Review

Emerging roles of extracellular vesicles in neurodegenerative disorders: focus on HIV-associated neurological complications

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Exosomes are membrane-enriched extracellular vesicles with a proposed diameter in the range of 30–100 nm. They are released during both normal homeostasis as well as under pathological conditions by most cell types. In recent years, there has been robust interest in the study of these vesicles as conduits for the delivery of information between cells in both analogous as well as disparate tissues. Their ability to transport specialized cargo including signaling mediators, proteins, messenger RNA and miRNAs characterizes these vesicles as primary facilitators of cell-to-cell communication and regulation. Exosomes have also been demonstrated to have important roles in the field of cancer biology and metastasis. More recently, their role in several neurodegenerative disorders has been gaining increased momentum as these particles have been shown to promote the spread of toxic factors such as amyloid beta and prions, adding further validity to their role as important regulators of disease pathogenesis. This review briefly summarizes current findings and thoughts on exosome biology in the context of neurodegenerative disorders and the manipulation of these particles for the development of potential therapeutic strategies.
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Facts

- Exosomes are globular, membrane-bound extracellular nanovesicles (30–100 nm in diameter) that are released by almost all types of cells.
- ARF6 and PLD2 have important roles in extracellular vesicle release through the regulation of the budding of ILVs into MVBs.
- Extracellular vesicular molecules (including ADAM17, TNFα and Nef) released from HIV-infected cells induce activation, apoptosis and HIV susceptibility in the recipient cells.
- Extracellular vesicles released from CD8^{+} T cells contain antiviral membrane-bound factors that inhibit HIV-1 transcription.

Open questions

- Are HIV proteins such as Tat /gp120 released in the extracellular vesicles and if so, do they disseminate CNS toxicity?
- What is the role of EVs in propagation of pathogenic proteins in the neurodegenerative disorders?
- How can extracellular vesicle therapeutics be applied in the context of neurodegenerative diseases?

Cellular cross talk underlies most pathological conditions including those within the central nervous system (CNS). Although various factors have been identified as instigators of disease pathogenesis, it is now becoming clear that unrestrained neuroinflammation and, subsequent cellular toxicity are the key hallmark features of various neurological disorders. In this light, the notion that disease pathogenesis may be accelerated or mediated by exosomes and their associated cargos is recently gaining momentum. Exosomes are globular; membrane-bound extracellular nanovesicles (30–100 nm in diameter) that are released by almost all types of cells during normal cellular functioning and specifically, in response to cellular stressors. These small vesicles originally thought to contain 'junk' cellular debris were first described by Trams *et al.*[1] when they observed smaller membrane-bound vesicles within the larger endosomes (later termed multi-vesicular bodies (MVBs). An electron micrographic study related the

exosomes to sheep reticulocytes.[2] The release of these small vesicles into the extracellular environment was proposed as a mechanism by which reticulocytes could secrete transferrin receptor. This proposed mechanism was further supported by *in vitro* analysis of sheep reticulocytes, which demonstrated the selective loss of certain proteins from maturing cells.[3] An understanding of the role of exosomes in various cell types has evolved greatly. They are no longer viewed as waste bags; instead, exosomes are thought to have an important role as cargo-carrying vesicles mediating communication among different cells and tissues including the CNS.[4] Exosomes are known to carry nucleic acids (RNA, microRNAs (miRNA) and DNA), functional proteins (including those of viral origin) and other cellular products. In the literature extracellular vesicle (EV) subtypes have often been given names such as exosomes, microvesicles, ectosomes or microparticles based on their biogenesis, physical characteristics (such as size), or function.

A growing body of evidence suggests the involvement of exosomes in many neuroinflammatory diseases. These small vesicles are important in CNS communication as most CNS cells secrete these particles.[4] Cell–cell communication via exosomes can be envisioned to have an important role in pathogenesis through their ability to transmit disease-causing agents from one cell to the other. Indeed, exosomes have been associated with numerous neuro-inflammatory diseases including Parkinson's, Alzheimer's and Creutzfeldt–Jakob diseases. Further research into the role of these vesicles in disease progression is important for the development of effective preventative and therapeutic options. The focus of this review is to examine the role of exosomes in the progression of various neurodegenerative disorders.

Exosome Cargo

Exosomes are generated via inward budding of the late endosomal membrane with the newly formed intraluminal vesicles (ILVs) destined for one of the two outcomes: either the late endosome merges with a lysosome, which degrades the ILVs along with their cytoplasmically derived cargo or the late endosome binds to the plasma membrane and releases the ILVs as exosomes with their cargo into the extracellular environment. Specifically, lysosome-directed vesicle formation occurs via the endosomal sorting complexes required for transport (ESCRT) machinery, whereas exosome budding is directed by the sphingolipid ceramide on the membrane of the endosome.[5] Recent studies have shown that the small GTPase ADP ribosylation factor 6 (ARF6) and its effector phospholipase D2 (PLD2) regulate exosome release.[6] ARF6 and PLD2 function together to regulate the budding of ILVs into MVBs.[6] After formation of ILVs, exosomes are released from the cell upon fusion of the late endosome with the plasma membrane. These vesicles are then free to carry their cargo throughout the surrounding environment and are associated with juxtacrine, paracrine and endocrine uptake in the host tissue (see the detailed reviews discussing exosome biogenesis:[7,8] (Figure 1a). The specific composition of an exosome largely depends upon the originating cell and can vary widely depending on the cellular and environmental factors. Large-scale proteomic and phosphoproteomic studies of exosomes derived from various cell types suggest that these vesicles shuttle a wide array of biologically relevant molecules, including lipids, carbohydrates, RNAs and proteins.[9] For example, the study from Knepper's group identified 1132 proteins contained within exosomes isolated from urine. In addition, unique phosphorylation sites have also been identified on exosomal proteins.[10] Interestingly, miRNAs are abundantly present in the exosomes.[11] MiRNAs regulate gene expression at the post-transcriptional level by binding to the 3'-UTR and/or the coding regions of their target mRNAs.[12] In fact, hundreds of miRNAs have been found in exosomes.[13] Many cell types, including reticulocytes, epithelial cells, neurons and tumor cells, have been reported to deliver exosomal miRNAs to recipient cells.[14,15] In addition, Epstein–Barr virus (EBV)-infected cells have been shown to secrete exosomes containing EBV-encoded miRNAs.[16] Importantly, exosomal miRNAs can repress mRNAs in target cells and subsequently influence target cell function. Furthermore, these exosomal miRNAs have been implicated in a number of cellular processes and human diseases including cell migration, cell differentiation, cell viability, aging, neurodegeneration, cancer and immune disorders.[8] These studies support the notion that exosomes obtained from body fluids have the potential to serve as biomarkers of disease development and/or progression. In this regard, Witwer *et al.*[17] have suggested the need for standardization of specimen handling, appropriate normative controls, and isolation and analysis techniques for EVs/exosomes to facilitate comparison of results.

