [35]. Furthermore, inflammatory responses, including glial cell activation and peripheral immune cell infiltration, are also associated with the pathogenesis of PD. The release of exosomes seems to be closely related to the inflammatory response. It has been shown that exosomes from inflamed macrophages can induce the activation of microglia and astrocytes, stimulate proinflammatory cytokine expression and participate in the pathological progression of PD [36]. Furthermore, inflammatory factors could enhance α-syn aggregation induced by exosome, leading to enhanced α-syn toxicity [17]. Han et al. observed a significant increase in TNF-α and IL-1β levels in serum exosomes of PD patients. Then,

Table 1
Summary of exosome-associated biomarkers in Parkinson's disease.

Potential Biomarkers		Exosomes Source	Patients	ROC analysis	Reference
Protein	α-syn	CSF	PD = 76 HC = 58	AUC = 0.741	[38]
		Plasma	PD = 267 HC = 215	AUC = 0.654	[39]
		Plasma	PD = 36 HC = 36	AUC = 0.710	[40]
		Plasma	PD = 43 RBD = 20 HC = 21	AUC = 0.800	[41]
		Plasma	PD = 78 RBD = 153 HC = 63	AUC = 0.761	[42]
		Plasma	PD = 93 HC = 85	AUC = 0.616	[43]
		Plasma	PD = 51 HC = 50	AUC = 0.674	[44]
		Serum	PD = 38 ET = 21 HC = 18	AUC = 0.675	[45]
		Serum	PD = 275 HC = 144	AUC = 0.860	[46]
		Saliva	PD = 18 HC = ?	AUC = ?	[47]
	DJ-1	Plasma	PD = 39 HC = 40	AUC = 0.703	[48]
		Urine	PD = 27 HC = 27	AUC = ?	[49]
	LRRK2	Urine/CSF	PD = 65 HC = 67	AUC = ?	[50]
		Urine	PD = 79 HC = 79	AUC = ?	[51]
		Urine	PD = 41 HC = 35	AUC = 0.844	[52]
	Tau	Plasma	PD = 91 AD = 106 HC = 106	AUC = 0.607	[53]
	AChE	Plasma	PD = 34 HC = 29	AUC = 0.709	[54]
MiRNA	MiR-153 MiR-409–3p	CSF	PD = 78 AD = 53 HC = 35	[a]AUC = 0.990	[55]
	MiR-375 MiR-1468–5p MiR-576–5p MiR-197–3p Let-7e-5p	Plasma	PD = 7 AD = 5 HC = 35	AUC = ?	[56]
	MiR-331–5p MiR-505	Plasma	PD = 52 HC = 48	[b]AUC = 0.898	[57]
	MiR-19b MiR-195 MiR-24	Serum	PD = 109 HC = 40	[c]AUC = 0.946	[58]
LncRNA	Linc-POU3F3	Plasma	PD = 93 HC = 85	AUC = 0.763	[43]
	Lnc- MKRN2-42:1	Plasma	PD = 24 HC = 11	AUC = ?	[59]

AD Alzheimer's disease; AUC area under the curve; CSF cerebrospinal fluid; ET essential tremor; HC healthy control; PD Parkinson's disease; RBD rapid eye movement behavior disorder; ROC receiver operating characteristic.

[a] Model based on miR-153 and miR-409–3p.

[b] Model based on miR-505.

[c] Model based on miR-19b miR-195 and miR-24.

injection of serum exosomes from PD patients into the striatum of mice caused α-syn accumulation, microglia activation, and dopaminergic neuron degeneration, providing evidence that exosome-mediated inflammatory cytokine transport further damages dopaminergic neurons [37].

In this review, we focus on the role of exosomes in the diagnosis of PD and its research progress. And whether exosomes can distinguish PD from atypical Parkinsonian syndrome is essential for the accuracy of PD diagnosis. Table 1 summarizes the biomarkers in exosomes isolated from different biofluids.

3. Role of exosomes in PD diagnosis

3.1. Protein

3.1.1. α-syn

α-Syn is a highly soluble unfolded protein, however it aggregates into filaments and becomes insoluble in PD. Aggregation of α-syn, also called Lewy bodies, is a hallmark pathological feature of PD and other α-synucleinopathies. It is reported that misfolded α-syn may also exhibit prion-like properties [60]. While exosomes may be one of the tools for its spread. Recent studies have indicated that misfolded α-syn can be encapsulated in exosomes, released from neurons to extracellular fluid, and spread among neurons, which has a great impact on the pathological progress of PD [61]. Therefore, α-syn in exosomes has become one of the candidate biomarkers for the diagnosis of PD.

