REVIEW

OPEN

Exosomes in HIV infection

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Purpose of review
The exosomes play a critical role in HIV infection, which constitute a pathway to release intracellular material and exchange material and information between cells. Exosomes have become a hotspot in the field of AIDS research. This review introduces the formation process of HIV particles and exosomes, and summarizes the role of exosomes in the progression of HIV disease from multiple aspects.

Recent findings
Many components of the exosomes involved in HIV transfer and replication affect the occurrence, development, and outcome of AIDS, and are closely related to HIV infection. Exosomes can have a dual impact on HIV infection, and play an important role in activating the latent reservoir of HIV and affecting the chronic inflammation of HIV. The biological information carried by exosomes is also of great significance for the prediction of HIV disease.

Summary
The present review summarizes the role of exosomes in HIV disease progression in various aspects in order to further understand the underlying mechanism affecting the infection and providing a new idea for the clinical diagnosis and treatment of AIDS.

Keywords
AIDS, exosome, progression of HIV

INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is a primary global public health concern. The human immunodeficiency virus (HIV) is the pathogen of AIDS. Since AIDS has been identified, >37 million people worldwide have been infected [1]. Presently, highly active antiretroviral therapy (HAART) inhibits HIV replication and improves patient prognosis, turning AIDS into a chronic viral infectious disease [2]. However, due to the existence of HIV reservoir [3], HAART cannot eliminate the virus completely from the body [4]. After the treatment is stopped, the latent HIV can be reactivated [5]. Hence, AIDS patients cannot yet be cured. Therefore, understanding the factors that affect HIV disease progression and cause cytopathic effects is the key to eliminating HIV completely and curing AIDS permanently.

Several studies have demonstrated that the generation process of exosomes has considerable overlap with viral assembly and outflow pathways of HIV, suggesting that exosomes play a significant role in the HIV infection process. Typically, the exosomes can transport viruses from the infected cells to uninfected cells, regulating the host's immune response to virus infection [6,7]. Moreover, exosomes can transmit disease-causing information, which affects the outcome of virus infections [8▪]. HIV is the first RNA virus used for exosome research. Although some breakthroughs have been made with respect to the correlation between HIV and exosomes, the specific biological function has not yet been clarified. This study reviews the correlation between exosomes and HIV and the application of exosomes in HIV infection.

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KEY POINTS

- As a carrier of material exchange and information transmission, exosomes play an important role in the progression of HIV disease.
- Exosomes have dual effects on HIV infection, which can inhibit HIV infection and promote HIV infection.
- Exosomes can activate the resting HIV-infected $CD4^+$ T lymphocytes, thereby activating the latent HIV reservoir.
- Exosomes play an important role in HIV-associated chronic inflammation.
- Biological information carried by exosomes can be used as markers in the process of HIV infection, which plays an important role in the diagnosis and treatment of HIV.

SOURCES AND BIOLOGICAL CHARACTERISTICS OF EXOSOMES

Exosomes are small extracellular vesicles derived from various cell types under physiological or pathological conditions. Polymorphic vesicles or exosomes were first discovered in sheep reticulocytes by Johnstone *et al.* [9]. Originally, exosomes were thought of as a way for cells to get rid of metabolic waste. Later, studies found that exosomes secreted by B lymphocytes contain histocompatibility class II antigen complexes, which can induce the immune response of T lymphocytes [10]. Therefore, exosomes gradually attracted people's attention. Exosomes have a lipid bilayer structure with a diameter of 30–150 nm and a density of about 1.13–1.19 g/ml [11]. In the presence of sufficient water, exosomes are round when viewed under a low-temperature microscope [12,13]. At present, it has been found that exosomes have morphological diversity in body fluids, and some studies have classified exosomes derived from human mast cells 1 (HMC-1) into nine categories according to their different morphologies. Previous studies have shown that exosomes come from a wide range of sources and can be released into the microenvironment by a variety of different cells [14]. They are found in a variety of biological fluids, including blood [15], urine [16], breast milk [17], and semen [18]. Exosomes contain abundant macromolecular substances, such as proteins, nucleic acids, and lipids [19–21], and their surfaces contain specific protein markers, such as CD9, CD63, and CD81 [22], which can be used as specific markers for the identification of exosomes. Exosomes serve as the carriers of cell signaling molecules and mediate cell-to-cell transmission, promote the transport of proteins and nucleic acids and effectuate disease development [23]. In addition to participating in multiple processes such as intercellular signal transduction, extracellular mechanism generation, and tumor interstitial communication [24], exosomes also play an important role in viral infection. For example, exosomes can both deliver antiviral agents between different cells [25] and target viral antagonists to allow the virus to evade host immunity [24,26].

