

Review

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[Krithik N Kamath](#) * and [Alin Bose](#)

Posted Date: 6 December 2023

doi: 10.20944/preprints202312.0303.v1

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Review

Unlocking the Secrets of Exosomes: Neurobiological Signalling, Adult Neurogenesis, and Influence of Exosome in Progression of Parkinson's Disease

Krithik Kamath N¹ and Alin Bose²

^{1,2} Department of Pharmaceutical Biotechnology JSS College of Pharmacy, JSS Academy of Higher Education & Research, Ooty, The Nilgiris, Tamil Nadu, 643001, India

* Correspondence: kknin25@gmail.com; Tel.: +919945471936

Abstract: Exosomes, a subset of extracellular vesicles, have emerged as pivotal mediators of intercellular communication, revolutionizing our comprehension of cellular interactions. Their diverse cargo, comprising proteins, nucleic acids, and bioactive molecules, plays a crucial role in various physiological and pathological processes. In the intricate landscape of adult neurogenesis, exosomes wield significant influence, actively participating in the generation of new neurons within the adult mammalian brain. Originating predominantly from non-niche cells, these vesicles contribute substantially to the regulation of neuroinflammation, thereby impacting neurogenesis and offering therapeutic promise for neurological disorders. In the specific context of Parkinson's disease (PD), exosomes assume a multifaceted role, prominently implicated in the propagation of α -synuclein oligomers and the orchestration of neuroinflammation. Stem cell-derived exosomes present themselves as promising therapeutic agents, capable of reshaping the microenvironment, repairing neuronal damage, and mitigating apoptosis. The ongoing exploration of exosomes continues to unravel intricate biological processes, paving the way for innovative medical interventions and treatments, particularly within the challenging landscape of neurodegenerative diseases such as PD. The comprehensive understanding of exosomal functions holds the potential to advance our therapeutic approaches, offering new avenues for intervention and treatment strategies in the realm of neurodegenerative disorders.

Keywords: Parkinson's disease; Neurogenesis; Exosomes; Stem cell-derived exosomes. Non-niche cells

Introduction

Extracellular vehicles (EVs) represent a diverse group of membrane-bound vesicles that are actively secreted by cells, originating either from endosomal or plasma membrane compartments. The existence of these membranous structures in the extracellular milieu has been a well-established and long-recognized phenomenon in the field of cellular and molecular biology. This realization has substantially reshaped our understanding of intercellular communication and cellular interactions.[1–3] The currently defined exosomes (40–100nm) were first found in sheep reticulocytes in 1983[4,5] In the study conducted by Johnstone and colleagues [6], they investigated the dynamics of transferrin receptors throughout reticulocyte maturation and identified exosome formation as the underlying mechanism responsible for the reduction of transferrin receptors in mature red blood cells. The complex composition of exosomes, including their content of nucleic acids, proteins, lipids, cytokines, transcription factors, receptors, and other bioactive molecules, has been revealed through extensive research efforts. The recognition of this multifaceted cargo within exosomes has been made possible despite the methodological challenges associated with their isolation and characterization. These findings have fundamentally expanded our comprehension of the biological significance and functional versatility of exosomes, shaping our understanding of intercellular communication and their potential roles in various physiological and pathological processes [7,8]. Exosomal protein

components can be categorized into two distinct groups, each with its own unique functions. The first group consists of “public components,” which are universally present in exosomes and play a crucial role in the processes of vesicle formation, secretion, and intercellular communication. These components include membrane transport and fusion-related proteins like Rab GTPases, heat shock proteins (e.g., HSP70 and HSP90), proteins from the four-transmembrane protein superfamily (e.g., CD63 and CD81), proteins associated with the Endosomal Sorting Complex Required for Transport (ESCRT) complex (e.g., Tsg101 and Alix), and integrins, among others. The second group comprises “specific components” that are unique to the exosomes’ progenitor cells and, as a result, are cell-specific. These components, such as CD45 and MHC-II, are closely linked to the identity and functions of the originating cells, especially antigen-presenting cells. As research on exosomes continues to advance, their applications are expanding across a wide range of physiological and pathological processes. Exosomes serve as vital mediators for intercellular communication and the exchange of biological materials. Moreover, they offer a safe and effective means to transport a variety of bioactive substances, including therapeutic agents that may have limited stability or retention when administered independently. Exosomes can transport these substances through multiple pathways and to specific sites, allowing them to play critical roles in diverse processes such as tissue repair, tumor diagnosis and treatment, and immune regulation. This versatility positions exosomes as promising tools for innovative approaches to medical interventions and treatments. [9–17]. In this review, our primary emphasis is on exploring the origins and categorization of exosomes, delving into the biological composition of exosomes, elucidating the intricate process of exosome biogenesis, uncovering the connections between exosomes and adult neurogenesis, and investigating the impact of exosomes on Parkinson’s disease.