CSF is a kind of clear, colorless fluid that bathes the CNS as an ideal source of biomarkers for PD. Stuendl et al. found lower levels of α-syn in CSF exosomes from patients with early PD compared to healthy controls, which may be useful as a potential biomarker [38]. They also demonstrated CSF exosomes from PD patients contain a pathogenic α-syn that can act as a seed to spread between cells, which could induce oligomerization of soluble α-syn in target cells and participates in disease progression.

However, CSF collection can be invasive compared to other body fluids. Therefore, blood may be a better choice. L1cell adhesion molecular (L1CAM) is a cell adhesion molecule expressed primarily in the nervous system and present on the surface of neural-derived exosomes [62]. Shi et al. established an immunoaffinity capturing protocol to isolate L1CAM-containing exosomes in plasma, providing a theoretical basis for searching CNS exosome in blood [39]. They also indicated that the level of α-syn in CNS-derived exosomes in the plasma was significantly increased in PD patients compared with controls and the diagnostic performance was comparable to that of CSF α-syn. In addition, CNS-derived exosomal α-syn showed a significant association with disease severity, whereas CSF α-syn did not, suggesting that CNS-derived exosomal α-syn, rather than CSF α-syn, may be useful for monitoring or predicting disease progression. Zheng et al. suggested that the different types of α-syn (oligomeric α-syn and the Ser 129 phosphorylated α-syn (p-α-syn)) might be helpful in the diagnosis of PD [40]. Furthermore, Niu and colleagues, through a longitudinal study, demonstrated that the α-syn levels in plasma neuronal exosomes were correlated with progression of motor symptoms [41]. Idiopathic rapid eye movement sleep behavior disorder (iRBD) has been reported as a prodromal stage of PD, which may provide assistance in the early diagnosis of PD [63]. Recently, Yan et al. found that both plasma exosomal α-syn and plasma neural-derived exosomal α-syn were elevated in PD patients compared to healthy controls, whereas only plasma neural-derived exosomal α-syn were elevated in the RBD group [42]. Therefore, α-syn levels in plasma neural-derived exosomes are useful for the diagnosis of early PD. However, Shim et al. found that the levels of plasma exosomal α-syn had no significant difference between PD and healthy controls [54]. Similarly, Si et al. also found that the level of α-syn in CNS-derived exosomes in serum samples was lower in PD patients than healthy controls [45].

In addition to the use of exosomal α-syn to distinguish PD from healthy controls, a more detailed study was conducted by Si et al. They evaluated the levels of α-syn in CNS-derived exosomes in serum samples of PD with different motor types [45]. They divided patients with PD into the tremor-dominant (TD) group and the non-tremor-dominant (NTD) group. As a result, CSF-derived exosomal α-syn was significantly lower in the NTD group compared to the TD group. Furthermore, the performance of CNS-derived exosomal α-syn was found to moderately aid in PD diagnosis and had a potential to diagnose NTD. Therefore, CNS-derived exosomal α-syn in the serum may help in the early diagnosis of PD patients and the identification of different motor types in PD.

Besides exosomal α-syn in blood, exosomal α-syn in saliva can also be used as a diagnostic marker for PD. Rani et al. found that salivary exosomal p-α-syn levels are significantly higher in PD patients than healthy controls with the higher abundance of neuronal origin salivary exosomes in PD patients [47]. These novel findings suggested the utility of exosomes in the saliva as an accessible source of biomarker discovery for PD. However, the mechanism of exosomal α-syn release from salivary glands needs to be investigated.

Since α-syn is a pathological hallmark of PD, it can be very helpful in the diagnosis of PD. However, there are still differences in the current assay outcomes, probably due to variations in the type of biofluid or the technique of exosome isolation. Therefore, we need to conduct more studies to address these issues. Exosomes of neural origin are still considered a better choice because it can reflect the state of the brain. In summary, α-syn in plasma neuronal exosomes can be used as a promising biomarker for PD diagnosis.