FORMATIONS OF EXOSOMES AND HIV PARTICLES

The features of HIV particles overlap with exosomes. Both are composed of a lipid bilayer membrane and can carry genetic material. Also, the density of HIV (1.16–1.18 g/ml [27]) and diameter (100 nm) are similar to those of exosomes. However, these similarities render isolating exosomes from the HIV virus rather challenging.

Exosomes are a series of membranous nanovesicles and generated as follows. First, the cell membrane invaginates to form an endosome. Then, the endosomal membrane is dented, budding inwards to form multivesicular bodies (MVBs) containing intraluminal vesicles (ILVs). Finally, some MVBs fuse with lysosomes for degradation [28], whereas others whose membrane surface contains CD63 and lysosomal associated membrane protein 1 (LAMP1) mediate the fusion of MVBs with the cell membrane to release ILVs in the extracellular space. These vesicles then convert into exosomes [29]. The biogenesis of ILVs and MVBs is driven by two mechanisms: Endosomal Sorting Complex Required for Transport machinery (ESCRT-dependent) and ESCRT-independent. ESCRT consists of four protein complexes (ESCRT-0, -I, -II, and -III) and some associated proteins (Alix and VPS4) [30], each with varied functions. ESCRT-0 recognizes and aggregates ubiquitinated proteins on the endosomal membrane [31] and recruits ESCRT-I. ESCRT-I merges with ESCRT-II to promote endosomal membrane invagination and bring RNA and proteins into the newly formed vesicle. Subsequently, ESCRT-II recruits ESCRT-III to sever the connection between the endosomal membrane and the vesicle to separate the vesicle [32]. Moreover, the ESCRT-I-related protein, tumor susceptibility gene 101 protein (TSG101), and ESCRT-III-related proteins, Alix, and CHMP4, play critical roles in developing exosomes. Some studies have shown that exosomes decrease when the levels of these proteins are low [33,34]. In addition, ILVs and MVBs can be generated even when the cell is depleted of the four ESCRT protein complexes [35], indicating that the biogenesis of ILVs and MVBs can be driven in an ESCRT-independent manner where four transmembrane proteins and some lipid molecules, such

as ceramide, are involved [19]. Current studies have demonstrated that Ral GTPase regulates the biogenesis of MVBs and the secretion of exosomes [36]. This finding is valuable to the study of exosomes.

The formation of HIV particles has many features overlapping those of exosomes. The budding of HIV requires ESCRT, as well as TSG101 and Alix [37]. HIV is constituted of three structural proteins: Gag, Pol, and Env. Among these, Gag is the only necessary protein for HIV formation. It assembles at the plasma membrane to generate the virus particles [38], interacts with TSG101 protein, and recruits ESCRT-I to the plasma membrane. Then, ESCRT-I recruits ESCRT-II to the plasma membrane. TSG101 protein merges with AIP1 protein and interacts with CHMP4 in ESCRT-III. ESCRT-III binds to the plasma membrane to form a complex cellular protein [39] that nourishes HIV budding. ESCRT-III with the plasma membrane forms vesicles to encase the virus particles. Subsequently, the vesicles separate after plasma membrane fission [40,41] and fuse with the cell membrane to release the virus. The cellular protein Vps4 is crucial for this process. It is recruited to the plasma membrane to facilitate the release of ESCRT components from the vesicles after virus budding and effectuate the recycling of ESCRT components [42]. The similarity between the formation process of exosomes and the budding process of HIV complicates the research on exosomes. Figure 1 describes the formation of exosomes and the budding process of HIV.

ROLE OF EXOSOMES IN HIV DISEASE PROGRESSION

Exosomes are crucial to the development of HIV. The pathogenesis of HIV is complex, and the molecular substances in the virus are important to the disease progression. Being the carriers of material exchange, exosomes play a key role in cell communication [43]. Studies have shown that exosomes can transfer the substances from the HIV virus among cells, which affects the occurrence and development of the disease. In the following sections, the present paper will introduce the impact of exosomes on HIV disease progression and the application of exosomes in HIV treatment.

