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REVIEW

Roles and mechanisms of exosomal microRNAs in viral infections

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Abstract
Exosomes are small extracellular vesicles with a diameter of 30-150 nm that originate from endosomes and fuse with the plasma membrane. They are secreted by almost all kinds of cells and can stably transfer different kinds of cargo from donor to recipient cells, thereby altering cellular functions for assisting cell-to-cell communication. Exosomes derived from virus-infected cells during viral infections are likely to contain different microRNAs (miRNAs) that can be transferred to recipient cells. Exosomes can either promote or suppress viral infections and therefore play a dual role in viral infection. In this review, we summarize the current knowledge about the role of exosomal miRNAs during infection by six important viruses (hepatitis C virus, enterovirus A71, Epstein-Barr virus, human immunodeficiency virus, severe acute respiratory syndrome coronavirus 2, and Zika virus), each of which causes a significant global public health problem. We describe how these exosomal miRNAs, including both donor-cell-derived and virus-encoded miRNAs, modulate the functions of the recipient cell. Lastly, we briefly discuss their potential value for the diagnosis and treatment of viral infections.

Introduction

Extracellular vesicles (EVs) can be classified into three categories based on their size. The first type includes exosomes (30–150 nm), which are released during the fusion of multivesicular bodies (MVBs) with the plasma membrane. EVs of the second type are microvesicles (100–1000 nm) that detach from the cell membrane following activation, injury, or apoptosis. Apoptotic bodies (1 μm) constitute the third category of EVs, which are released from apoptotic cells and contain fragmented nuclei and intracellular organelles. The first two types of EVs, namely, exosomes and microvesicles, are active vesicles [1, 2]. Exosomes originate from the inward budding of the endosomal membrane, which forms intraluminal vesicles (ILVs) that enter into multivesicular bodies (MVBs). Following the fusion of MVBs with the plasma membrane, the ILVs are secreted into the extracellular space as exosomes [3]. The biogenesis of exosomes is primarily mediated by the endosomal sorting complex transport (ESCRT) machinery, which can influence the specific sorting of cargo into exosomes [4]. Cellular communication pathways coordinate and maintain biological functions, including the development and growth of organs and formation of various tissues [5, 6]. Exosomes may act as useful bridges in such cases for transporting biological molecules from donor to recipient cells, owing to their protective ability. Exosomes contain a wealth of cargo, including diverse proteins [7] and nucleic acids such as mRNA, miRNA, and DNA, which are protected by the double-membrane structure of the exosome and can be transported to recipient cells, where these specific RNAs sometimes act on specific targets [8, 9].miRNAs are small (~22 nt), endogenous, non-coding nucleic acids that regulate post-transcriptional gene expression. Functionally, miRNAs can trigger the degradation and/or translational repression of their target mRNAs by binding to the 3′ untranslated region (UTR) or open reading frame (ORF) of these mRNAs [10, 11]. Viral miRNAs are virus-encoded miRNAs that can regulate virus-encoded transcripts or networks of host genes to participate in the infectious

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cycle of the virus [12–14]. Most virus-infected cells can release nanovesicles or microvesicles that contain proteins and RNA or miRNA from donor cells [15], which can play vital roles in the recipient cells as well as in the infected donor cells.

Exosomal miRNAs (exo-miRNAs) have been found to exist in various body fluids and can play essential roles in viral infections. This is attributed to the fact that the miRNAs, including host and viral miRNAs, within the exosomes are protected from *in vitro* degradation by RNase [16, 17]. The loading of miRNAs into exosomes remains to be clearly elucidated. Some hypotheses on the mechanisms of miRNA sorting state that RNA-binding proteins (RBPs) [18–20], the KRAS status [21, 22], and neutral sphingomyelinase 2 [23] facilitate the sorting of miRNAs into exosomes.

This review discusses the functions and mechanisms of exo-miRNAs during viral infections. Six viruses, namely, hepatitis C virus (HCV), enterovirus A71 (EV A71), Epstein-Barr virus (EBV), human immunodeficiency virus (HIV), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and Zika virus (ZIKA) were selected, first, because these viruses are important infectious agents, especially SARS-CoV-2, which has become a recent global threat, and second, because the functions of exo-miRNAs have been explored in depth for these viruses. Understanding the dual role (Fig. 1) and mechanisms of action of these exo-miRNAs will improve our knowledge of the development of viral infections, which may in turn provide novel insights for the development of new methods or strategies for the diagnosis and treatment of viral infections.