Ev Categorization

Extracellular vesicles (EVs) are classified according to their size and the mechanisms governing their release. Among these subtypes, exosomes are the most extensively studied. Exosomes originate as intraluminal vesicles (ILVs) within multivesicular bodies (MVBs). Upon fusion of the MVB with the plasma membrane, these ILVs are released into the extracellular space. [18]. Micro vesicles, also known as ectosomes or microparticles, are generated when the plasma membrane undergoes outward blebbing, followed by the division of these plasma membrane blebs [19]. Micro vesicles originating from human cancer cells have garnered significant attention due to their capacity to engage in the horizontal transfer of signalling proteins among cancer cells, thereby enhancing their invasive behaviour. Additionally, extracellular vesicles (EVs) can be discharged from nanotubular structures that extend from the plasma membrane [20]. The diverse sizes of extracellular vesicles (EVs) have been taken into account when categorizing different subtypes of EVs. However, it’s important to recognize that these classifications can appear somewhat incongruent with the inherent heterogeneity within vesicle classes, encompassing variations in both cargo composition and functional properties [21,22]. Extracellular vesicles (EVs) encompass vesicles of varying sizes, ranging from 30 to several microns in diameter, each carrying a diverse cargo. Notably, there is a degree of overlap in size distribution between different types of vesicles. For instance, microvesicles (MVs) typically fall within the range of 200 nm to over 1 micron, whereas exosomes are relatively smaller, with a diameter typically falling between 50 and 100nm [23]. Apoptotic bodies, arising from cellular blebbing and fragmentation during the process of cell death, typically exhibit dimensions on the order of several microns [24]. Up to this point, the classification of vesicles has been a matter of some ambiguity and has not been without debate. Hence, it is advisable to exercise caution when seeking absolute definitions for different types of vesicles. In the classification of various EV types, it appears that the mode of biogenesis and the specific cargo carried by these vesicles may hold greater relevance and significance.

Table 1. Extravesical subtype character.

Characteristic	Exosome	Micro vesicle	Apoptotic Body
Origin	Derived from endocytic pathways	Originate from plasma membrane via blebbing	Formed through plasma membrane budding and blebbing
Size	Typically, 30–200 nm	Ranging from 100–1000 nm	Mostly larger than 1000 nm
Density	Exhibits a density range of 1.13–1.19 g/ml	Shows a density range of 1.04–1.07 g/ml	Has a relatively high-density range of 1.16–1.28 g/ml
Shape	Generally spherical in shape	Irregular shape	Variable in shape
Composition	Composed of proteins, nucleic acids, lipids, and metabolites	Comprises proteins, nucleic acids, lipids, and metabolites	Contains DNA fragments, histone, chromatin remnants, cytosol portions, and degraded proteins
Typical Constituent Proteins	Includes tetraspanins, ESCRT proteins (Alix, TSG101), integrins, and heat shock proteins	Contains integrins, selectins, CD40 ligand, flotillin-2, and adenosine diphosphate ribosylation factor 6, phosphatidylserine	Characterized by annexin V and phosphatidylserine
References	[43–47]	[43,44,48,185,186]	[43,49–52]

Biological composition of exosomes

Exosomes, as membrane-bound vesicles, can encapsulate a range of contents, such as proteins, nucleic acids, and metabolites, within their cargo [25]. The composition of exosome cargo reflects the characteristics of the donor cell and its current physiological condition [26]. While in solution, exosomes typically exhibit a spherical shape, but when subjected to artificial drying during the preparation process, they can take on a bi-concave or cup-shaped appearance [27]. Exosomes primarily include a range of membrane-bound and cytosolic proteins, featuring key representatives from the tetraspanin family (such as CD9, CD63, and CD81), as well as essential components from the endosomal sorting complex required for transport (ESCRT) proteins like Alix and TSG101. Additionally, integrins, heat shock proteins (Hsp), actin, and flotillins are commonly found among their cargo. [28–42]

Table 2. Exosome composition and roles of main component.

