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REVIEW

Exosomes in Parkinson's Disease

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Abstract Exosomes, nano-sized extracellular vesicles secreted by most cell types, are found in all kinds of biological fluids and tissues, including the central nervous system (CNS). The proposed functions of these vesicles include roles in cell–cell signaling, removal of cellular debris, and transfer of pathogens between cells. Many studies have revealed that exosomes derived from the CNS occur in the cerebrospinal fluid and peripheral body fluids, and their contents are altered during disease, making them an appealing target for biomarker development in Parkinson's disease (PD). Exosomes have been shown to spread toxic α-synuclein (αsyn) between cells and induce apoptosis, which suggests a key mechanism underlying the spread of αsyn aggregates in the brain and the acceleration of pathology in PD. However, potential neuroprotective roles of exosomes in PD have also been reported. On the treatment side, as drug delivery vehicles, exosomes have been used to deliver small interfering RNAs and catalase to the brain, and have shown clear therapeutic effects in a mouse model of PD. These features of exosomes in PD make them extremely interesting from the point of view of developing novel diagnostic and therapeutic approaches.

Keywords Exosomes · Parkinson's disease · Biomarker · Pathology · Therapy

Introduction

Exosomes are small membranous vesicles that originate from endosomes. They are secreted by virtually all cell types, including neurons, and can be isolated from conditioned cell media or bodily fluids such as cerebrospinal fluid (CSF), plasma, and urine. The exosome contents change during disease, which makes them particularly attractive targets for novel diagnostic approaches, and the important functions of exosomes such as cellular communication allow them to act as notable contributors to both health and disease. In this review, we discuss the biogenesis of exosomes and their roles in the central nervous system (CNS), and reveal their multiple roles in Parkinson's disease (PD), the second most common neurodegenerative disease after Alzheimer's disease (AD).

The Biogenesis of Exosomes and Their Roles in the CNS

Exosomes are generated within multivesicular bodies (MVBs) that contain intraluminal vesicles in the endosomal system. The endosomal system consists of primary endocytic vesicles, early endosomes (EEs), and MVBs. EEs are located near the cell membrane and act as the first port of call for primary endocytosed vesicles. After leaving the EE, the cargo is recycled to the plasma membrane or targeted to MVBs. MVBs then fuse with plasma and endosomal membranes to release exosomes into the extracellular space [1, 2].

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Exosomes typically have a diameter of 40–150 nm. They present a cup-shaped morphology under the transmission electron microscope, but cryo-electron microscopy of unfixed exosomes has revealed a spherical morphology [3]. Exosomes are secreted *in vitro* and *in vivo* by various types of cells, including those of the CNS, under physiological and pathological conditions. They have been isolated from blood, CSF, urine, saliva, and milk [4–7]. Due to their different cellular ancestries, exosomes carry cell-type-specific proteins and lipids. For instance, exosomes derived from oligodendrocytes carry myelin proteins [8]. Several proteins are specifically found on the surface of all exosomes and thereby serve as exosomal markers. They include the multivesicular endosome proteins Alix and tsg101, integrins, the tetraspanins CD63, CD89, CD81, CD9, and CD82, and the heat-shock proteins hsp70 and hsp90, as well as the endosomal and endosome maturation-related proteins flotillin and annexin [9]. On the other hand, proteins such as transferrin receptor and GM130 derived from the nucleus, mitochondria, Golgi apparatus, or endoplasmic reticulum are not detectable in exosomes and thereby serve as negative markers. Apart from various types of proteins, nucleic acid constituents including messenger RNA (mRNA), small non-coding microRNAs (miRNAs), and double-stranded DNA have also been detected in exosomes and may function in cardiovascular protection and repair [10, 11].

Recently, many types of cells in the CNS, including oligodendrocytes [8, 12], neurons [13, 14], and astrocytes [15] have been confirmed to secrete exosomes. It is believed that exosomes as potential carriers can disseminate disease pathology in neurodegenerative disorders. Exosomes secreted from PrP (prion protein)-expressing neurons are reported to contain PrPsc (scrapie isoform) and PrPc (cellular isoform) when parental cells are infected with PrPsc [16, 17]. Moreover, there is evidence that exosomes facilitate the unique transmissible nature of prions. For instance, the exosomes from prion-infected cells can cause prion propagation in uninfected recipient cells and produce prion disease-like clinical features when inoculated into mice [18]. While prion disease has generally been thought to be the only neurodegenerative disease that is transmissible, misfolded forms of proteins involved in other neurodegenerative disorders such as AD, PD, and amyotrophic lateral sclerosis may also spread to different brain regions similar to prions [19, 20]. The exosomes from either neurons or brain tissues contain amyloid-β precursor protein (APP) and APP-processing products, C-terminal fragments and amyloid-β [21, 22]. Furthermore, neuronal exosomes accelerate the propensity of soluble amyloid peptides to form fibrils [23]. Misfolded Cu/Zn superoxide dismutase (SOD1) propagation between cells can be mediated through exosomes. This may help to explain why SOD1 aggregates propagate in a prion-like manner in neurons and how sporadic amyotrophic lateral sclerosis spreads systemically from region to region in a progressive manner [24, 25].