3.1.2. DJ-1

DJ-1 is an antioxidant protein that is automatically oxidized upon exposure to oxidative stress, protecting cell contents and regulating gene expression for antioxidant defense [64]. DJ-1 has previously been considered to be linked to the mechanism of PD [65]. Subsequently, in order to explore its potential as a biomarker, many researchers began to investigate the variation of DJ-1 levels in different biological fluids, such as cerebrospinal fluid [66], plasma [67], serum [68] and urine [69]. For example, J. Jang et al. observed significantly higher, 2-fold, oxidized DJ-1 (OxiDJ-1) levels in the urine of Korean PD patients than in non-PD controls [69].

Zhao et al. measured plasma levels of DJ-I in PD patients and healthy controls. However, there was no significant difference between the two groups [48]. Then, they isolated neural-derived exosomes from plasma and found that the concentrations of DJ-1 were

significantly higher in PD patients compared with healthy controls. Moreover, there were significant positive correlations between DJ-1 and α-syn in plasma neural-derived exosomes in PD patients and healthy controls. However, there was no relationship between the two proteins and disease progression. The receiver operating characteristic (ROC) curve analysis performance of DJ-1 in plasma neural-derived exosomes was identified to be moderate (area under the curve (AUC) = 0.703, sensitivity = 79.5 %, specificity = 57.5 %). However, the combination of these two proteins did not perform a significant discrimination (AUC = 0.714, sensitivity = 82.1 %, specificity = 52.5 %). Currently, studies on DJ-1 in exosomes are lacking. Further studies should be conducted with larger patient cohorts in order to corroborate the significance of these findings and the relationship of these biomarkers and disease progression.

In addition, proteomic analysis of urinary exosomes also reported the presence of DJ-1 [70,71]. Dong Hwan Ho et al. investigated the potential use of exosomes in urine as a diagnostic tool for PD. They compared levels of DJ-1 in urine exosomes isolated from Korean PD patients and non-PD controls [49]. The results showed that DJ-1 protein was increased ~1.7-fold in male PD patients compared to male non-PD controls. Thus, only in males, DJ-1 levels in urinary exosomes have potential as a diagnostic biomarker for PD.

Overall, DJ-1 is not yet considered to be a useful biomarker. On the one hand, DJ-1 has a moderate diagnostic performance, and has not been found to be associated with disease progression. On the other hand, we do not know the exact reason for the gender differences of DJ-1 at present. To address these issues, further research should be conducted in a larger patient cohort.

3.1.3. *LRRK2*

In 90 % patients of PD, no genetic or environmental causes have been identified, these patients are known as idiopathic PD (iPD), the remaining 10 % have a definite genetic involvement and show a tendency for familial onset. Among them, mutations in leucine-rich repeat kinase 2 (*LRRK2*) are the most commonly known cause of late-onset PD [72]. *LRRK2* is also the single gene most associated with PD, having only been identified by researchers in 2004 [73]. Researchers have believed that the most common missense mutation in *LRRK2* causes overactivity of LRRK2 protein kinase, which is associated with the onset of PD [74].

In 2013, Fraser et al. have demonstrated that LRRK2 protein can be detected in urinary and CSF exosomes. However, LRRK2 levels in exosomes are unable to differentiate between PD patients and healthy controls [75]. In 2016, this team found in the first small pilot cohort of patients that PD patients with the *G2019S-LRRK2* mutation (*LRRK2*+/PD+) had approximately 5-fold elevated Ser(P)-1292 LRRK2 protein in urinary exosomes compared to healthy controls and PD patients without the *G0219S-LRRK2* mutation (*LRRK2*-/PD+) [52]. Later, in a larger cohort of patients, the same trend was found in male *LRRK2*+/PD + patients, but age-matched male mutation carriers without PD (*LRRK2*+/PD-) were only 2.2-fold higher than healthy controls and lower than *LRRK2*+/PD + patients ($p < 0.001$). And Ser(P)-1292 LRRK2 levels in urinary exosomes were predictive of the diagnosis of PD in mutation carriers (AUC = 0.844) [52]. These results suggest that elevated Ser(P)-1292 LRRK2 in urinary exosomes predicts *LRRK2* mutation status and PD risk in *LRRK2* mutation carriers.