HCV

HCV is a positive-sense, single-stranded RNA virus that belongs to the family *Flaviviridae* and has a 9.6-kb genome that encodes a polyprotein that is cleaved into a series of non-structural and structural proteins [24, 25]. HCV infections frequently cause chronic liver disease, hepatocellular carcinoma (HCC), hepatic fibrosis, and cirrhosis [26–29].

In a previous study, exosomes extracted from supernatants of HCV-infected Huh7.5 cells or sera from patients with chronic hepatitis were shown to contain replication-competent viral RNA in complex with Ago2-miR122-HSP90 and to be able to deliver the contents to uninfected recipient cells to initiate infection even when the HCV receptors CD81, SB-RI, and ApoE were blocked. This suggests that exosomes can transmit HCV via a receptor-independent mechanism [30]. Previous studies have demonstrated that this mechanism involves exosomal miR122, a host factor that enhances HCV replication [31] and has been found in exosomes derived from HCV-infected cells and individuals infected with HCV. Other studies have demonstrated that HCV RNA is associated with miR-122, HSP90, and the RISC protein Ago2, which aid in stabilizing HCV RNA and transferring it to naïve Huh7.5 cells, thereby enhancing viral replication [32, 33]. Traditional therapeutic methods against HCV usually employ antibodies that target receptors to inhibit HCV entry. However, this strategy is not comprehensive, and the administration of miR-122 and HSP90 inhibitors to inhibit HCV transmission is a novel strategy for inhibiting exosome-mediated, HCV-receptor-independent transmission of the

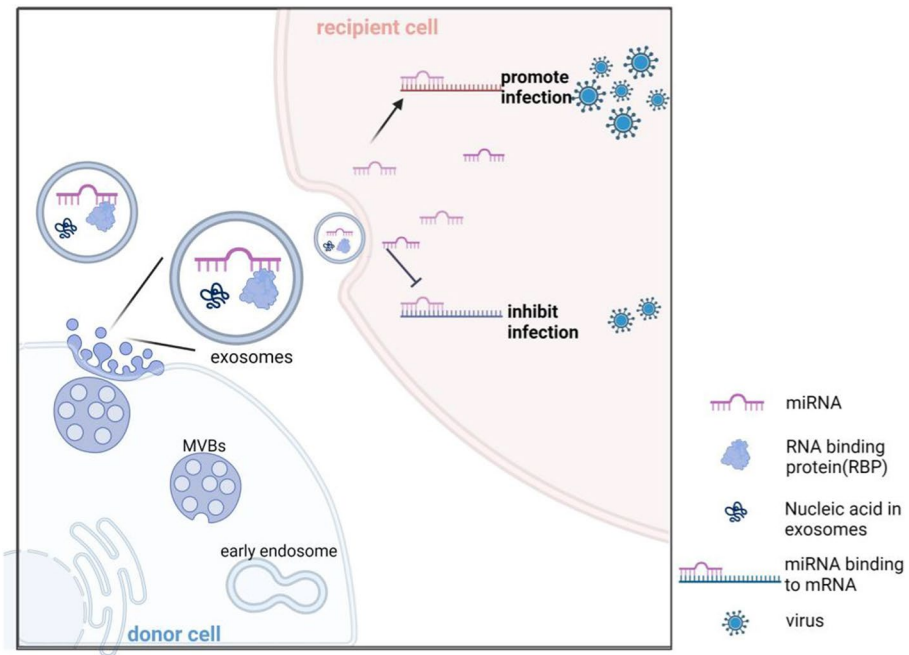

Fig. 1 The dual role of exosomal miRNAs in recipient cells. The exosomes released into the extracellular milieu protect the miRNAs and allow them to be transferred from donor cells to recipient cells. In the cytoplasm of the recipient cell, the miRNA can alter the response to a viral infection in either a positive or negative manner by targeting host and viral mRNA transcripts. Specific mechanisms by which exosomal miRNAs function during viral infection are described in Table 1. The figure was created by the authors using BioRender.com. Abbreviations: miRNA, microRNA; RBP, RNA-binding protein; MVBs, multivesicular bodies; mRNA, messenger RNA