Several properties of exosomes make them excellent candidates for carrying biomarkers. Their cargos of cell-type-specific proteins and nucleic acids are likely to reflect the core of pathogenic intracellular processes. Moreover, exosomes can cross the blood-brain barrier (BBB) so that CNS-derived exosomes can reach the peripheral blood and provide protein biomarkers of CNS disease. For instance, altered levels of autolysosomal proteins such as cathepsin D, lysosome-associated membrane protein 1, and ubiquitinylated proteins, as well as AD-associated proteins including P-S396-tau, P-T181-tau, and Aβ1-42, are found in CNS-derived blood exosomes in patients with AD and appear to reflect the pathology up to 10 years before clinical onset [26, 27].

Exosomes in Parkinson's Disease

PD, a progressive movement disorder, is the second most common neurodegenerative disease after AD and affects ~2% of the population aged over 65 years [28]. The common clinical manifestations are the characteristic motor symptoms of resting tremor, bradykinesia, muscular rigidity, and postural instability. In PD, degeneration of dopaminergic neurons in the substantia nigra leads to an imbalance of excitatory (acetylcholine) and inhibitory (dopamine, DA) neurotransmitters, which causes the motor symptoms [29].

One of the major pathological characteristics of PD is the presence of Lewy bodies [30]. The misfolding and fibrillar aggregation of α-synuclein (αsyn) constitutes the main component of Lewy bodies and Lewy neurites in both the genetic and sporadic forms of PD [30]. Other mechanisms include reactive oxygen species, neuroinflammation, excitotoxicity, apoptosis, and loss of trophic factors. However, none of these is the only contributor to the generation of PD; rather, molecular pathways act together to induce the degeneration of dopaminergic neurons.

At present, there is no cure for PD, and the goals of treatment are to alleviate the symptoms for the comfort of patients and to minimize the dyskinesia. Levodopa is the primary line of DA replacement therapy, which restores deficits resulting from the loss of dopaminergic neurons. This provides the greatest symptomatic benefit and alleviates the motor symptoms [31]. However, as the effectiveness diminishes with time, clinicians have to delay prescription of levodopa as much as possible. Reports have shown that only ~25% of patients treated with levodopa for 5 years continue to have a good response [32, 33]. In

addition, adverse effects of levodopa (dyskinesia, psychosis, hallucinations, and hypotension) have been noted [34–36]. Furthermore, although there are other therapeutic options such as Sinemet (carbidopa-levodopa), Rasagiline, antioxidants, and factors that inhibit apoptosis, as well as operative treatments such as deep brain stimulation, their side-effects are also of serious concern [37–41].

There is a presymptomatic phase during PD progression, in which clinical signs do not appear until 70%–80% of the dopaminergic terminals in the striatum and >50% of the dopaminergic neurons in the substantia nigra have been lost [42]. This highlights the importance of early detection and intervention, which may prevent the degeneration of dopaminergic neurons. Moreover, numerous studies have shown that the development of disease-modifying treatments for PD is hampered by the lack of sensitive and specific biomarkers.

Exosomes as Biomarkers in PD

A biomarker has been defined as "a role that is objectively measured and evaluated as an indicator of physiological processes, pathogenic processes or pharmacologic response to a therapeutic intervention" [43]. At present, the diagnosis of PD is typically based on observation of the motor symptoms but the misdiagnosis rate is appreciably high, particularly in the early stages and in the presymptomatic phase [44]. Biomarkers of PD have gained increasing attention, and the discovery of disease-related proteins in exosomes isolated from plasma/CSF samples from PD patients has inspired research into the use of exosomes as biomarkers.