Mechanism(s) of Exosome Interactions with Recipient Cells

A primary function of exosomes is their ability to deposit their cargo inside a recipient cell. Although many roles of exosomes have been extensively reported in the literature, detailed interactions between target cells and exosomes remain to be elucidated. Currently, it is hypothesized that the uptake of exosomes into a target cell occurs by one of the three mechanisms: phagocytosis, receptor-mediated endocytosis (RME) or direct fusion of exosomes with the plasma membrane of the recipient cell[15,18,19] (Figure 1b). The latter mechanism involves the release of exosomal cargo directly into a cell following the fusion of the exosomes with the plasma membrane of the recipient cell. RME, on the other hand, consists of binding of exosomal surface proteins to proteins on the plasma membrane of recipient cells, thereby facilitating the targeting of exosomes to distinct cell types. The exosomes can then either fuse directly with the plasma membrane or follow a different endocytic pathway consisting of fusing with the delimiting membrane of an endocytic compartment (i.e., endosome, lysosome, etc.).[20,21]

The uptake of exosomes by RME in any given cell is largely dependent on the exosomal surface protein and lipid composition, which in turn, is primarily based on the type and condition of the secreting cell. The types of exosomal surface proteins and the kinds of receptors on the target cell determine the interaction between the exosome and the recipient cell. In addition, it has been hypothesized that lipid receptors could
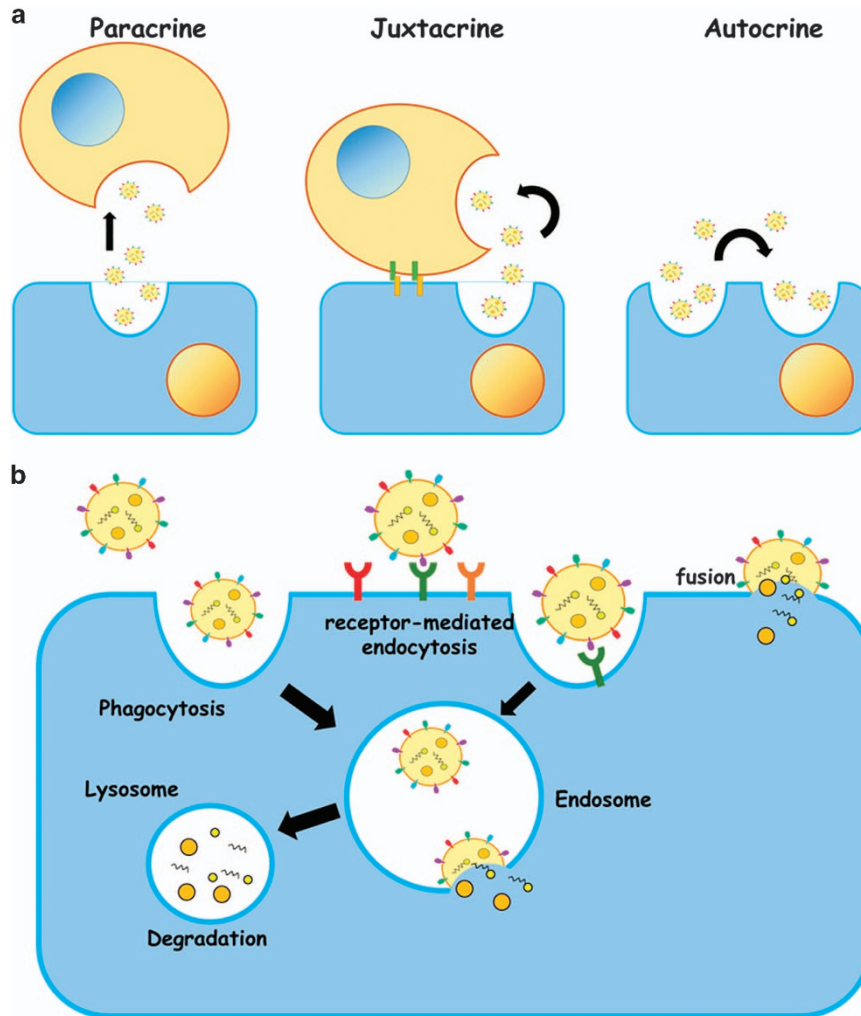
Figure 1 (**a**) Exosomes delivery via various signaling pathways. Exosomes can deliver molecules to distant cells (paracrine), adjacent cells (juxtacrine) or neighboring cells (autocrine). (**b**) The uptake of exosomes into a target cell occurs by one of the three mechanisms: phagocytosis, receptor-mediated endocytosis (RME) or direct fusion of exosomes with the plasma membrane of the recipient cell