INHIBITION OF EXOSOMES IN HIV INFECTION

The exosomes released by T cells contain a large number of $CD4^+$ molecules, whose exosomes compete with host cells to bind to the HIV envelope protein, inhibiting the HIV infection of target cells [44]. The exosomes are crucial to cell-to-cell communication as they can mediate intercellular communication by delivering functional factors to recipient cells [45]. Previous studies have shown that exosomes contain multiple antiviral factors that inhibit replication in HIV [46,47]. Human cytidine dehydrogenase APOBEC3G (A3G) is a part of the defense system against HIV. Exosomes carrying A3G reduce HIV replication [48]. In addition, Human intestinal epithelial cells (IECs) and human brain microvascular endothelial cells (HBMECs) are activated by TLR3 and release exosomes containing anti-HIV viral factors, including several vital interferon-induced genes (*ISG15*, *ISG56*, and *Mx2*) and anti-HIV miRNA, that inhibit HIV replication in macrophages [46,49]. Exosomes derived from body fluids can also inhibit HIV infection, while those in semen promote HIV transcriptional silencing by disrupting the NF-κB/Sp1/Tat pathway [50]. Moreover, human semen contains exosomes with anti-HIV activity. Exosomes from healthy human semen (SE) contain antiviral mRNA, which has been shown to inhibit HIV replication and weaken the infectivity of the progeny virus [51]. Madison *et al.* [52] demonstrated that SE in healthy individuals could prevent HIV spread from vaginal epithelial cells to target cells, and SE can inhibit reverse transcriptase activity of the offspring LP-BM5 murine HIV, and limit the transmission of murine HIV in the vagina. Similarly, exosomes in semen from the HIV infector also inhibit HIV replication in vitro, deliver antiretroviral drugs in the body [53▪▪], and provide novel treatment approaches. Furthermore, exosomes in vaginal fluid and breast milk also inhibit HIV infection [25,54]. For example, exosomes in breast milk combine with dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN) to inhibit HIV infection and HIV spread in the dendritic cells derived from monocytes, indicating that the risk of mother-to-child transmission is reduced [25].

PROMOTION BY EXOSOMES IN HIV INFECTION

Some studies showed that exosomes promote HIV infection. HIV infects the cells by engaging $CD4^+$ and coreceptors (CCR5 and/or CXCR4) at the cell surface and fusing the viral envelope to the cell membrane, promoting the internalization of the HIV particles. In addition, exosomes derived from monocytes and macrophages can transfer CCR5 and CXCR4 to the cells that lack coreceptors, thereby increasing the number of susceptible cells [55,56]. T cell immunoglobulins and T-cell Immunoglobulin and Mucin-domain containing proteins (TIMs) play a critical role in the immune system, invaded by enveloped viruses through TIMs [57,58]. HIV