Protein Category	Example Proteins	Role	References
Tetraspanins	CD9, CD63, CD37, CD81, CD82, CD53	Exosome biogenesis, cargo selection, targeting and uptake	[53,54]
ESCRT Machinery/MVB Biogenesis	Alix, TSG-101	Exosome biogenesis	[53,55]
Heat Shock Proteins (Hsp)	Hsp90, Hsc70, Hsp60, Hsp20, Hsp27	Exosomes release, signalling	[56–58]
Membrane Transport and Fusion	GTPases, Annexins, Flotillin, Rab GTPases, dynamin, syntaxin	Exosome secretion and uptake	[59–62]
Major Histocompatibility Complex (MHC) Molecules	MHC Class I, MHC Class II	Antigen presentation to generate immunological response	[63,64]
Cytoskeletal Proteins	Actin, Cofilin, Tubulin	Exosome biogenesis and secretion	[64–66]
Adhesion Proteins Glycoproteins	Integrin- α , - β , P-selectin β -galactosidase, O-linked glycans, N-linked glycans	Exosome targeting and uptake Exosomes targeting and uptake	[64,65] [67,68]

Growth Factors and Cytokine		TNF- α , TGF- β , TRAIL	Exosome targeting and uptake, signalling	[69,70]
Other Receptors	Signalling	Fas ligand (FasL), TNF receptor, Transferrin receptor (TfR)	Exosome targeting and signalling including apoptosis induction and iron transport	[64,71,72]

Exosome biogenesis

Initially, there was a belief that exosomes were extracellular debris released by reticulocytes, which were thought to be subsequently degraded by lysosomes (Kruh Garcia et al., 2015). However, recent research has revealed that exosomes actually originate from the endocytic pathway (Lässer, 2015; Hessvik and Llorente, 2018). Furthermore, the conventional process of exosomal biogenesis involves the formation of intraluminal vesicles within multi-vesicular bodies before their release into the extracellular environment (Alenquer and Amorim, 2015; Yue et al., 2020). Exosomes are generated through three primary mechanisms (DM and SJ, 2019): (1) Vesicles bud into distinct endosomes, which subsequently mature into multivesicular bodies and release exosomes as they fuse with the plasma membranes. (2) Direct vesicle budding allows for immediate exosome release from the plasma membrane. (3) A delayed release occurs when exosomes bud within the intracellular plasma membrane-connected compartment (IPMC), followed by contraction of the IPMC neck. During exosome biogenesis, a wide range of proteins, including membrane transporters and heat shock proteins, are loaded into these vesicles (Jones et al., 2018). Exosomes also contain various non-coding RNAs, such as long non-coding RNAs (lncRNAs), microRNAs (miRNAs), and circular RNAs (circRNAs) (Li et al., 2021). They are commonly found in bodily fluids like urine, blood, milk, and saliva (Qin and Xu, 2014; Sun et al., 2018). Exosomes are transported throughout the body, akin to viral particles, via the bloodstream to exert their biological effects. They can be taken up by recipient cells through processes like macropinocytosis, phagocytosis, clathrin-dependent endocytosis, and clathrin-independent endocytosis (van Dongen et al., 2016). Target cells typically recognize and capture exosomes in three ways: (1) through the binding of exosomes or their released substances to ligands on the cell membrane's surface, (2) via endocytosis of exosomes by recipient cells, and (3) by fusion of exosomes with the membranes of target cells. Exosomes primarily induce biological effects by facilitating the transfer of bioactive materials, including proteins and miRNAs, between cells, including glia cells (Shin et al., 2014).

Correlation between exosomes and the process of adult neurogenesis

It is well-recognized that neural stem cells (NSCs) are located in specific regions of the adult mammalian brain where they undergo proliferation, leading to the generation of new neurons—a phenomenon referred to as adult neurogenesis (Matsubara et al., 2021). Approximately 9,000 new cells are believed to be produced every 25 hours in the rat hippocampus, as reported by Cameron and McKay in 2001. In humans, the initial direct evidence of adult neurogenesis was acquired through the examination of post-mortem brain tissue from cancer patients who had undergone treatment with the thymidine analog bromodeoxyuridine (BrdU or 5-bromo-2'-deoxyuridine), as described by (Eriksson et al. in 1998). The control of adult neurogenesis is a multifaceted process encompassing extracellular factors and intracellular mechanisms within the brain. This complexity makes it a prominent focus of research. In the majority of mammalian brain regions, neurons are established before birth, with each neuronal population being incorporated during distinct developmental stages, as highlighted by (Cameron and Glover in 2015). Neural stem cells (NSCs) are characterized by two key features: their ability to undergo cell proliferation and their capacity to generate three distinct neural lineages, namely, neurons, astrocytes, and oligodendrocytes, as outlined by Gonçalves et al. in 2016. Neural stem cells (NSCs) have the ability to divide either symmetrically or asymmetrically, with the latter process being the predominant mode of division. Studies have demonstrated that when a single NSC undergoes symmetrical division, it yields both progenitor cells and an immature neuron, as observed by (Bonaguidi et al. in 2011). Immature neurons migrate to either the dentate gyrus (DG) or the olfactory bulb (OB) where they undergo