virus. With regard to the most common HCV-related B cell lymphoproliferative disorders, including mixed cryoglobulinemia (MC), Liao et al. explored the relationship between exo-miRNAs derived from HCV-infected hepatocytes and the expression of B-cell activating factor (BAFF) in a recent study. The findings revealed that exosomal miR-122/let-7b/miR-206 upregulated the expression of BAFF and activated Toll-like receptor 7 (TLR7) in human macrophages. The induction of BAFF by exo-miR-122 stimulates the activation of B cells during HCV infection and may serve as a potential regulatory mechanism underlying the development of MC [34]. In contrast, exosomes can transfer miR-155 derived from B cells to hepatocytes to inhibit HCV activity [35, 36]. Rituximab, a chimeric monoclonal antibody that targets human CD20, is usually used for treating patients with rheumatoid arthritis (RA). However, patients treated with rituximab are more likely to develop HCV viremia, which is also associated with exosomal miR-155 [36]. This can be attributed to the fact that rituximab may decrease the production of miR-155 and the transmission of exosomal miR-155 by inhibiting B cell receptor signaling, thereby enhancing viral replication. Therefore, exosomal miR-155 may prove useful for therapeutic or diagnostic purposes, especially in patients with HCV who have received rituximab therapy. Although exosomes and miRNAs play an important role in HCV infections, the mechanism regulating the secretion of exosomes and exosomal miRNAs remains unknown. Kim et al. demonstrated that HCV infections can upregulate the secretion of exosomes and exosomal miR-122 and miR-146a. That study further revealed that the secretion of exosomes and exo-miRNAs is regulated by the caspase-3/Panx1/P2X4 pathway in Huh7.5.1 cells [37].

Hepatic fibrosis is one of the most common diseases caused by HCV infections that may be associated with exo-miRNAs. Exosomes derived from HCV-infected hepatocytes are abundant in miR-19a, which can be transferred to hepatic stellate cells (HSCs) to induce the expression of fibrogenic markers, including α-smooth muscle actin and transforming growth factor β1 (TGF-β1), which contribute to hepatic fibrosis [38]. Similarly, Kim et al. [39] reported that the levels of miR-192 are also elevated in exosomes derived from HCV-infected hepatocytes, leading to hepatic fibrosis. The transmission of exosomal miR-192 to HSCs upregulates the expression of fibrogenic markers by increasing the expression of TGF-β1. Targeting the expression of exosomal miR-192 and miR-19a may serve as a therapeutic strategy for HCV-induced fibrosis, and both exosomal miR-192 and miR-19a may serve as potential therapeutic targets in hepatic fibrosis induced by HCV infection.

Previous studies have demonstrated that exo-miRNAs can be useful for the treatment of HCV infections. For instance, exosomes derived from umbilical mesenchymal stem cells (uMSC-exo) can inhibit HCV infections but have no effect on other viruses, indicating the specificity of uMSC-exo. Further research confirmed that uMSC-exo carries a series of miRNAs, four of which, namely, miR-145, miR-199a, let-7f, and miR-221, cooperate with one another to inhibit HCV replication rather than inhibit viral entry, which was indicated by the fact that the suppression of one of these four miRNAs in exosomes was sufficient to inhibit their antiviral activity. Treatment with uMSC-exo may provide a novel effective anti-HCV strategy, especially when combined with the general antiviral drug VX-950, which inhibits interferon (IFN) α and the HCV NS3-NS4A protease [40].

EV A71

EV A71 is a single-stranded, positive sense RNA virus belonging to the family *Picornaviridae*. It is often associated with hand, foot, and mouth disease (HFMD). EV A71 is especially infectious in infants and toddlers. It is a non-enveloped virus that is released during cell lysis following infection. However, EV A71 virions and RNA have been found to be enveloped by exosomes and released in a non-lytic manner [41]. Moreover, exosomal EV A71 RNA (exo-EV A71-RNA) is capable of replicating in resistant L919 cells, indicating that exo-EV A71 is more efficient at infecting host cells than free viral particles [42].