Next, Wang et al. quantified levels of Ser(P)-1292 LRRK2, total LRRK2, and other exosome proteins in urine from 132 subjects and in CSF from 82 subjects in a novel cohort with and without the *G2019S-LRRK2* mutation, with and without PD [50]. They found that male *LRRK2*+/PD-patients had intermediate urine exosomes Ser(P)-1292 LRRK2 levels compared to male *LRRK2*+/PD + patients with PD and controls. However, this trend was not found in females. Moreover, in *LRRK2* mutation carriers, the levels of Ser(P)-1292 LRRK2 in CSF exosomes may be much higher, on average ten times, than in urinary exosomes. But LRRK2 levels in CSF exosomes failed to discriminate *LRRK2* mutation carriers from non-carriers, or PD from controls.

However, according to findings by researchers at the University of Pittsburgh, *LRRK2* plays a major role in the development of PD regardless of mutations, and patients with iPD without mutations also have over-activation of LRRK2 protein and impaired autophagy in neurons, leading to abnormal accumulation of α-syn, which is involved in the development of PD [76]. In 2016, Fraser et al. compared Ser(P)-1292 LRRK2 levels from biobanked urine samples with clinical data in PD and controls [51]. The results revealed that Ser(P)-1292 LRRK2 levels were higher in PD patients than in controls. And, gender affected Ser(P)-1292 LRRK2 levels, with male having higher Ser(P)-1292 LRRK2 levels than female. They also found that Ser(P)-1292 LRRK2 levels in urinary exosome correlated with the severity of cognitive impairment.

Although the findings mentioned showed increased levels of Ser(P)-1292 LRRK2 in urinary exosomes of PD patients, it is not yet certain that Ser(P)-1292 LRRK2 is an appropriate biomarker, as many questions remain to be answered. For example, the relationship between Ser(P)-1292 LRRK2 levels and gender, as well as the source of urinary exosomes is unknown. Studies are still to be done to validate whether Ser(P)-1292 LRRK2 in exosomes can be used to diagnose PD or to assess the severity of PD and how that correlates with motor and non-motor symptoms.

3.1.4. *Tau*

Tau is a microtubule-associated protein (MAP) that is abundant in the axons of neurons where it stabilizes microtubule bundles [77]. Tau protein plays a key role in tauopathies, especially Alzheimer's disease [78]. Notably, several studies have shown that tau gene (*MAPT*) are found to be associated with the risk of sporadic PD [79–81]. A study of the human postmortem striatum found that PD patients had higher levels of tau protein than healthy controls [79]. While loss of tau expression significantly delayed the progression of α-synucleinopathy disease, as evidenced by a significant reduction in histopathological markers of neurodegeneration [82].

In 2016, Shi et al. investigated potential of tau in plasma neural-derived exosomes as a diagnostic marker [53]. They administered radioactively labeled and unlabeled tau intracerebroventricularly in wild-type and tau knock-out mice, respectively. They have demonstrated that tau can be transported from the brain into blood, that tau can also be detected in mouse plasma neural-derived exosomes, and that the exosome-associated tau was mainly inside the exosomes, but not on the surface. They first demonstrated in animal experiments that tau can be transported to the plasma through exosomes. Then, they measured tau in whole plasma and plasma neural-derived exosomes from 91 PD, 106 AD and 106 healthy controls using Simoa assay. The results indicated that tau in plasma

neural-derived exosomes was significantly higher in PD patients compared to healthy controls. In contrast, whole plasma tau concentrations were significantly higher in AD compared to PD and healthy controls. ROC curve analysis showed that tau in plasma neural-derived exosomes was modestly predictive in distinguishing PD from healthy controls (AUC = 0.607, sensitivity = 57.8 %, specificity = 65.1 %).

To sum up, exosomal tau is not highly effective in the diagnosis of PD, and there is a scarcity of literature on the subject. The diagnostic effect of tau combined with other candidate species such as α-syn on PD can be investigated in the future.

3.1.5. Other proteins

Recently, it has been observed that degeneration of the nigrostriatal dopaminergic system is found to be linked to cholinergic denervation in patients with PD [83,84]. By using PET to measure acetylcholinesterase (AChE) activity, there is severe cholinergic denervation in the brains of PD patients compared to AD [85]. Moreover, the uptake of ^{11}C donepezil by peripheral organs was significantly reduced in PD patients, indicating a decrease in AChE in the peripheral system [86]. In conclusion, AChE has been extensively associated with pathophysiological processes of PD in the peripheral organs and the brain. As mentioned previously Kyu Hwan Shim et al. found that the levels of exosomal α-syn had no significant difference between PD and healthy controls. However, exosomal AChE activity was significantly lower in PD patients compared to healthy controls and was negatively correlated with disease severity [54]. Then, it seems that AChE may also have potential as a diagnostic biomarker for PD.