further differentiation and maturation into their respective neural cell types, as described by (Cope and Gould in 2019) Numerous investigations have established that the control of neurogenesis relies on the microenvironment, commonly referred to as the neurogenic niche, surrounding neural stem cells in the brain. This specialized microenvironment plays a pivotal role in promoting NSC proliferation and their differentiation into neurons. In contrast, recent research has shown that exosomes derived from non-niche cells also play a role in regulating neuroinflammation. This regulation can have positive effects on the environment that supports the neurogenic niche, bolster neurogenesis, and potentially hold therapeutic promise for various neurological disorders. This insight has been explored in studies by (Luarte et al. in 2016, Yang et al in 2017), and (Nasirishargh et al. in 2021) As an illustration, research has demonstrated that exosomes released by somatic cell-induced neural progenitor cells (NPCs) and normal NPCs play a role in governing neuronal differentiation and fostering neural regeneration through the action of miR-21a, as indicated by Ma et al. in 2019. Additionally, a separate study identified several miRNAs, including miR-125, miR-145, miR-18, and miR-21, which are closely associated with the process of adult neurogenesis within exosomes derived from human mesenchymal stem cells. This insight was highlighted in the work of (Lojewski et al. in 2014). The researchers conducted an investigation into the potential impacts of these exosomes on the differentiation of multipotent neural stem cells (NSCs). They observed that the mRNA levels of the neural progenitor cell (NPC) marker nestin increased in NSCs exposed to exosomes from various human mesenchymal stem cell sources. These findings underscore the substantial therapeutic promise of these exosomes for a wide range of neurological disorders.

The modulation of neurogenesis through exosomes in the context of Parkinson's disease (PD).

Parkinson's disease (PD), the second most prevalent neurodegenerative condition following Alzheimer's disease, is characterized by a clinical presentation featuring symptoms such as tremors, rigidity, bradykinesia, and postural instability. The pathological aspects of PD encompass the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc), the pronounced misfolding and abnormal accumulation of α -synuclein (α -syn) in the remaining dopaminergic neurons, and the formation of Lewy bodies, as documented by (Mor et al. in 2016). The periventricular region of the tegmental aqueduct (Aq-PVRs), situated in close proximity to the substantia nigra pars compacta (SNpc), is believed to harbor dormant neural progenitors with the potential to differentiate into dopaminergic neurons, as suggested by (Marchetti et al. in 2020). In instances of PD-related injury, adult neural stem cells (NSCs) from the Aq-PVR region can be activated and induced to develop into dopaminergic (DAergic) neurons, both in controlled laboratory conditions (in vitro) and within living organisms (in vivo), as demonstrated by (Marchetti et al. in 2020). This underscores the importance of adult hippocampal neurogenesis in the context of Parkinson's disease. The protein α -synuclein, well-known for its role in adult neurogenesis, plays a critical role in the onset of PD and Lewy body dementia, as detailed by (Hall et al. in 2014). α -synuclein, when released into the extracellular space, can be taken up by neurons, neural progenitor cells (NPCs), and astrocytes, as shown in the study by (Lee et al. in 2010). Research using transgenic rat models of PD has revealed that the accumulation of α -synuclein and disruptions in 5-HT neurotransmission adversely impact hippocampal neurogenesis in PD. Notably, these effects may occur prior to the emergence of protein aggregation and motor impairments associated with the disease, according to the findings reported by (Kohl et al. in 2016). Additionally, in the midbrain aqueduct region, there is a continued presence of markers such as Lmx1a and other progenitor markers, even after the typical period of dopaminergic neurogenesis. Studies have indicated that dopamine receptor antagonists can stimulate the proliferation of these markers and enhance neurogenesis, as evidenced in the research conducted by (Hedlund et al. in 2016). Furthermore, research has identified several factors that have been identified as contributors to impaired neurogenesis in Parkinson's disease (PD), including the abnormal accumulation of α -synuclein, oxidative stress, disruptions in calcium homeostasis, and impaired axonal transport, as highlighted in the research conducted by (Kline et al. in 2021). The active role of exosome-mediated communication between glial cells and neurons in these processes underscores the crucial role of this mechanism in regulating neurogenesis in PD. Compelling evidence suggests