αsyn in the CSF has been consistently reported to be significantly lower in PD patients than in controls [45–47]. However, as peripheral cells, especially red blood cells and platelets, can produce abundant αsyn [48, 49], the use of blood αsyn as a biomarker has been found to be readily accessible but inconsistent [50–52]. By using the anti-L1CAM (neural cell adhesion molecule L1) antibody which specifically identifies exosomes derived from the CNS, Shi *et al.* discovered that CNS-derived exosomes can efflux into blood and the level of αsyn from CNS-derived exosomes in plasma is substantially higher in PD patients and is associated with the severity of the disease [53]. Therefore, they concluded that plasma CNS-derived exosomal αsyn can serve as a PD biomarker with high sensitivity and specificity [53]. In CSF samples, Stuendl and coworkers found decreased exosomal αsyn levels in PD patients, consistent with the total αsyn levels in CSF [54]. It is likely that tau is transported from brain to blood by exosomes, and CNS-derived exosomal tau in plasma is significantly higher in PD patients than in controls and is correlated with CSF total tau and phosphorylated tau [55].

Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene cause late-onset PD in both familial and sporadic late-onset populations [56, 57]. In 2013, Fraser *et al.* reported that LRRK2 is secreted by exosomes from various kinds of cells where LRRK2 is natively expressed, including duct epithelial cells in the kidney, neurons, and macrophages. Thus, LRRK2 can be detected through the purification of exosomes from clinical samples of urine or CSF, which provides a foundation for using exosomal LRRK2 as a biomarker in clinical trials [58], and this has been confirmed by Ho and coworkers in Korean PD patients [59]. In addition, Ho *et al.* found that the protein levels of LRRK2 and DJ-1 (PD causative gene products) in urinary exosomes show clear gender-dependent differences. In male patients, the DJ-1 levels are significantly higher in PD than in controls and increase in an age-dependent manner in PD [59]. Another research team also reported gender differences of Ser(P)-1292 LRRK2 levels in urinary exosomes [60]. They showed that the exosomal Ser(P)-1292 LRRK2 levels are higher in idiopathic PD, especially in females, than in controls, and are correlated with the severity of cognitive impairment and the loss of activities of daily living, independent of age. Although the accuracy is not high, the Ser(P)-1292 LRRK2 levels in urinary exosomes appear to predict several aspects of PD severity [60]. In the same year, these authors focused on familial PD, and found higher ratios of phosphorylated Ser-1292 LRRK2 to total LRRK2 in urinary exosomes from LRRK2 mutation carriers than in those of non-carriers (with or without PD). Among the carriers, those with PD have a higher ratio of Ser(P)-1292 LRRK2 to total LRRK2 in urinary exosomes than those without PD. Moreover, an elevated ratio predicts the LRRK2 mutation status and PD risk among LRRK2 mutation carriers [61].

In addition to proteins, it has long been known that exosomes contain various RNA species. Gui and coworkers explored the exosomal miRNAs isolated from the CSF in PD and AD patients. They found that 16 exosomal miRNAs are upregulated while 11 miRNAs are downregulated in PD patients compared with controls. Among these, miR-1 and miR-19b-3p have been validated and are significantly reduced in PD CSF exosomes. On the contrary, miR-153, miR-409-3p, miR-10a-5p, and let-7g-3p are increased. On the other hand, different levels of mRNA transcripts such as APP, αsyn, and long non-coding RNAs (RP11-462G22.1 and PCA3) occur in CSF exosomes from PD compared to AD patients [62]. This evidence shows the potential value of CSF exosomal RNA for the diagnosis and assessment of PD.

The above studies support the idea that exosomes have potential value in PD diagnosis (summarized in Table 1). However, substantiation in large population studies is still needed, and the sensitivity and specificity of these