In a case-control study, Jiang et al. explored the proteomic profile of serum exosomes in healthy subjects and PD patients at different stages, and the results revealed the development of PD-specific proteins [87]. They applied mass spectrometry with label-free quantitative proteomics to analyze exosomal proteins and identified a total of 429 proteins, 14 of which were significantly different in patients with mild and severe PD. In PD patients, seven proteins, including clusterin, complement C1r subcomponent, apolipoprotein D (ApoD), were progressively increased from mild to severe PD. In contrast, seven proteins, including complement C1q subcomponent, myosin-reactive immunoglobulin kappa chain, Ig kappa chain VIII region, were progressively downregulated in serum exosomes from mild PD to severe PD. Similarly, Kitamura et al. reported that the levels of clusterin, complement C1r subcomponent, and apolipoprotein A1 in plasma exosomes in PD patients were significantly decreased compared to healthy controls, and apolipoprotein A1 was associated with disease progression [88]. Moreover, Anastasi et al. further extracted plasma neural-derived exosomes for proteomics analysis and identified 23 proteins associated with PD for their potential as biomarkers [89]. Among these 23 proteins, Parkinson's disease protein 7 (PARK7), amyloid P component, clusterin, and stromal cell-derived factor 1 (CXCL12) may be biomarker candidates for the diagnosis of PD.

Recently, Jang et al. used a novel method called magnetic transferrin nanoparticles (MTNs) assay to extract brain-derived exosomes from serum and performed proteomic analysis to explore the value of brain-derived exosomes protein profiles as biomarkers of neurodegenerative diseases [90]. The results showed that compared to dementia and multiple sclerosis (MS) groups, six proteins, such as Noelin and Fibrinogen alpha chain, were abundant in the PD group, highly involved in the hydrogen peroxide catabolic process, and 10 proteins including clusterin, Filamin-A, Tenascin, Cadherin-13) was less abundant in PD, closely associated with lipids metabolism and complement activation. These proteins may be useful in distinguishing PD from other neurodegenerative diseases.

The mentioned studies suggest that proteomic approaches are feasible in exosomes analysis and may identify new candidate biomarkers for PD. However, some challenges still remain, for example, non-specific proteins in body fluids that bind to exosomes can affect the results, and the low abundance of disease-associated proteins [91]. Therefore, more research needs to be invested in developing more advanced methods to overcome these obstacles in the future.

3.2. RNA

3.2.1. miRNA

MicroRNAs (miRNAs) are a class of small non-coding RNA molecules, about 21–24 nucleotides in length encoded by endogenous genes that down-regulate mRNA through RNA interference (RNAi) and have a variety of important regulatory roles in cells. MiRNAs play an important epigenetic role in many diseases and can be overexpressed or suppressed in different diseases [92]. MiRNAs are stable in plasma, serum and other body fluids and can bind to proteins or be encapsulated in vesicles, thus protecting them from degradation [93]. This also laid a foundation for detecting miRNAs in body fluids or vesicles as biomarkers.

In 2015 Gui et al. for the first time, demonstrated the presence of miRNAs in CSF exosomes from patients with PD and AD [55]. The results showed that 16 miRNA expressions were upregulated and 11 miRNA expressions were downregulated in PD patients compared to healthy controls. Validated in independent samples, miR-1 and miR-19b-3p were significantly reduced in CSF exosomes from PD patients, whereas miR-153, miR-409–3p, miR-10a-5p and let-7g-3p were significantly overexpressed. They also performed DIANA mirPath analysis of these differentially expressed miRNAs and found that neurotrophin signaling, mTOR signaling, ubiquitin mediated proteolysis, dopaminergic synapses and glutamatergic synapses were the most significant pathways enriched in PD-miRNA patterns, suggesting that these biological pathways are involved in the development of PD. ROC curve analysis showed that miR-409–3p could achieve the highest area under the curve for a single miRNA (AUC = 0.970). And, the combination of miR-153 and miR-409–3p significantly improved the performance of discrimination (AUC = 0.990).