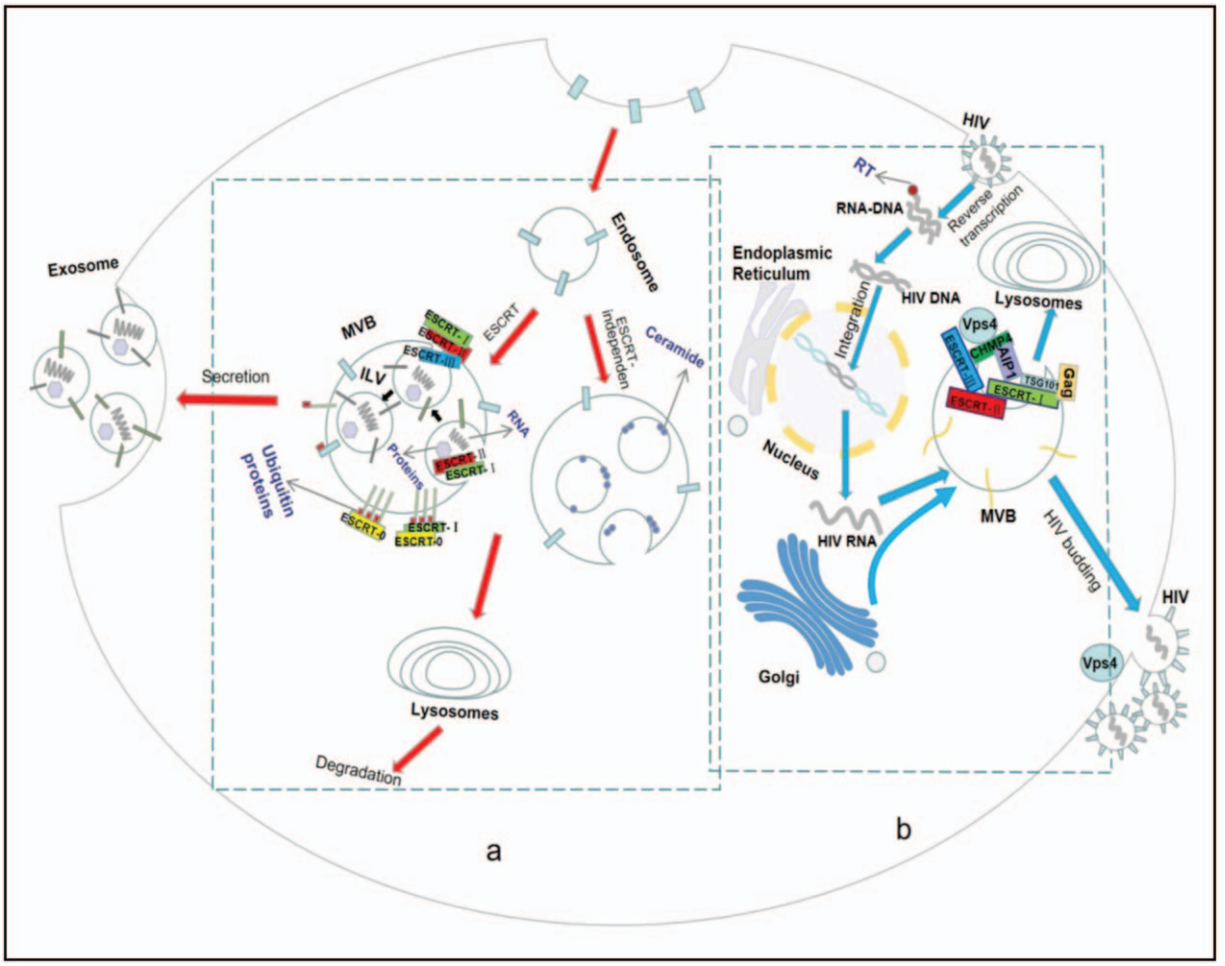

FIGURE 1. The formation of exosomes and the budding process of HIV. (a) The formation of exosomes. First, the membrane invagination forms the endosome. Subsequently, the endosomal membrane dented, budding inward to form MVBs containing ILVs. Finally, some MVBs fuse with the lysosome for degradation, while others fuse with the cell membrane, releasing ILVs in the extracellular space and eventually forming exosomes. There are two main mechanisms that induce the production of ILVs and MVBs. One is produced by a mechanism that ESCRT-dependent:ESCRT-0 recognizes and aggregates ubiquitinated proteins on the endosomal membrane and recruits ESCRT-I, ESCRT-I merges with ESCRT-II to promote endosomal membrane invagination and bring RNA and proteins into the newly formed vesicle. ESCRT-II recruits ESCRT-III to cut the connection between the endosomal membrane and the vesicle to separate the vesicle. The other is produced by an ESCRT-independent mechanism, which may involve four transmembrane proteins and some lipid molecules, such as ceramides. (b) The budding of HIV particles. The budding of the HIV virus also requires ESCRT, as well as TSG101 and Alix. Gag protein can interact with TSG101 protein and recruit ESCRT-I to the plasma membrane. Next, ESCRT-I recruits ESCRT-II to the plasma membrane. TSG101 protein merges with AIP1 protein and interacts with CHMP4 in ESCRT-III. ESCRT-III binds to the plasma membrane to form a complex cellular protein that nourishes HIV budding. ESCRT-III can make the plasma membranes in which the viruses are encased to form vesicles, and drive the cut to separate the vesicles after plasma membrane fission. And the vesicles fuse with the cell membrane to release the virus. VPS4 is recruited to the plasma membrane and promotes the release of the ESCRT complex from the vesicle for recycling after the viral budding process. ESCRT, Endosomal Sorting Complex Required for Transport; ILV, intra-luminal vesicle; MVB, multivesicular body.