Table 1 Summary of literatures assessing exosomes as biomarkers in PD

Exosomal source	Target	Patients	Findings	Author, year	Ref
Plasma	αsyn	267 PD, 215 control	Exosomal αsyn and exo/total ratio are higher in PD compared to controls ($P < 0.0001$)	Shi *et al.* 2014	[53]
	Tau	91 PD, 106 control	Exosomal tau is higher in PD than in control ($P = 0.038$)	Shi *et al.* 2016	[55]
CSF	αsyn	76 PD, 58 control	Exosomal αsyn is lower in the PD ($P < 0.05$)	Stuendl *et al.* 2016	[54]
	LRRK2	–	LRRK2-positive exosomes circulate in CSF	Fraser *et al.* 2013	[58]
	miR-1, miR-19b-3p, miR-153, miR-409-3p, miR-10a-5p, let-7g-3p	47 PD, 27 control	Sixteen exosomal miRNAs (including miR-153, miR-409-3p, miR-10a-5p, and let-7g-3p are validated) are up-regulated and 11 miRNAs (including miR-1 and miR-19b-3p are validated) are down-regulated in PD (relative fold > 2, $P < 0.05$).	Gui *et al.* 2015	[62]
Urine	LRRK2	20 PD, 15 control	LRRK2 levels are overall comparable but highly variable in urinary exosomes derived from PD	Fraser *et al.* 2013	[58]
		PD (14 males, 12 females), control (10 males, 11 females)	Levels of LRRK2 are lower in females, whereas the DJ-1 level is the opposite.	Ho *et al.* 2014	[59]
	DJ-1	14 PD, 10 control	In males, DJ-1 level in exosomes is higher (1.7-fold) in PD than in control	Ho *et al.* 2014	[59]
	Ser(P)-1292 LRRK2	79 PD, 79 control	Ser(P)-1292 LRRK2 levels are higher in males than in females ($P < 0.0001$) and elevated in PD patients when compared with controls ($P = 0.0014$).	Fraser *et al.* 2016	[60]
	Ser(P)-1292/total LRRK2	14 males (7 LRRK2+/PD+; 4 LRRK2−/PD+; 3 LRRK2−/PD−) 62 males (16 LRRK2−/PD−; 16 LRRK2+/PD−; 14 LRRK2+/PD+; 16 LRRK2−/PD+)	LRRK2+/PD+ have a higher Ser(P)-1292 LRRK2-to-total ratio than LRRK2−/PD− (4.8-fold, $P < 0.001$) and LRRK2−/PD+ (4.6-fold, $P < 0.001$). Among mutation carriers, those with PD have a higher Ser(P)-1292 LRRK2-to-total ratio than those without PD (2.2-fold, $P < 0.001$).	Fraser *et al.* 2016	[61]

PD: parkinson's disease, αsyn: α-synuclein, exo/total ratio: exosomal αsyn/total αsyn ratio, CSF: cerebrospinal fluid, LRRK2: leucine-rich repeat kinase 2, Ser(P)-1292 LRRK2: phosphorylated Ser-1292 LRRK2.

biomarkers need to be further confirmed by clinical studies. At the same time, there is a pressing need to develop exosome isolation methods that exclude lipoprotein aggregates and contaminating proteins. They are of particular importance for improving the diagnostic value of exosomes.

Exosomes as Both a Curse and a Blessing in the Progress of PD

Lewy bodies often initially occur in the periphery, gradually affect the brain stem, and eventually appear in the cortex [63]. In addition, Lewy bodies have been observed in mesencephalic stem-cell transplants in recipients with PD, suggesting host-to-graft transfer of αsyn pathology [64], and seeded propagation of αsyn is a leading hypothesis to explain the interneuronal transmission of pathology [65–67]. Exosomes are biologically active vesicles that participate in intercellular communication [68, 69]. They have the exceptional property of interacting with target cell membranes and thereby act as nanocarriers of biological information. Ghidoni *et al.* have proposed the "Trojan horse" hypothesis of exosomes in neurodegeneration; a mechanism leading to the death of cells by shipping toxic agents in exosomes from cell to cell [70]. In fact, many researchers believe that exosomes act as potential intercellular carriers of pathogenic proteins and cause impaired neuronal function [9, 19, 20, 71].

It is widely accepted that αsyn oligomers cause neuronal death [72]. αsyn in exosomes may not only serve as a biomarker for PD, but also as a potentially pivotal player in the propagation of the neurotoxic form of αsyn and its spread in the brain. αsyn has been detected in exosomes separated from the conditioned media of SH-SY5Y cells with wild-type αsyn and inducible β-galactosidase, and the exosome-associated αsyn impacts the viability of neighboring neurons [73]. In 2011, Alvarez *et al.* published a study in which they demonstrated that exosomes from SH-SY5Y cells overexpressing αsyn efficiently transfer αsyn to normal SH-SY5Y cells. Moreover, when the release of αsyn from exosomes increases, its transmission to recipient cells increases correspondingly [74]. Soon after that, Danzer *et al.* performed a similar study and found that exosome-associated αsyn oligomers *in vitro* are preferentially internalized by recipient cells and are more toxic than free αsyn oligomers [71]. Moreover, the aggregation of exogenous αsyn can be accelerated by exosomes, caused by the exosome lipid [75]. αsyn can induce an increase of exosomal secretion by microglial BV-2 cells, and these activated exosomes cause increased apoptosis of cortical neurons. The authors concluded that exosomes from activated microglia may be important mediators of αsyn-induced neurodegeneration in PD [76]. Some years later, Kunadt *et al.* provided evidence for exosomal αsyn in the CNS *in vivo* [77]. The next year, these authors published a study showing that CSF exosomes derived from PD patients contain a pathogenic species of αsyn, and these exosomes can initiate the oligomerization of soluble α-synuclein in target cells in a dose-dependent manner and confer disease pathology [54]. These studies support the notion that exosomes contain αsyn "seeds" or "strains" that spread αsyn aggregates in the brain and aid pathology.