Nie et al. reported a wide range of altered exosomal miRNA expression levels detected in the plasma of both AD and PD patients by small RNA sequencing [56]. They found that 8 miRNAs were differentially expressed between AD and PD, 6 miRNAs were elevated in PD and 2 miRNAs were increased in AD. Among them, let-7e-5p increased in PD samples and decreased in AD samples, suggesting that let-7e-5p may be a helpful biomarker to distinguish neurodegenerative disease subtypes. Another study by Yao and colleagues analyzed the potential value of plasma exosomal miRNAs as biomarkers of PD and showed that miRNAs including miR-331–5p (AUC

= 0.849) and miR-505 (AUC = 0.898) may serve as biomarkers of PD [57].

Many studies have been performed to find miRNA-based biomarkers for diagnosing PD, which identified several miRNAs that are significantly altered in PD. Nevertheless, there are some discrepancies between the results of these studies [94–96]. Cao et al. selected 24 previously reported miRNAs in serum or plasma that could serve as clinical biomarkers for PD, examined the levels of these miRNAs in serum exosomes, and reassessed their feasibility for clinical application, exploring their potential as biomarkers for PD [58]. They collected serum samples from 109 PD patients and 40 age- and sex-matched healthy controls to detect miRNA in serum exosomes. They only observed consistent results for three miRNAs, with miR-19b downregulated as well as miR-195 and miR-24 upregulated in serum exosomes from PD patients. Then, the validated gene targets for miR-195, miR-24 and miR-19b were searched using the Targetscan tool, and among these targets, Parkin RBR E3 ubiquitin protein ligase (*PARK2*; miR-19b), *LRRK2/PARK8* (miR-19b) and *ATP13A2/PARK9* (miR-24 and miR-195) were found to be closely associated with neuronal apoptosis, regeneration and the neurodegenerative processes of PD. ROC curve analysis showed that the AUC for miR-19b, miR-24 and miR-195 were 0.753, 0.908 and 0.697, respectively, compared to the controls. The AUC for the three-miRNA panel was 0.946 (sensitivity = 85.3 %, specificity = 90.0 %). This also confirms that as a potential serum-based biomarker, the expression levels of miR-19b, miR-24 and miR-195 may contribute to the diagnosis of PD patients.

Many studies on miRNAs in exosomes as biomarkers remains in progress. However, the large discrepancy between study results may be due to the limitations and variability of all techniques leading to the fact that no standard procedure for exosomal miRNA extraction is currently established, making it difficult to identify potential biomarkers for PD. On the other hand, few studies have examined the differences between miRNAs in exosomes of central nervous system origin in PD and healthy controls. Thus, more detailed studies and more advanced technology are in need and a standardized method and source is necessary before it is possible to consider exosomal miRNA as biomarkers of PD in the clinical setting.

3.2.2. lncRNA

LncRNA is one of the non-coding RNAs with a length generally defined as longer than 200 nucleotides. In particular, lncRNA has been shown to play important roles in epigenetic control, transcription, translation, regulation of RNA metabolism, as well as in stem cell maintenance and differentiation, cell autophagy and apoptosis, and embryonic development [97]. In addition, lncRNA plays an important role in brain development, neuronal function and maintenance, and are closely related to the pathophysiology of neurodegenerative diseases such as PD, AD, HD and ALS [98]. Here, we focus on the diagnostic role of lncRNA in exosomes in PD.

In 2020, Zou et al. recruited 93 PD patients and 85 controls to evaluate the severity of PD using several scales, isolated L1CAM-containing exosomes from human plasma using antibody-coated superparamagnetic microbeads, and analyzed the lncRNA in the exosomes by microarray [43]. The results showed that Linc-POU3F3 expression was highly upregulated in PD patients and had the most stable detection density. β-Glucocerebrosidase (GCase), a lysosomal enzyme encoded by the *GBA1* gene, has been investigated to be associated with α-syn protein stability and plays a key role in the pathogenesis of PD [99]. And there was a significant correlation among L1CAM exosomal Linc-POU3F3 levels, plasma GCase activity, and PD severity. However, based on the difference in Linc-POU3F3, the separation between PD patients and controls was low (AUC = 0.763). When binding LINC-POU3F3 and α-syn in L1CAM exosomes and plasma GCase activity, the separation was increased (AUC = 0.824). Therefore, the L1CAM exosomal Linc-POU3F3 may be a diagnostic biomarker for PD.