also have a vital role in exosome recognition by the target cells. For example, phosphatidylserine (PS) on exosomal outer surface can interact with PS receptors, TIM1 and TIM4,[22] inducing accumulation of neutral lipids in the recipient cells.[23] In regard to the role of proteins in this uptake, an elegant study by Escrevente *et al.*[24] has shown that pretreatment of exosomes and recipient cells with a broad specificity protease K significantly decreased exosomal uptake efficiency. This study concluded that proteins from both exosomes and cells were required for uptake.[24] It has also been shown that heat-shock proteins such as Hsp90/Hsp70, which are chaperone proteins enriched in exosomes can interact with receptors such as low-density lipoprotein receptor-related protein 1 (LRP1), and that this interaction is critical for recognition of exosomes by target cells.[25] These findings further validated the notion that the interaction between exosomal membrane proteins and recipient cell-surface proteins is fundamental for the uptake of exosomes via RME. Upon further inquiry, it was shown that blocking CD9

and CD81 tetraspanins, which are commonly expressed on exosomes, resulted in a significant decrease in the exosomal uptake efficiency by dendritic cells.[26]

It has been demonstrated that phagocytic cells including RAW 264.7 macrophages and U937 monocyte-derived macrophages (MDM) internalize exosomes via phagocytosis.[27] More specifically, Feng *et al.*[27] demonstrated that macrophages internalized exosomes more efficiently than other non-phagocytic cells. Using electron microscopy, it was observed that exosomes remained localized on the surface of non-phagocytic cells, whereas exosomes in the vicinity of phagocytic cells were enveloped into large cellular extensions. Furthermore, the colocalization of fluorescent phagocytic tracers and the PKH26-dyed exosomes further confirmed that phagocytosis was the primary exosomal uptake mechanism employed by the macrophages. Furthermore, actin polymerization, which is necessary for phagocytosis, when inhibited by either cytochalasin D or latrunculin B, resulted in significantly reduced efficiency of exosomal uptake. This

further reinforced the idea that phagocytosis is an important mechanism for exosome internalization in the macrophages and likely also in other phagocytic cell types.

It has also been documented that oligodendroglia-derived exosomes can be taken up by microglia via macropinocytosis.[28] Furthermore, the authors also demonstrated that oligodendroglia-derived exosomes colocalized with Lamp1, a lysosome or late endosome marker, indicating thereby that an endocytic pathway was involved in the uptake of these exosomes. In order to deduce that macropinocytosis was indeed the mechanism in question, the microglia were treated with several reagents that interfered with specific steps in the macropinocytosis pathway. Following inhibition of macropinocytosis, microglial uptake of exosomes was inhibited, thereby underscoring the role of macropinocytosis in internalization of exosomes by microglia.[28]

HIV Budding and Exosome Biogenesis

HIV acquires its envelope and propagates infection by budding through the limiting membranes of infected cells. HIV usurps a cellular pathway – that of formation of ILVs into MVB, to facilitate budding, indicating thereby that HIV budding and exosome release share a common mechanism. Indeed, published data on HIV-infected macrophages demonstrate the presence of HIV-containing vacuoles in macrophages that are reminiscent of MVBs.[29] Using human MDMs, the study by Nguyen *et al.*[30] revealed that the host protein profile of macrophage-derived exosomes and that of the HIV particles have a strong concordance, supporting the hypothesis that retroviral budding results from the exploitation of a pre-existing cellular pathway of intercellular vesicle trafficking. Proteomic analyses revealed that MDM-derived HIV virions contained 26 of 37 cellular proteins previously found in exosomes, consistent with the idea that HIV uses the late endosome/MVB pathway during virion budding from macrophages.[21] In addition, Bieniasz *et al.*[31] demonstrated that ESCRT components are recruited by HIV gag at the plasma membrane site of HIV budding. Nabhan *et al.*[32] revealed a role for ESCRT components in ectosomes budding at the plasma membrane. It is thus speculated that the budding of virus and EVs could utilize similar cellular pathways at the plasma membrane and inside the MVBs. An excellent review on the similarities between EVs and viral entry has been published and could provide further insights on this phenomenon.[33] Furthermore, it is also interesting to note that the HIV-1 virion has a similar size to that of the exosomes, an evidence supporting a similar origin of the two. This, however, also poses a potential confound for the isolation of exosomes from HIV-1-infected materials.[34] In addition, studies on T cells and exosomes have been inconsistent and confounding. For example, some studies implicate that HIV-1 budding does not involve either endosomes or exosomes,[34–36] whereas other reports have shown that HIV-1 budding from T cells is closely associated with exosomes.[37] In this regard, Park and He[34] have shown that high-speed centrifugation with 20% sucrose cushion during the last step can yield exosome-free HIV-1 virions compared with centrifugation only. Herein the authors provided a technical platform that could be employed to define the relationship between exosome biogenesis and budding of

HIV-1.[34] Though the small GTPase ARF6 and its effector PLD2 have an important role in exosome release, ARF6 is not involved in HIV-1 budding.[6] Early studies on HIV budding demonstrated that loss of the viral envelope Gag p6 domain caused a severe defect in virus budding.[38] However, recent studies showed that the N-terminal 433 amino acids of HIV Gag-Pol were sufficient to cause budding from cells, supporting the hypothesis that HIV budding was mediated by the exosome/microvesicle biogenesis pathway.[39] In other cell types such as the dendritic cells, exosomes can be internalized and transfer signaling molecules to the recipient cells. HIV-1 particles exploit this exosome-dissemination pathway to spread infection to the dendritic cells, thereby underscoring a potentially new viral dissemination pathway (Figure 2 and Table 1). Taken together, it remains an exciting question of whether HIV-1 budding and exosomal biogenesis are closely related processes and/or whether they mutually influence the respective processes.