Though the intercellular transfer of αsyn by exosomes may cause the spread of pathology, neurons can benefit from the reduction of its intracellular level by αsyn externalization *via* exosomes. The surviving DA neurons in the substantia nigra of PD patients express higher levels of ATP13A2 mRNA and protein than controls [78, 79]. Gitler *et al.* further showed inhibitory effects of ATP13A2 on the αsyn toxicity in primary DA neurons [80]. Then, Kong and co-workers reported that elevated ATP13A2 expression increases the externalization of exosome-associated αsyn and thereby reduces the intracellular αsyn levels, while reduced ATP13A2 expression correspondingly leads to decreased levels of exosomal αsyn [81]. Further functional evidence for the neuroprotective efficacy of exosomes came from a study by Tomlinson *et al.* showing that PD-derived microvesicles are protective in models of neuronal stress [82]. These results indicate a potential neuroprotective role of exosomes in PD.

The evidence shows contradictory roles of exosomes in PD, which may be due to the properties of exosome complexes that are not completely clear. But different exosomes from different cellular ancestries in the brain have unique characteristics, so their roles in PD pathology may be distinctly different. In future, studies of exosomes may need to take into consideration their sources.

Exosomes as Drug-Delivery Vehicles for PD Therapy

It has been reported that ~98% of all potent drugs that may be therapeutic for many diseases in the CNS failed in clinic trials because of their inability to cross the BBB [83]. As naturally-occurring nano-sized vesicles, exosomes can cross the BBB and have attracted considerable attention as drug-delivery vehicles [84, 85]. The incorporation of therapeutic agents into exosomes preserves the therapeutic activity, increases the circulation time, and improves delivery to the brain. For instance, exosomes have been harnessed for the systemic delivery of therapeutic agents such as curcumin and exogenous siRNA across the BBB [84, 86, 87]. In PD, when macrophages are transfected with plasmid DNA encoding catalase, the exosomes secreted from these macrophages are packed with catalase genetic material including plasmid DNA and mRNA, active catalase, and nuclear factor κ-B, a transcription factor involved in encoded gene expression. What is more, these contents can be efficiently transferred by exosomes to neurons and thereby result in *de novo* protein synthesis in target cells. Ultimately, this approach results in reduced inflammation and neuroprotection in PD mice [88]. Peripheral injection of modified exosomes expressing rabies virus glycoprotein

loaded with siRNA to αsyn leads to decreased αsyn mRNA and protein levels in S129D αsyn transgenic mice [89]. Exosomes secreted by monocytes and macrophages loaded with catalase efficiently accumulate in neurons and microglial cells in the brain, decrease brain inflammation, and significantly increase neuronal survival in PD mice [90].

Accumulating evidence suggests that exosomes can be engineered to target neurons or even specific neuronal populations and be effectively used for the treatment of various neurodegenerative disorders. However, several problems must be solved before they are used in the clinical setting. First, exosomes are complex systems containing various molecular constituents that raise multiple safety issues. Thus, future studies will focus on the engineering of exosome-mimetic delivery systems containing only the desired therapeutic molecules. On the other hand, more experiments are needed to evaluate possible adverse effects related to the therapeutic administration of exosomes. It is known that exosomes vary considerably among different cellular sources, so it is important to identify the best sources. The role of exosomes as potential novel therapeutic tools against neurodegenerative diseases has recently (2016) been discussed in detail in a review article by Jarmalaviciute and Pivoriunas [91].

Conclusions

The field of exosome studies has recently been attracting increasing interest. In recent years, exosomes have risen from being considered mere cellular "trash-cans" to possible biomarkers and multipotent therapeutic targets of various diseases, among which PD is one of the most important. Though exosomes have been shown to possess the ability to propagate PD pathology as well as to hinder it, we believe that eventually exosomes can be harnessed for the benefit of PD patients.

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