Wang et al. have examined the expression differences for lncRNA in plasma exosomes of patients with PD compared with healthy individuals, looking for lncRNAs that may be involved in the pathogenesis of PD [59]. They evaluated lncRNA levels extracted from plasma exosomes by next-generation sequencing and real-time quantitative PCR, resulting in 15 up-regulated and 24 down-regulated exosomal lncRNAs in the PD group. According to the lncRNA differential expression results, MSTRG.336210.1 and lnc-MKRN2-42:1 were highly expressed in healthy controls, whereas MSTRG.242001.1 and MSTRG.169261.1 were highly expressed in PD patients. Based on bioinformatic analysis, lnc-MKRN2-42:1 was selected for further study because some of its target genes were also down-regulated in the plasma of 24 PD patients and 11 healthy controls. Moreover, the expression level of lnc-MKRN2-42:1 was positively correlated with the severity of dyskinesia and dysarthria, but not with other clinical symptoms, suggesting that lnc-MKRN2-42:1 may be associated with the development of PD. However, the sample size of the study was too small and the results were not convincing and need to be validated in a large sample. In addition, the mechanism of lnc-MKRN2-42:1 in PD can be studied in cellular or animal models.

Currently, miRNAs have been the main RNA biomarkers studied in exosomes for PD, while lncRNAs have been less investigated. The above findings suggest that LncRNA expression levels differ between PD patients and healthy controls and may be a candidate marker for PD. However, the results are relatively few and a large number of studies are still needed to confirm this deduction. In addition, similar to miRNA, standard extraction methods and exosome sources are required, reducing the variability of study results.

4. Role of exosomes in differentiating PD from atypical parkinsonian syndrome

MSA is a primarily sporadic, adult-onset, fatal neurodegenerative disease, the etiology of which is still unknown [100]. Neuropathological hallmark of MSA is the appearance of misfolded α-syn in oligodendrocytes [101]. There are two types of MSA: MSA-P, which is predominantly Parkinsonism, and MSA-C, which is predominantly cerebellar in character, and they are associated with two major morphological variants of striatonigral degeneration (SND) and olivopontocerebellar atrophy (OPCA), respectively [102]. In the early stages of the disease, symptoms of PD and MSA may overlap [103]. In clinic, early identification of the both is a challenge [104]. Recently, Dutta et al. investigated whether α-syn in neuron-derived and oligodendrocyte-derived exosomes isolated from the serum or plasma could distinguish among healthy controls, PD patients, and MSA patients [44]. As a result, the order of increase of

α-syn in both the neuronal and oligodendroglial exosomes were control < PD < MSA, which is reversed from the findings of Jiang et al. [46]. In addition, they found that the ratio between α-syn concentrations in oligodendroglial exosomes compared to neuronal exosomes was a particularly sensitive biomarker for differentiating PD from MSA.

PSP is one of the most common APS and is characterized by vertical gaze palsy, dysarthria, dysphagia and frontal lobe syndrome [105]. The differential diagnosis between PD and PSP is often challenging, especially in the early stages of the disease. Recently, Ida Manna et al. investigated whether miRNA in serum exosomes could be used as a biomarker to distinguish PD from PSP [106]. They found that the best discriminated PSP from PD was a set of six miRNAs (miR-21–3p, miR-199a-5p, miR-425–5p, miR-483–5p, miR-22–3p and miR-29a-3p) with an AUC = 0.91 and a diagnostic sensitivity and specificity of 0.89 and 0.90, respectively. Thus, the researchers suggested that exosomal miRNA combinations can provide good diagnostic discrimination and will be validated in larger cohorts in the future and can be used to support the clinical differential diagnosis of PSP and PD.

The typical DLB patient presents with early dementia, usually with visual hallucinations. Extrapyramidal motor signs and symptoms characterized by PD usually appear simultaneously or quickly. Progressive cognitive decline starts early, usually after age 55 [107]. The cognitive fields affected in DLB and Parkinson's disease dementia (PDD) largely overlap, with significant executive dysfunction and visuospatial abnormalities, as well as variable impairment in memory capacity [108]. According to international consensus, DLB is diagnosed when cognitive impairment precedes parkinsonian motor signs or begins within 1 year of its onset [109]. In contrast, in PDD, cognitive impairment develops in the context of diagnosed PD [110]. Despite the different chronology of motor and cognitive deficits and some clinical differences, the two disorders show a large degree of convergence [111]. Accurate differentiation between PD, DLB is challenging due to the overlap of clinical symptoms and neuropathological changes. Stuendl et al. found that CSF exosomal α-syn has potential as a biomarker for differentiating PD from DLB [38]. CFS samples from DLB patients contained fewer exosomes and lower levels of exosomal α-syn compared to PD patients and controls. ROC curve analysis also showed high sensitivity and specificity of exosomal α-syn for identifying DLB, PD and controls.