that α -synuclein aggregates can prompt microglia to release exosomes containing α -synuclein oligomers. These exosome-associated α -synuclein oligomers are more likely to be taken up by neighboring cells and exert higher levels of toxicity compared to free oligomers, as initially observed by (Danzon et al. in 2012). Moreover, subsequent research has indicated that pro-inflammatory cytokines have the capacity to stimulate microglia to release increased amounts of exosome-bound α -synuclein, thereby intensifying the inflammatory response in the brain, as demonstrated by (Guo et al. in 2021). In such instances, exosomes may potentially play a detrimental role in facilitating the intercellular spread of α -synuclein oligomers, thereby impeding native neurogenesis due to the toxic nature of exosomal α -synuclein oligomers. To gain a comprehensive understanding of the role of exosome-associated α -synuclein in regulating adult neurogenesis in PD, further in-depth studies are warranted. Researchers have also explored the utility of exosomes derived from various stem cells in vitro to mitigate the aggregation of α -synuclein and impede the degenerative process in dopaminergic neurons. For example, specific studies have suggested that exosomes hold significant therapeutic potential for enhancing neurogenesis within the neural niche. Exosomes sourced from bone marrow mesenchymal stem cells have demonstrated the ability to reverse the pathological characteristics of PD by reshaping the inflammatory microenvironment in the substantia nigra pars compacta (SNpc) region and repairing damage to dopaminergic nerve cells, as evidenced in the research conducted by (Li et al. in 2022). Furthermore, research findings have indicated that astrocyte atrophy in the early stages of Parkinson's disease (PD) may be linked to disruptions in exosome biogenesis within the neural niche. Nevertheless, the exact role of astrocyte atrophy in disease progression remains incompletely understood, as discussed by (Gómez-Gonzalo et al. in 2017). Previous studies involved the generation of exosomes and microvesicles from dental pulp stem cells, aiming to assess their potential in safeguarding human dopaminergic neurons from oxidative stress induced by 6-hydroxydopamine treatment, as carried out by (Jarmalavičiūtė et al. in 2015). Exosomes derived from dental pulp stem cells are regarded as promising therapeutic tools for Parkinson's disease (PD). This optimism is based on the findings that when human dopaminergic neurons were exposed to these exosomes, a significant reduction in apoptosis triggered by 6-hydroxydopamine was observed, highlighting their potential as an innovative approach to treating PD.

Table 3. Exosome protein as biomarkers for Parkinson disease.

Diseases	Exosome Sources	Biomarkers	References
PD (Parkinson's Disease)	Blood, CSF	α -syn (alpha-synuclein), DJ-1	Shi et al., 2014; Zhao et al., 2018

Conclusion

In conclusion, the remarkable role of exosomes in intercellular communication has transformed our understanding of cellular interactions, particularly in the intricate landscape of adult neurogenesis. These extracellular vesicles, with their diverse cargo of proteins, nucleic acids, and bioactive molecules, wield significant influence in the generation of new neurons within the adult mammalian brain. Their origins from non-niche cells highlight their substantial contribution to the regulation of neuroinflammation, thereby impacting neurogenesis and holding therapeutic promise for neurological disorders. In the specific context of Parkinson's disease (PD), exosomes play a multifaceted role, being prominently implicated in the propagation of α -synuclein oligomers and the orchestration of neuroinflammation. Notably, stem cell-derived exosomes emerge as promising therapeutic agents, capable of reshaping the microenvironment, repairing neuronal damage, and mitigating apoptosis. As we delve deeper into the exploration of exosomes, ongoing research continues to unravel intricate biological processes, paving the way for innovative medical interventions and treatments, particularly in the challenging landscape of neurodegenerative

diseases like PD. The comprehensive understanding of exosomal functions opens up new avenues for intervention and treatment strategies in the realm of neurodegenerative disorders. This knowledge not only enriches our understanding of the physiological and pathological processes underlying these conditions but also holds the potential to advance therapeutic approaches. As we continue to unveil the mysteries of exosomes, the future promises innovative medical interventions that could significantly impact the lives of those affected by neurodegenerative diseases, offering hope for improved outcomes and quality of life.

Conflicts of Interest: No conflict of interest.

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