Further investigation of the miRNAs present in exosomes derived from EV-A71-infected HT29 cells using RNA-seq revealed that miR-146a is the most enriched miRNA in these exosomes. MiR-146a can be transferred to uninfected recipient cells via exosomes to accelerate viral replication by inhibiting the production of type I IFNs. Inhibition of VP1 expression following the inhibition of miR-146a confirmed the role of miR-146a in the replication of EV A71 [42]. It has been demonstrated that miRNA effector complex Ago2 proteins promote viral replication by interacting with the viral internal ribosomal entry site [43], which can interact with GW182 and is co-immunoprecipitated with miR-146a and EV A71 RNA to form complexes in exosomes, demonstrating the mechanism of sorting of miR-146a into exosomes [42]. As EV A71 infections often cause HFMD, exosomes from human oral epithelial (OE) cells infected with EV A71 were analyzed using deep small RNA-seq, which revealed that the levels of miR-30a are elevated in exosomes derived from infected OE cells. Further analysis with human antiviral response RT profiler PCR array profiles revealed that myeloid differentiation factor 88 (MyD88), which is involved in the production of type I IFNs, was the target gene of miR-30a. Further studies demonstrated that the levels of miR-30a are elevated in infected OE cells, and exosomal miR-30a can be transferred to macrophages to facilitate viral replication by binding to the 3′-UTR of MyD88 to suppress the type I IFN response [44]. Another

study showed that exosomes derived from EV-A71-infected RD cells are enriched in miR-155, which can be transferred to SK-N-SH cells to inhibit viral replication. The mechanism involves the suppression of phosphatidylinositol clathrin assembly (PICALM) protein expression mediated via the binding of miR-155 to the 3′-UTR of PICALM mRNA [45]. PICALM plays a significant role in viral invasion because it is essential for clathrin-mediated endocytosis, a major pathway that is utilized by most viruses for cellular invasion [46, 47]. These studies demonstrated that exo-miRNAs can play a dual role in EV A71 infection. Exosomal miR-146a and miR-30a can facilitate EV A71 replication, whereas exosomal miR-155 suppresses EV replication. Targeting these exo-miRNAs may aid in improving therapeutic strategies against HFMD and other EV-A71-related diseases.

EBV

EBV is a member of the genus *Lymphocryptovirus* of the family *Herpesviridae*. More than 95% of adults harbor EBV. It is a human tumorigenic virus that can cause cancers and is strongly associated with the occurrence of nasopharyngeal carcinoma (NPC) and childhood lymphoma. EBV miRNAs carried by exosomes are protected from degradation by RNase and can be delivered to recipient cells [48]. For instance, human B cells infected with EBV can stimulate the production of cellular miR-155. Yoon et al. reported that EBV-positive Burkitt's lymphoma (BL) cells can transfer miR-155 to retinal pigment epithelial (ARPE-19) cells via exosomes. This results in an increase in the levels of vascular endothelial growth factor (VEGF), which regulates the formation of tumor blood vessels [49]. Exosomal miR-155 can serve as a potential target for preventing the development, spread, and metastasis of tumor tissues [50].

In addition to host miRNAs, viral miRNAs can also be found in exosomes. EBV encodes three kinds of viral miRNAs, namely, BART cluster 1, BART cluster 2, and BHRF 1 [51]. The levels of EBV-miR-BART13-3p are increased in the salivary glands (SGs) of patients with sicca syndrome (SS); however, as SG cells lack the CD21 EBV receptor, they cannot be infected in a receptor-dependent manner, and infection must therefore occur via another mechanism. Subsequent studies have demonstrated that miR-BART13-3p is transferred from EBV-infected B lymphocytes to SG cells via exosomes. miR-BART13-3p subsequently downregulates the levels of STIM1 protein to inhibit the entrance of Ca^{2+} ions essential for fluid secretion and also decreases the expression levels of aquaporin 5 (AQP5), which is also essential for fluid secretion in acinar cells [52]. As EBV-miR-BART13-3p is associated with the loss of saliva in patients with SS and could be transferred from B cells to salivary epithelial cells through exosomes, targeting miR-BART13-3p may be a novel therapeutic strategy for alleviating xerostomia [53].

It has also been demonstrated that NPC cells release certain EBV BART miRNAs into the extracellular environment via exosomes. The miRNAs subsequently diffuse from the tumor site and enter the bloodstream, and miR-BART 7-3p has been found to be the most abundant of these miRNAs [54]. Comparison of the BART miRNAs of patients and uninfected control individuals may aid in the diagnosis of NPC. As angiogenesis is one of the characteristics of NPC, Wang et al. [55] analyzed the related BART miRNAs that could be associated with angiogenesis from NPC samples. The findings revealed that the levels of EBV-miR-BART10-5p and hsa-miR-18a were increased in NPC samples and that these miRNAs could promote angiogenesis by upregulating VEGF and inhibiting the tumor suppressor Spry3. Antagonists of these miRNAs can be transferred to endothelial cells via exosomes to inhibit angiogenesis in NPC and thus provide a novel strategy for the treatment of NPC or other EBV-associated tumors [55].