HIV Infection Alters Exosomal Release and Composition

Both exosome release as well as exosomal composition are regulated through cell signaling pathways that are activated by many factors, including but not limited to, HIV infection and subsequent immune activation. For example, it has been shown by Kadiu *et al.* that exosome numbers are increased in MDMs following HIV-1 infection.[40] Furthermore, HIV-1 was shown to accelerate infection and viral dissemination by surrounding itself with exosomes.[40] HIV infection not only affects exosome release, but also impacts exosomal cargo. Indeed, large-scale proteomics studies have revealed that compared with uninfected cells, exosomes released from HIV-1-infected cells harbor distinct regulatory molecules and are composed of a unique and quantitatively different protein signature.[41,42] Fourteen proteins out of 770 were identified to be differentially expressed in the exosomal fractions of HIV-1-infected cells compared with the uninfected cells. Three immunomodulatory molecules included ADP-ribosyl cyclase 1 (CD38), L-lactate dehydrogenase B chain and Annexin A5.[41] Recent studies have found that HIV-1 RNAs, such as HIV miRNA TAR, can also be incorporated into exosomes released from HIV-infected cells.[43,44] Furthermore, TAR RNA is sorted into exosomes in a chromosome region maintenance 1-dependent manner.[44] Importantly, exosomal TAR inhibited apoptosis by downregulating Bim and Cdk9 protein levels in recipient cells,[44] and stimulated proinflammatory cytokines, including IL-6 and TNF-β in primary macrophages.[45] In a separate study, it was also shown that specific miRs such as miR-29b are transported via the EVs from HIV Tat and morphine-treated astrocytes to neurons and that, this transfer resulted in downregulation of PDGF-B (miR-29b target) in neurons, leading to neuronal apoptosis[46] (Figure 3). A recent study by Yelamanchili *et al.*[47] has also suggested increased expression of miR-21 in EVs derived from SIV-infected brains compared with the uninfected controls. Herein the authors demonstrated that in the brains of macaques with SIV-encephalitis, EV-miR-21 from donor macrophages/microglia resulted in neurotoxicity via activation of a TLR7-dependent downstream cell death pathway[47] (Figure 3). HIV-1 infection of MDMs resulted in significant upregulation of a
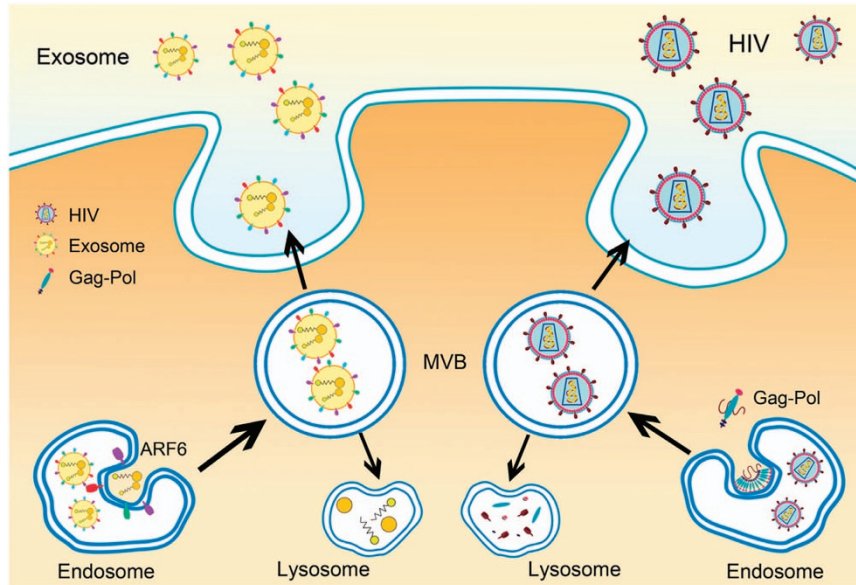
Figure 2 HIV budding is mediated by the exosome/microvesicle biogenesis pathway. HIV budding (left) and exosome release (right) share similar pathways. HIV Gag binds the viral RNA and drags it into the cytoplasmic face of intracellular vesicles, then the virus particles bud into the MVB. Subsequently, the virus-containing vesicles traffic to and fuse with the cell membrane, resulting in the release of virus particles

distinct class of miRNAs in exosomes isolated from the infected cells.[48] Secretion of various HIV proteins has also been reported in the exosomes from infected cells. Specifically, the viral proteins Nef and Gag have also been found in released exosomes.[37,49–54] It was also shown that Nef-containing exosomes were able to fuse with HIV-1 virions and deliver functional Nef to the virions as well as fuse with bystander cells to induce apoptosis in these cells.[50] In addition, it has been shown that HIV Tat is also secreted and is present in exosomes derived from Tat-expressing astrocytes, and HIV-infected cells, and can be taken up by the neurons, leading to neuronal injury and death.[55] A recent study demonstrated that exosomes are also enriched in cytokines in the plasma of HIV-positive individuals relative to the negative controls.[56] These studies provide a basis for exosomes as biomarkers for HIV infection. Elegant work from the study by Sampey *et al*.[137] using hydrogel nanotrap particles as affinity baits, has demonstrated the capture of HIV-1 virions, HIV proteins and exosomes-containing TAR-RNA in the patient serum. This could have ramifications for the future development of HIV diagnostics.[57,58] Further studies aimed at exploring the role of exosomes in the potentiation of HIV-associated complications are important for the development of effective therapies against these disorders.