CBD is a chronic progressive neurodegenerative disease characterized by asymmetric akinetic-rigid syndrome, dysfunction, dystonia, and postural abnormalities. The pathology is characterized by the accumulation of abnormal tau proteins in neurons and glial cells. In the early stages of CBD, it is often diagnosed incorrectly as PD or other degenerative diseases [112]. In recent study, Meloni et al. isolated neural-derived exosomes from serum of PD patients and patients with APS, including CBD and PSP patients, to verify whether exosomal α-syn and Tau aggregates have a differential diagnostic effect [113]. As might be expected, exosomal oligomeric α-syn is significantly elevated in PD compared to APS, whereas exosomal Tau aggregates are significantly augmented in APS compared to PD. Combination of both biomarkers separated PD from APS (PD vs. PSP with AUC = 0.880, PD vs. CBD with AUC = 0.902). Of note, there was a positive correlation between exosomal oligomeric α-syn and disease severity (disease duration, Modified H&Y, UPDRS motor scores) in PD patients. Therefore, α-syn and tau aggregates in neural-derived exosomes could be promising biomarkers.

In 2020 Jiang et al. expanded the types of cases and explored the clinical application of serum neuronal exosomes as biomarkers for PD, MSA, and other proteinopathies. They analyzed serum from 664 participants from three regions with RBD, PD, DLB, MSA, FTD, PSP, CBD and healthy controls [46]. They found that α-syn was increased in RBD, PD and DLB exosomes by ~ twofold compared with controls, MSA or other proteinopathies. Besides α-syn, clusterin concentrations in serum neuronal exosomes were also significantly different between disease groups. The combination of α-syn and clusterin allowed to distinguish PD from MSA (AUC = 0.94) and other proteinopathies (AUC = 0.98).

In 2021, Jiang et al. validated their early findings with a number of additional samples and combined them with their previous data in a joint analysis, bringing the total sample size to 735 individuals (PD (n = 290), MSA (n = 50), PSP (n = 116), CBD (n = 88), healthy controls (n = 191)) [114]. This is the largest study of exosomal α-syn in PD and related disorders. This study confirmed that α-syn levels in L1CAM-immunocaptured exosomes above 14 pg/mL are a robust biomarker for differentiating PD from MSA (AUC, 0.90 vs 0.98) or 4-repeat tauopathies (AUC, 0.93 vs 0.94). And when combined with exosomal clusterin, it improves the discrimination between PD and 4-repeat tauopathy. This remarkable finding suggests that future studies should include clusterin among the markers used to distinguish APS.

This section describes the differentiating diagnostic role of exosomes between PD and various APS. Notably, most studies have examined exosomes of neurological origin, while few comparative studies have been conducted on exosomes from different brain cell types, for example, exosomes derived from neurons, oligodendrocytes, and astrocytes. At the same time, we propose a larger idea of whether exosomes of nigrostriatal dopaminergic neuronal origin can be extracted for analysis in the future, which will make a greater contribution to the diagnosis of PD. In addition, due to the different pathological proteins of APS, the diagnosis of PD and APS can be greatly assisted by the method of combining multiple biomarkers.

5. Conclusion

Overall, the field of exosome research has evolved rapidly over the past decade. Since exosomes are carriers of intercellular communication, they can influence gene expression and protein activity in recipient cells by carrying "cargo" such as proteins and miRNAs. Therefore, it can lead to the propagation of misfolded proteins, such as α-syn. The use of targeted exosomes as diagnostic tools for biomarker detection has received much attention. Many studies have shown the feasibility of utilizing exosomes, especially those originating from the central nervous system, as diagnostic biomarkers for PD and APS. Isolation of exosomes derived from specific brain cell types, such as astrocytes and oligodendrocytes, may provide a more specific and valuable source of diagnostic and progression biomarkers. However, the isolation of exosomes derived from specific brain cell types and the validation of the cell type of origin are still to be strictly addressed.

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

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Declaration of competing interest

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