HIV

HIV, which causes acquired immunodeficiency syndrome (AIDS), is a retrovirus that suppresses the functions of the immune system in humans. Studies on HIV have primarily focused on HIV-1. miRNAs are known to play vital roles in regulating immune suppression and the replication of HIV. It has been demonstrated that exo-miRNAs serve as potential prognostic markers for the treatment of HIV-1 [56], and miRNAs transferred by exosomes are used for the treatment of HIV-associated diseases. For instance, the children of mothers infected with HIV-1 are protected against HIV infections, which could be attributed to the presence of an antiviral factor in human milk (HM). In a study in which the profiles of exosomes in HM obtained from mothers infected with HIV-1 were analyzed [57], the expression of 19 miRNAs was altered in the HM-derived exosomes of infected individuals compared to that of uninfected mothers. Of these, 13 miRNAs were found to be upregulated and could therefore aid in the treatment of HIV, and two of these miRNAs, miR-378g and miR-630, could also serve as biomarkers of HIV infection.

There is a possible association between HIV and cervical cancer, and a previous study demonstrated that women infected with HIV are more likely to develop aggressive cervical cancer [58]. The findings revealed that miR-155-5p was responsible for the development of aggressive cervical cancer in these individuals. Exosomal miR-155-5p from HIV-1-infected T cells can be transferred to cervical cancer cells to accelerate invasion by inhibiting the expression of

the target *ARID2* gene via the excision repair cross-complementation group 5 (ERCC5)-NF-κB signaling pathway. Exosomal miR-155 may serve as a potential target for the treatment and prevention of HIV-associated cervical cancer [59]. Apart from host miRNAs, the HIV genome encodes viral miRNAs, most of which belong to the trans-activation response element (TAR) group [60, 61]. Narayanan et al. [62] showed that TAR miRNA was present in exosomes derived from HIV-1-infected cells or patient's sera. From these viral miRNAs, TAR miRNA can suppress apoptosis to protect against cell death by downregulating the expression of the pro-apoptotic proteins Bim and Cdk9. That study also demonstrated that one exosomal TAR miRNA, 3′-TAR miRNA, could enhance susceptibility to HIV-1 infection in naïve cells. The study of exosomes derived from cells infected with HIV-1 can provide a better understanding of host-virus interactions.

The use of exo-miRNAs can also aid in improving combination antiretroviral therapy (cART) and precision targeted therapy for cancer. Some studies have found chronic neuroinflammation in HIV-1-infected patients following cART, which is attributed to exosomal TAR miRNA. Exosomal TAR miRNA can upregulate the expression of inflammatory factors, especially IL-6 and tumor necrosis factor (TNF) β, which can result from the binding of Toll-like receptors (TLRs) to TAR miRNA. This subsequently activates the NF-κB pathway to accelerate the production of cytokines [63]. Additionally, the presence of transactivating (Tat) regulatory proteins of HIV-1 in lymph nodes and brain tissues following cART therapy can induce chronic glial activation in HIV-associated neurological disorders (HANDs). It has been reported that the levels of miR-9 are elevated in exosomes derived from Tat-stimulated astrocytic A172 cells. Exosomal miR-9 miRNA is subsequently internalized by BV-2 microglial cells, resulting in microglial migration, a characteristic feature of inflammatory response within the central nervous system (CNS) [64]. miR-9 mediates microglial migration by targeting phosphatase and tensin homolog (PTEN), an essential suppressor of cell motility that participates in most cellular processes. The application of anti-miRNAs can provide a novel strategy for the inhibition of HAND following cART [65].