contains a large amount of Phosphatidylserine (PtdSer), which can fuse with the PtdSer-receptor TIMs. Some studies have shown that exosomes contain TIMs, which can fuse with the PtdSer on the surface of HIV to facilitate its entry into T cells and macrophages. After the invasion, the virus is blocked by anti-TIM-4 [59]. Moreover, exosomes promote the maturation of DCs that upregulate the expression of costimulatory molecules on DCs. In this case, a large number of DC/T lymphocyte clusters are induced, and ultimately leads to higher levels of HIV metastasis to T lymphocytes [60].

Exosomes also carry some cytokines, such as interleukin (IL)-3, IL-4, IL-8, and IL-17 [61], which affect the virus's life cycle and enhance HIV infection [62,63]. Some studies demonstrated that exosomes promote HIV infection by affecting HIV-derived proteins. The HIV accessory protein Nef is one of the most abundantly expressed viral proteins. The released exosomes contain HIV Nef protein [64], and the exosomes containing Nef protein increase the susceptibility of T cells to HIV infection [24,64]. In addition, exosomes containing Nef proteins have also been shown to accelerate T cell apoptosis, which may lead to depletion of $CD4^+$ T cells in the AIDS mechanism [65]. Some studies have shown that the exosomes released by HIV-infected cells contain the viral envelope (Env) protein gp120, which promotes the virus to attach and fuse to the target cells and promote HIV infection in various indirect ways [66]. Figure 2 illustrates the dual function of exosomes in HIV infection.

ACTIVATION OF EXOSOMES FOR HIV LATENT RESERVOIR

HAART controls the HIV level of AIDS patients below the detection limit [67–69]. However, due to the existence of an HIV reservoir, HAART cannot eliminate the virus completely [70], and AIDS patients cannot yet be cured. The latent reservoirs are mainly generated when the viruses enter the activated $CD4^+$ T lymphocytes and restore them to a resting state [71]. Supposedly, exosomes activate the resting HIV-infected $CD4^+$ T lymphocytes via different mechanisms, which might cure AIDS. Exosomes from HIV-infected cells activate the resting $CD4^+$ T lymphocytes via ADAM17 and TNF-α-dependent mechanisms [24,72]. Among these, Nef is the determinant factor for the exosomes to activate HIV [24,72]. It induces the exosomes to upregulate activated ADAM17 in target cells, following which mature tumor necrosis factor (TNF)-α is released [24,26,73] and the latent HIV is activated. HIV-coded Tat protein is an effective viral