Functional Effects of EVs in the Context of HIV Infection

It has been reported that the release of exosomes from HIV-1-infected lymphocytes is associated with HIV-1 replication in co-cultured quiescent CD4$^+$ T lymphocytes.[59] Exosomes from HIV-1-infected cells expressing a functionally defective viral mutant can still induce cell activation and lead to HIV-1 susceptibility in unstimulated CD4$^+$ T lymphocytes.[60] A Nef domain, 62EEEE65 acidic cluster, has been identified as a

contributor of these effects.[59,60] Furthermore, active ADAM17 associates with exosomes from HIV-1-infected cells and induces HIV-1 replication in resting CD4+ T lymphocytes, thus stimulating viral spread.[59,60] (Figure 4a). In addition, exosomes released from HIV-1-infected cells can also impact many cellular processes in the recipient cells including proliferation and apoptosis.[41] Specifically, exosomal Nef can enter the target cells and cause activation-induced cell death in resting CD4$^+$ T lymphocytes.[51] This is not surprising since Nef itself has been shown to induce dramatic dysregulation of cellular and exosomal miRNAs in human monocytic cells.[61] Interestingly, exosomes purified from a transformed CD8$^+$ T-cell line have been shown to an antiviral membrane-bound factor that inhibits HIV-1 transcription in both acute and chronic models of infection[62,63] (Figure 4b). Furthermore, a recent study demonstrating the association of cytokines with exosomes in the plasma of HIV-seropositive individuals suggests the role of exosomes in inflammation and viral propagation via bystander cell activation.[56] In summary, alterations in exosomal cargo, following HIV infection contribute to HIV pathogenesis via multiple mechanisms including viral dissemination, cell apoptosis and inflammation.

Alzheimer's Disease and EVs

Alzheimer disease (AD) is the most common neurodegenerative disorder with 46.8 million people affected worldwide, clinically characterized as an ongoing cognitive impairment.[64] Aggregation of hyperphosphorylated tau in the neurofibrillary tangles and accumulation of amyloid beta (Aβ) plaques are the two salient pathological features of AD.[65] Accumulating evidence suggests the involvement of EVs in the pathogenesis of AD.[66–68] Many reports also implicate spread of the pathogenic AD proteins via the EV cargo.[67,69,70] For example,

Table 1 Similarities and differences among HIV and EVs

	HIV	Exosome	Microvesicle, Ectosome	Apoptotic body
Size	90–160 nm	30–100 nm	100–1000 nm	50–5000 nm
Origin cell	Infected cell	Resting or activated cell	Activated or tumor cell	Apoptotic cell
Marker/Protein	Gag, Pol, gp120, Tat, Rev, Nef, Vpr, Vif and Vpu	CD63, CD9, Alix, Tsg101 and HSP70	Annexin V, flotillin-2, selectin, integrin and CD40 metalloproteinase	Annexin V, DNA and histones
Budding mechanism	Exocytosis from MVB	Exocytosis from MVB	Budding directly from a plasma membrane (outward budding)	Programmed cell death (blebbing)
Involved components	ESCRT components, Tsg101, Alix and HIV-1 Gag	nSMASe2, ceramide, ESCRT components and ARRDC1, Tsg101, Alix and tetraspanin	aSMAse2, ceramide, ESCRT components, Tsg101, Vps4 and tetraspanin	Caspase-3, rho-associated coiled-coil-forming kinase I and actin polymerization
Entry mechanism	HIV viral envelope, gp120 and gp41, binding CD4	Direct fusion, phagocytosis and RME (Figure 1).	Direct fusion, phagocytosis and RME (Figure 1).	Phagocytosis
Occur position	Plasma membrane and MVB	Plasma membrane and MVB	Plasma membrane	Plasma membrane

exosomal proteins such as Alix and flotillins have been reported to be localized within the amyloid plaques in the brains of the Tg2576 mice (AD model) as well as in the postmortem tissues of human AD patients.[69,70] In support of this is also another clinical report indicating upregulation of AD pathogenic proteins including P-T181-tau, P-S396-tau and $A\beta_{1-42}$ in the plasma exosomes isolated from AD patients, compared with cognitively normal-matched healthy individuals.[67] Furthermore, mechanistic studies have also implicated that both cleavage and endocytic transportation of amyloid precursor protein (APP) have cardinal roles in packaging $A\beta$ into exosomes for dispersion.[70–72] $A\beta$ is a cleaved product resulting from the cleavage of APP by the β- and γ-secretases.[73] A finding by Rajendran *et al.*[70] demonstrated that $A\beta$ was sorted into MVBs in both HeLa and N2a cells following β-cleavage in the early endosomes. Interestingly, Sharples *et al.*[72] also reported that inhibition of γ-secretase stimulated α- and β-cleavage, leading in turn, to C-terminal fragments of APP (APP-CTFs) in the exosomes. Furthermore, γ-secretase has also been shown to be required for the clearance of APP-CTFs from the endocytic recycling compartments, which constitute a series of perinuclear tubular and vesicular membranes, that regulate recycling of APP-CTFs to the plasma membrane.[74,75] In addition, deficits in retromer, a multimeric complex that mediates retrograde protein transportation from endosome to the trans-Golgi network, has also been shown to promote amyloidogenic APP processing by enhancing interactions between APP and secretase enzymes in the late endosomes.[71] As endocytic trafficking is becoming increasingly recognized as a possible mechanism(s) of AD pathogenesis, examining the role of other endocytic trafficking regulators such as diacylglycerol kinase, Eps15 homology domain and molecules interacting with CasL-like1 (MICAL-L1) in both processing and release of APP via the exosomes can be developed as future areas of research that will provide valuable insights into the pathogenesis of AD.[76–78] Interestingly, aggregated tau protein has also been found to be present in the exosomes in both the *in vitro* taupathy models as well as in the cerebrospinal fluids of early Alzheimer's patients.[68] It is worth noting that microglia have an important role in spreading both $A\beta$ and tau through the EVs.[79,80] The advent and widespread application of new imaging techniques such as super-resolution microscopy and quantitative methodology are also avenues that continue to contribute in our understanding of the molecular mechanism(s) involved in the processing of APP and tau and their roles in the pathogenesis of AD.[75,81]