SARS-CoV-2

SARS-CoV-2, a new member of the genus *Betacoronavirus*, family *Coronaviridae*, was first isolated from patients with pneumonia in the city of Wuhan, China, in December 2019. The current COVID-19 outbreak caused by this virus has been declared a global pandemic by the World Health Organization (WHO) [66]. SARS-CoV-2 infections can cause neuropathic disease even when the viral load is low [67], and this phenomenon has been attributed to the viral spike protein. Exosomes derived from spike-transfected HEK-293T cells (S-exo) are loaded with miR-148a and miR-590, which can be transferred to human microglia to inhibit the expression of ubiquitin-specific peptidase 33 (USP33) and IFN regulatory factor 9 (IRF9), respectively [68]. IRF9 has been reported to act downstream of USP33 and to function as a protective factor in inflammation [69]. Therefore, the hyperactivation of the USP33-IRF9 axis regulated by exosomal miRNA in human microglia uncovers a bystander pathway of SARS-CoV-2-mediated CNS damage. The suppression of exosomal miR-148a and miR-590 could upregulate IRF9, and this initial insight may suggest potential strategies for the treatment of neuropathogenesis associated with SARS-CoV-2 infection.

Exo-miRNAs can be used to treat inflammation during COVID-19. MSC-derived EVs contain exosomes and have been reported to carry abundant miRNAs, including miR-125a-3p and miR-125b-3p [70]. These miRNAs have a common region for targeting multiple sites in the 3′-UTRs of certain mRNAs and function cooperatively to reduce the production of inflammatory cytokines and chemokines. Furthermore, mice exposed to exosomes derived from lung epithelial cells infected with SARS-CoV-2 were shown to develop pulmonary inflammation due to activation of the NF-κB pathway, mediated via the exosomal transmission of nonstructural protein 12 (NSP12) to lung macrophages, followed by the induction of certain inflammatory factors [71]. Teng et al. observed that aly-miR396a-5p miRNA in ginger exosome-like nanoparticle (GELNs), which is present in the tissues of edible plants [72, 73], can inhibit the cytopathic effect caused by SARS-CoV-2 by suppressing the expression of genes encoding NSP12 and the spike protein [71]. Similarly, edible nanoparticles (ENPs) derived from other edible plants, including tomato, grapefruit, and pear, contain varieties of miRNAs that can differentially target SARS-CoV-2 at the corresponding sites. These ENP miRNAs are generally easier to isolate than the exosomes from animal tissues, and they may serve as a non-toxic therapeutic strategy for the treatment of inflammation associated with COVID-19 or other diseases [74].

ZIKV

ZIKV is a flavivirus that is usually transmitted by the bite of a mosquito. It is an important arthropod-borne virus (arbovirus) that can cause Zika fever and severe neurological complications, including Guillain-Barré syndrome (GBS) in adults and microcephaly in neonates. It has been shown that EVs/exosomes contribute to the pathogenesis of ZIKV infection in human hosts. ZIKV-infected *Aedes* sp. mosquito cells (C6/36) can release EVs/exosomes containing

viral RNA and the ZIKV E protein, which are transported from the infected mosquito to the host. These ZIKA C6/36 EVs/exosomes can establish a productive infection in human monocytes and vascular endothelial cells, which are the main targets of ZIKV infection. These ZIKA C6/36 EVs can also promote the activation and differentiation of naïve monocytes, participate in endothelial vascular cell damage, and promote a pro-inflammatory state [75]. York et al. investigated the mechanism by which ZIKV influences the biogenesis of EVs and cargo sorting and found that ZIKV infection can promote the secretion of EVs, which is regulated by the tetraspanin protein CD63, which is enriched in EVs [75]. Owing to the importance of EVs during ZIKV infections, Abari et al. investigated the EV-derived and intracellular miRNAs and mRNA transcriptomes in neural stem cells infected with ZIKV by next-generation sequencing. The results demonstrated that ZIKV infections alter the secretion of host miRNAs in EVs, including miR-4792, which are involved in oxidative stress and neurodevelopmental processes [76]. The role of EV-associated host miRNAs in the pathogenesis of ZIKV needs to be investigated in depth in future studies.

Conclusion

Non-coding RNAs, especially host and viral miRNAs, influence viral life cycles and host immune responses. miRNAs are key regulators of gene expression, controlling different physiological and pathological processes. They also possess therapeutic potential because of their ability to regulate gene expression by suppressing mRNA translation, cleavage, and decay, initiated by miRNA-guided rapid deadenylation and the reduction of mRNA stability [77]. Depending on the function of a given miRNA, two possible therapeutic strategies can be developed, including miRNA replacement (via miRNA mimics) and miRNA inhibition (via miRNA inhibitors). Different delivery systems, including nanoconstructs [78, 79], viral vectors, nonviral vectors (polymers [80], liposomes [81], and exosomes [82]), and other systems, are being investigated as miRNA delivery systems for the treatment of clinical diseases.