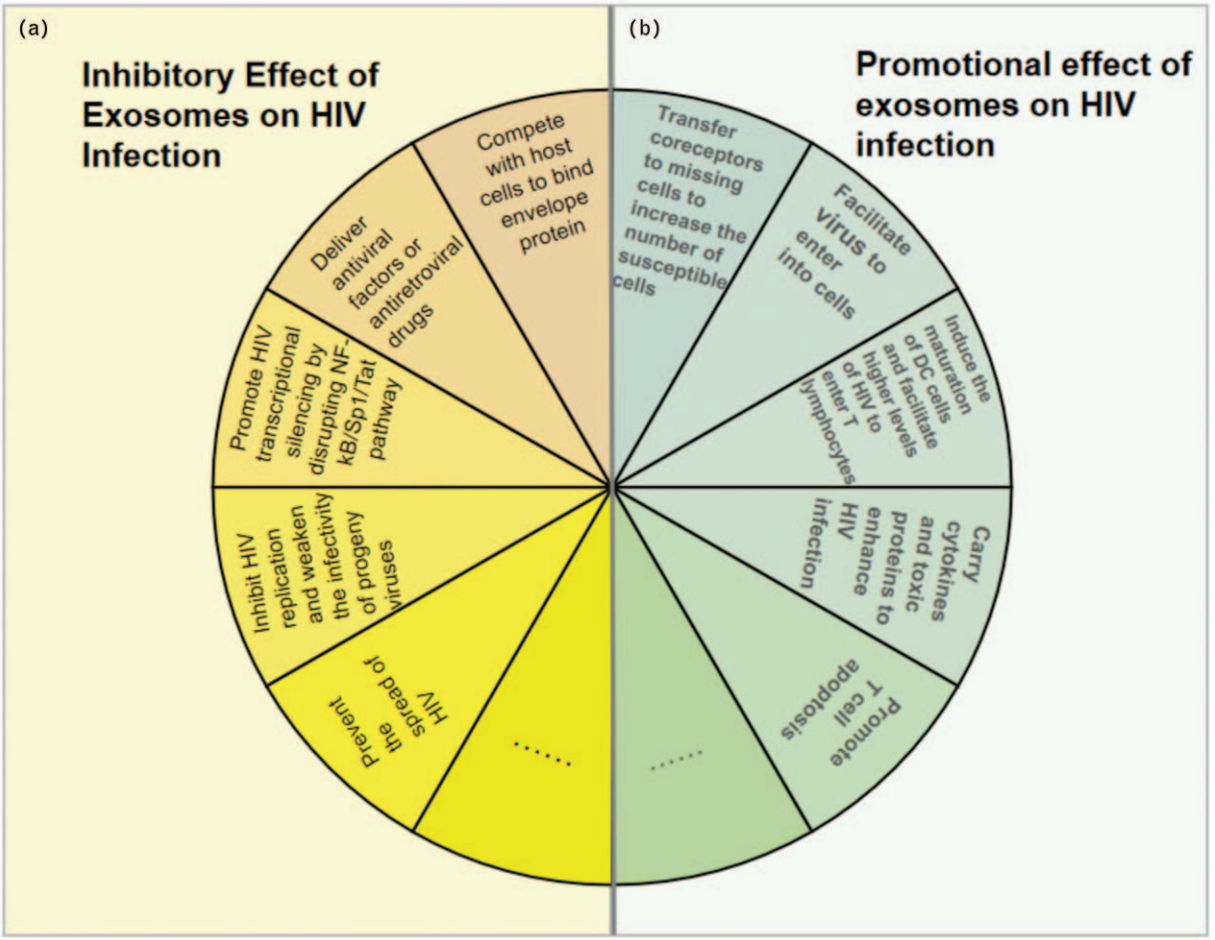

FIGURE 2. The dual function of exosomes in HIV infection. (a) The inhibitory effect of exosomes on HIV infection. (b) The promotional effect of exosomes on HIV infection.

transcription transactivator. Tat protein is encapsulated in exosomes for targeted delivery to latently infected $CD4^+$ T lymphocytes, which reactivate the virus. When combined with an HIV latency reversal agent (LRA), the expression of HIV mRNA increases by >30-fold [74]. In addition, the activation of T cells triggers the release of exosomes [75,76]. Also, exosomes derived from IL-2-activated $CD4^+$ T cells can be preferentially internalized by their own cells and reactivate the resting $CD4^+$ T cells of SIV-infected monkeys [77], which is a prerequisite for the potential application of exosomes in reactivating latent HIV.

ROLE OF EXOSOMES IN HIV CHRONIC INFLAMMATION

Chronic inflammation underlies pathological changes [78]. Accumulating evidence demonstrated that exosomes play a critical role in HIV-related chronic inflammation. The exosomes contain several proinflammatory factors derived from viruses or the host, affecting the development of the inflammation. Among these, the HIV-derived Nef protein is encapsulated in exosomes and transported to target cells, promoting the release of TNF-α [79]. In addition, the transactivated RNA (TAR RNA) in stem–loop structures is detected in the exosomes released by HIV-infected cells [80]. Some studies proved that TAR RNA could regulate the gene expression of proinflammatory cytokines, such as *IL-6* and *TNF-β*, through the TLR-3 pathway [81]. Similarly, exosomes produced during HIV infection have also been shown to contain various inflammatory mediators derived from the host. Additionally, the exosomes of HIV-infected patients contain a variety of miRNAs that promote inflammation, such as miR-155, miR-223 [82], and miR-29a (which enhance the inflammatory response of macrophages) [83]. Moreover, HIF-1α facilitates IL-1β transcription [84], which directly promotes the development of inflammation. Accumulating evidence stated that exosomes from the plasma of HIV patients promote HIF-1α activity of lymphocytes and macrophages [85], which could be because exosomes deliver HIF-1α protein [86,87] or a long noncoding RNA to prevent the degradation of *HIF-1α* mRNA [88]. Chronic inflammation may occur in the central nervous system during HIV infection [89]. In this process, exosomes mediate the communication among cells, such as astrocytes, microglia, and other cells, which exert regulatory functions on neuroinflammation through a variety of mechanisms [90,91] and affect the inflammation of the nervous system by transferring proteins, nucleic acids, and other substances.