Parkinson's Disease and EVs

Parkinson's disease (PD), clinically characterized by hypokinesia, rigidity and tremor, is the second most common neurodegenerative disorder. The pathological features of PD comprise of widespread degeneration of dopaminergic neurons and aggregation of Lewy bodies and cytoplasm inclusion bodies of α-synuclein.[82] Interestingly, similar to the spread of pathogenic tau and $A\beta$ proteins, EVs also have crucial roles in the aggregation and the spread of α-synuclein, thereby propagating disease pathogenesis in PD.[83–85]
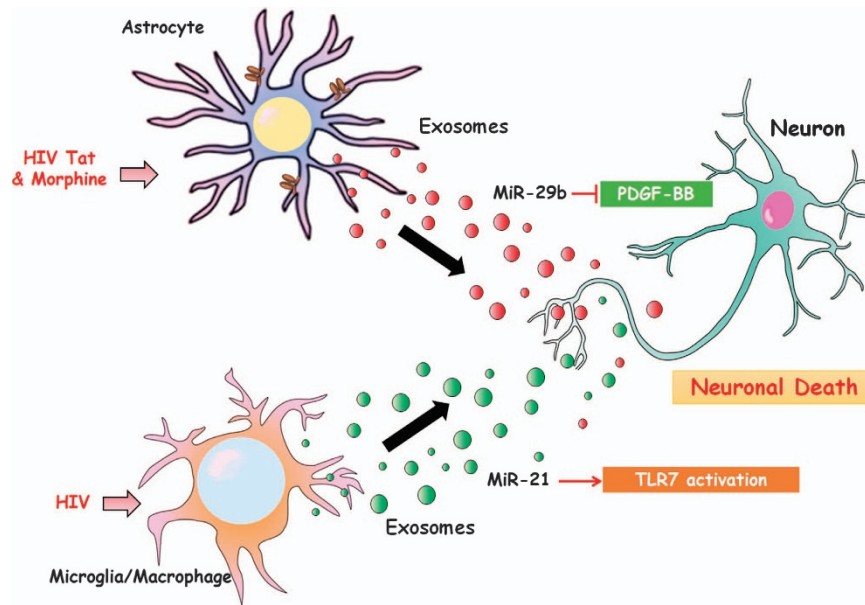
Figure 3 Exosome released from astrocytes treated with morphine and Tat carry miR-29b, which can be taken up by neurons, resulting in neuronal death. MiR-21 in EVs leads to neurotoxicity via the TLR7 signaling pathway

Increased levels of α-synuclein have been reported in the exosomes isolated from both plasma and CSF of PD patients with a significant correlation observed between disease severity and plasma exosomal α-synuclein levels.[85] It has been reported that spread of α-synuclein between neurons via the exosome route confers cytotoxicity to the recipient cells, leading to increased accumulation of Lewy body throughout the various brain regions.[84] Although it is not well understood how this process is regulated, both Tsunemi et al.[86] and Kong et al.[87] suggest the role of P-type ATPase ion pump (PARK9/ATP13A2) in regulating both the biogenesis of exosomes, as well as secretion of exosomal α-synuclein. In case of juvenile-onset PD, that is attributed to loss-of-function mutations in PARK9, it was demonstrated that knocking down of PARK9 resulted in the inhibition of exosomal secretion of α-synuclein, and that, reciprocally, overexpression of PARK9 resulted in localization of PARK9 in MVBs and was associated with release of α-synuclein from the exosomes.[86,87]

Another molecular mechanism regulating the exosomal release of α-synuclein is the autophagy-lysosomal pathway (ALP) that degrades the enclosed cargo in the lysosome.[88–90] It can be speculated that exosomal release of α-synuclein is likely an adaptive response to insufficient autophagic activity needed for the elimination of α-synuclein.[89] Using bafilomycin A1, a pharmacological inhibitor that disrupts ALP by blocking fusion between autophagosomes and lysosomes, Alvarez-Erviti et al.[91] demonstrated that disruption of lysosomal functions resulted in enhanced exosomal release and uptake of α-synuclein in SH-SY5Y cells. In line with this, Poehler et al.[88] also observed that ameliorating cytosolic accumulation of α-synuclein in bafilomycin A1-treated α-synuclein-transgenic mice, resulted in enhanced exosomal release of α-synuclein, which in turn, resulted in neuroinflammation and

cellular damage. It is also worth noting that there is evidence suggesting that the gangliosides present in the exosomes can contribute to a catalytic environment leading to increased aggregation of α-synuclein.[83]

The role of glial cells in mediating neuroinflammatory responses leading to neurodegenerative disorders has been well documented.[92–94] It is not surprising, therefore, that microglial/monocytes also have a role in the PD through the regulation of exosomal activities.[95] For example, Chang et al.[95] found that exosomes derived from α-synuclein-exposed BV-2 microglia contained high levels of MHC class II and membrane TNF-α and, that treatment of rat cortical neurons with these activated exosomes resulted in the neuronal apoptosis.

Amyotrophic Lateral Sclerosis and EVs

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder often accompanied with frontotemporal dementia.[96] In the United States ALS affects ~3.9 cases per 100 000 individuals, with increased prevalence in persons aged 60–69 years.[97] Loss of spinal cord motor neurons is the prominent pathological feature of ALS that manifests as muscle weakness and respiratory failure.[98,99] Several genes have been found to be associated with ALS including superoxide dismutase-1 protein (SOD1), RNA-binding protein fused in sarcoma and TAR DNA-binding protein 43 (TARDBP).[100,101]

Similar to Aβ, mutations of SOD1 in ALS result in aggregation of intracellular misfolded SOD1 protein and its spread.[102] Both mutant and wild-type SOD1 have been shown to be transmitted through both micropinocytosis of the released protein aggregates from dying cells as well as by uptake of SOD1 in the EVs by the recipient cells.[102] Secretion
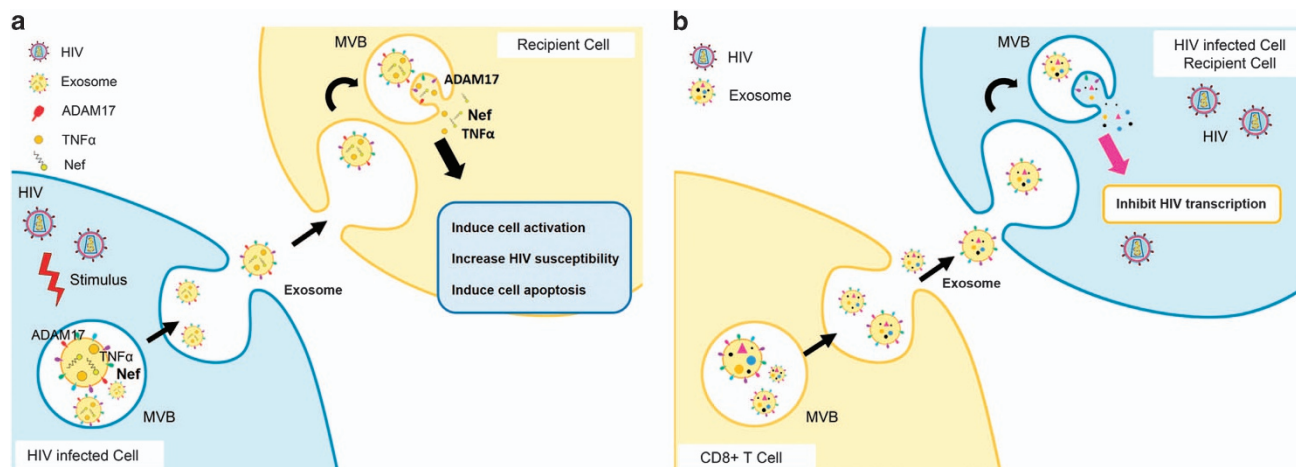
Figure 4 The exosome response to HIV infection. (**a**) Exosomes from HIV-infected cells contain various molecules including ADAM17, TNFα, Nef and so on. These molecules upon entering the recipient cells then induce the target cell activation, apoptosis and HIV susceptibility. (**b**) Exosomes released from CD8$^+$ T cells contain an antiviral membrane-bound factor that inhibits HIV-1 transcription

of exosomal SOD1 has also been observed in a widely used *in vitro* model of ALS that of mouse motor neuron-like NSC-34 cells overexpressing the mutant human SOD1 (G93A).[103,104] Moreover, it has also been reported that mutant SOD1-containing exosomes released from the astrocytes exerted neurotoxicicty.[105] Mechanisms by which SOD1 is packaged into the exosomes, however, remain elusive. Intriguingly, the expression of mutant SOD1 (G127X, G85R) has been reported on the surface of exosomes,[104] whereas that of the wild-type SOD1 is in the lumen of the exosomes.[106,107] Upon uptake by recipient cells, mutant SOD1 proteins function as templates for further misfolding and aggregation of the natively folded counterparts.[102] Studies by Grad *et al.*[104] reported inhibition of the conversion of SOD1 into its misfolded form in the recipient cells via the treatment of exosomes with antibodies specific for mutant SOD1, indicating thereby the therapeutic potential of drug delivery by exosomes.

Similarly, TDP-43 aggregates have also implicated to be released in the EVs and to initiate the intracellular aggregation of TDP-43 in the recipient cells.[108] Although TDP-43 aggregates are present in both microvesicles and exosomes, microvesicular TDP-43 has been shown to be the favored source for uptake by the recipient cells, leading in turn, to increased toxicity compared with the free TDP-43.[109] In the same study it has also been suggested that TDP-43 could also spread trans-synaptically in both the anterograde and retrograde manner. In addition, it was also shown that neurons exposed to either TDP-43-containing media (derived from cultured cells) or to brain lysates from ALS patients did take up TDP-43.