SIGNIFICANCE OF BIOLOGICAL INFORMATION CARRIED BY EXOSOMES FOR HIV DISEASE

The identification of biomarkers during antiretroviral therapy could lead to early identification of $CD4^+$ cell recovery after treatment, which could be important for monitoring the treatment of HIV patients [92,93]. Some studies demonstrated that exosome-derived microRNA 192 (miR-192), IL-6, and soluble CD14 (SCD14) are related to the recovery of $CD4^+$ cells at the beginning of antiretroviral therapy, and exosome-derived miR-144 is associated with the recovery of $CD4^+$ cells after 96 weeks of antiretroviral therapy [94▪▪]. In addition, HIV exosomes are also shown to promote the spread of toxic factors, such as β-amyloid and prions. Furthermore, HIV exosomes are regulatory factors for neurodegenerative diseases [95]. Sun *et al.* [96] showed that

Table 1. The role of some components of exosomes as markers in HIV disease

Components	Functions	First author(s)	Year	References
miR-192, IL-6, SCD14	Related to the recovery of $CD4^+$ cells at the beginning of antiretroviral therapy	Francisco Hernández-Walias	2020	[94▪▪]
miR-144	Related to the recovery of $CD4^+$ cells at W96 of antiretroviral therapy	Francisco Hernández-Walias	2020	[94▪▪]
HMGB1, NF-L, Aβ	Biomarkers for HIV cognitive impairment	Sun B	2017	[96]
miR-378, miR-630	Monitor HIV status of infected mother and prevent mother-to-child transmission of HIV	Zahoor MA	2020	[97▪]
miR-20a, miR-21	Biomarkers of the risk of development of classic Hodgkin lymphoma in HIV-infected patients	Hernández-Walias FJ	2020	[98▪]
miR-4516	Biomarkers of related neurological diseases in HIV-infected patients	Asahchop EL	2016	[99]

IL, interleukin; HMGB1, high mobility group protein B1; miR-192, microRNA 192; NF-L, nerve filament light chain; SCD14, soluble CD14.

proteins associated with neuronal damage in plasma neuron-derived exosomes (NDE) of HIV-infected patients could be used as the biomarkers for HIV cognitive impairment. High mobility group protein B1 (HMGB1), nerve filament light chain (NF-L), and Aβ proteins are altered in plasma NDE after HIV infection and are crucial to cognitive impairment and treatment response after HIV infection. Table 1 summarizes the components in exosomes that sever as the biomarkers for HIV disease.

CONCLUSION

Exosomes play a critical role in AIDS progression and affect the occurrence, development, and outcome of HIV through multiple mechanisms and aspects (such as cell-to-cell transmission and interaction with HIV molecules and receptor cells). This review introduced the role of exosomes in HIV progression and described the application of exosomes in HIV disease. Due to the existence of an HIV reservoir, AIDS cannot be cured yet. However, if latent HIV can be eliminated completely, AIDS can be cured permanently. In recent years, some progress has been made in exosomes to activate the latent HIV; hence, the exosomes have become a hotspot in clinical studies for AIDS and are expected to have wide application prospects for AIDS treatment. Although several studies were carried out on the correlation between exosomes and HIV, a specific mechanism is not yet elucidated. Also, since exosomes are similar to virus particles in size, density, membrane composition, and biogenesis, the separation, purification, and identification of exosomes are challenging. Presently, iodixanol density gradient centrifugation [100] and size exclusion chromatography (SEC) [101,102] are two effective methods that obtain higher purity exosomes from virus-containing plasma, and the two approaches may complement each other [78]. However, all the current methods are incomplete due to their limitations and require improvement. In conclusion, a comprehensive study is required to elucidate the correlations between exosomes and HIV, and thus, eliminate HIV completely and cure AIDS permanently.

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Conflicts of interest

There are no conflicts of interest.

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