Prion Disease and EVs

Prion diseases are a spectrum of transmissible lethal neurodegenerative diseases characterized by cognitive impairment, motor dysfunction, spongiosis, astrogliosis and cerebral deposition of insoluble PRNP (prion protein).[110,111]

In prion diseases, the native form of prion protein PRNP^C is converted into PRNP^{SC}, a pathogenic form that is protease-resistant and prone to aggregation.[112]

PRNP has been identified in the EVs derived from both CSF[113] and blood.[114] Accumulating evidence further suggests a key role of EVs in both the pathogenesis and propagation of prion diseases.[115–117] One of the initial studies has demonstrated that PRNP-expressing cell line (RK13) robustly secreted PRNP through exosomes.[118] Vella *et al.*[119] further showed that exosomes released from PRNP-infected neuronal cell line (GT1-7) also induced prion propagation in both neuronal as well as non-neuronal cells. Guo *et al.*[120] have shown that inhibiting exosome release reduces the intercellular transmission of PRNP in different PRNP-expressing cell lines *in vitro*. Taking advantage of pharmacological inhibitors and genetic approaches, the same group also demonstrated that neutral sphingomyelinase pathway has a crucial role in regulating the packaging of PRNP into exosomes.[121] Consistent with these *in vitro* findings, it has also been shown that EVs derived from plasma of mice infected with variant Creutzfeldt–Jakob disease (vCJD), one of the prion diseases, contains PRNP.[122] Due to the fact that transmission of vCJD has also been clinically reported to be associated with blood transfusion,[123] it is plausible to hypothesize that EVs might have a significant role in the transmission of prion diseases. Interestingly, upregulated miRNAs including let-7b, miR-146a, miR-103, miR-125a-5p and miR-342-3p were found to be present in the EVs isolated from human tissue samples affected with prion diseases,[124] suggesting thereby that PRNP-infected EVs also mediate the pathogenesis of prion disease through their contents in addition to spreading of PRNP.

However, much still remains to be discovered about the role of exosomes in prion diseases. It has been demonstrated that different strains of PRNP are secreted differentially from RK13 cell line possibly through various disparate cellular mechanisms.[125] A better understanding of the role of EVs in prion diseases is thus critical in dissecting mechanism(s)

underlying disease pathogenesis while also providing insights for other prion-like diseases such as AD and PD.[126]

Exosomes as Therapeutic Conduits

Exosomes via their ability to deliver specific cargo are critical for cellular communication and physiology. This property of exosomes can also be exploited as a treatment strategy to deliver specific therapeutic molecules to the diseased tissues. An elegant study by Zhuang *et al.*[127] analyzed the potential for exosome therapeutics, through a noninvasive nasal delivery method, for the treatment of neuroinflammatory diseases. Herein the authors showed that exosome-mediated delivery of curcumin and an inhibitor of signal transducer and activator of transcription selectively and rapidly targeted the microglia, and significantly mitigated disease pathogenesis in three experimental models of neuroinflammation. This study highlights a novel and a noninvasive therapeutic approach for drug delivery into the CNS. A review by Andaloussi *et al.*[128] covers the potential for exosomes as a delivery system for transporting siRNAs across biological barriers such as the blood–brain barrier. Exosomes-containing siRNAs could provide a means of targeted therapeutic delivery into areas that are difficult to traverse, such as the brain.[129,130] The ability to non-invasively deliver therapeutic exosomes to the CNS, with cell-specific targeting, could provide a potential therapeutic approach for the treatment and eradication of HIV-associated neurocognitive disorders by targeting the latent reservoirs in the CNS. Indeed, the application of exosomal delivery of natural HIV-defense molecules to host cells has been shown to establish HIV resistance to uninfected cells of *vif*-deficient HIV infection.[131] Combining noninvasive therapies with siRNA targeting to HIV-1/HIV proteins could, thus, provide new therapeutic approaches to treat HIV-associated end-organ pathologies. Delivery of HIV gene-specific siRNA to infected cells could control virus infection and could be considered as adjunctive treatment(s) in combination with other therapeutic modalities. An elegant review by Vlassov *et al.*[132] covering a broader view of exosome therapeutics, including their use against tumors, sets the stage for future work. Further studies on specific applications of exosome-mediated therapeutic delivery to specific cells are warranted in the field.

Intriguingly, assessment of the specific, signature contents of disease-specific exosomes could provide useful information for future development of exosome-based therapeutics. Indeed, exosomes from milk, but not plasma, have been shown to contain inhibitory factors for HIV-1 infection of monocyte-derived dendritic cells (MDDCs) and for the subsequent viral transfer to CD4 T cells through binding of MDDCs via DC-SIGN.[133] In addition, there are several reports highlighting the potential for delivery of modified miRNAs and normal siRNAs to specific targets via the exosomes.[129,130,134,135] For more details please refer to the comprehensive review on the specific potential of targeting HIV and exosomal miRNAs as therapeutic approaches.[8,136,137]

Besides the targeting of exosomal contents, the exosome itself could be a potential therapeutic target. It is well documented that accumulation of reactive oxygen species underlies PD-associated neuroinflammation and neurotoxicity.[138] Exosomes loaded with either antioxidant enzyme catalase or a plasmid DNA-encoding catalase have been used to reduce neuroinflammatory responses and exert neuroprotective effects.[139,140] Similarly, neurotropic factors that improve neuronal functioning can also be developed as potential therapeutic cargos for exosomal delivery. Along these lines Zhao *et al.* have demonstrated that delivery of macrophage exosomes containing glial cell line-derived neurotropic factor ameliorated neurodegeneration and neuroinflammation in PD mice.[141]

Conclusions

In summary the exponential growth in our understanding of exosome biogenesis, composition, function and their use continues to provide new insights into the normal physiology as well as disease processes. These small vesicles are secreted by many cell types, including all of the CNS cells, and are a key component for cell–cell communication. They have crucial roles in various diseases including cancer metastasis. This review focuses mainly on the possible link between exosomes, HIV-1 pathogenesis and HIV-associated CNS disease. The ability to target exosomes involved in HIV-1 pathogenesis could provide a new means of controlling infection, which in turn, could help to curtail many of the commonly associated complications of the CNS. Further studies are needed concerning the application of exosome therapeutics, involving the use of these vesicles as a drug delivery conduits and as therapeutic targets themselves, in the context of battling HIV-1 infection and its associated end-organ pathologies.

Conflict of Interest

The authors declare no conflict of interest.

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