Emerging evidence suggests that exo-miRNAs are associated with the mechanism of viral pathogenesis. Analysis of exo-miRNAs during viral infections may clarify fundamental mechanisms of disease, facilitate clinical diagnosis, and provide insights for the development of effective therapeutic strategies. Table 1 summarizes the functions and mechanism of action of miRNAs present in exosomes during viral infections. miRNAs derived from virus-infected cells can be transferred to recipient cells to alter their physiological functions. The potential applications of these exo-miRNAs in clinical diagnostics and in the development of novel therapeutic measures are also summarized in Table 1.

Previous studies have suggested that viral infections alter the levels of exo-miRNAs [83]. Because they provide a natural protective barrier, exosomes function as carriers of miRNAs to recipient cells during viral infections. The dysregulated miRNAs in the exosomes subsequently participate in viral propagation, modulation of the host immune response, and other pathological process. Interestingly, it has been reported that, rather than promoting viral infections, some exo-miRNAs are involved in suppressing viral infections. Exosomes are known to contain several miRNA species that are simultaneously transported via exosomes. The overall effect of these exo-miRNAs evidently depends on their miRNA composition. The effects of different combinations of exo-miRNAs and the specificity of exo-miRNA uptake in recipient cells require further investigation. Likewise, the mechanism by which the various miRNAs are sorted remains to be elucidated.

In addition to alterations in the exo-miRNAs themselves, the production of EVs has also been shown to be modulated during viral infections. Fu et al. demonstrated increased secretion of EVs following EV A71 infection [42]. Likewise, studies have shown that ZIKV infection promotes the release of exosomes from primary human fetal astrocytes, while treatment with the neutral sphingomyelinase 2 inhibitor GW4869 suppresses the release of exosomes [84, 85]. It has also been reported that HSV-1 and EBV infections can upregulate the secretion of exosomes [85, 86]. By examining clinical specimens, Hu et al. demonstrated that HIV-positive individuals had elevated levels of EVs/exosomes in their cerebrospinal fluid, which was correlated with HIV-related damage of the CNS [87]. The expansionary effects of viruses on the secretion of exosomes might be related to viral pathogenesis.

Given the important roles of exo-miRNAs in viral infections, the circulating exosomes and their cargo miRNAs may serve as useful biomarkers of viral diseases. For instance, circulating miRNAs and exo-miRNAs are regarded as putative early prognostic indicators of liver fibrosis and cancer during acute and chronic hepatitis virus infections. Notably, the guidelines of the International Society for Extracellular Vesicles (ISEV2018) [7] do not recommend a standard method for the isolation and identification of EVs. Exosomes and viruses share some degree of similarity in terms of structure and size, which necessitates researchers to use "low-recovery, high-specificity" techniques for the isolation of exosomes. This would aid in improving the reliability and reproducibility of studies on exosomes, and the protocols and methodologies need to be specified in such reports. Studies on exosomes have employed different methods for their isolation and characterization; therefore, the findings and conclusions of such studies require careful evaluation.

Table 1 Exo-miRNAs identified in viral infections

Virus	Donor cells	Exo-miRNA	Recipient cells	Function	Mechanism of action	Clinical significance	Reference
HCV	Huh7.5 cells	miR-122	Hepatocytes	Transmits HCV infection by cooperating with viral RNA and HSP90 in exosomes	Exosomal Ago2 and miR122 protect the HCV 5′-IRES and enhance HCV replication	Offers potential therapeutic approaches for inhibiting exosome-mediated HCV transmission	[30]
	Hepatocytes	miR-122	Macrophages	Causes B cell lymphoproliferative disorders, such as MC	Increased levels of exosomal miR-122 induce secretory BAFF, which stimulates B cell activation	A possible therapeutic target for HCV infections and extrahepatic diseases	[34]
	B cells	miR-155	Hepatocytes	Inhibits viral replication	Inhibits HCV replication by regulating the expression of anti-HCV inflammatory cytokines	A potential diagnostic biomarker or therapeutic target of chronic HCV infection	[35]
	Hepatocytes	miR-19a miR-192	HSC	Activates HSCs by inducing fibrogenic markers	Targets SOCS3 to activate the STAT3-mediated TGF-β signaling pathway	Exosomal miR-19a and miR-192 can serve as potential therapeutic targets for HCV-mediated hepatic fibrosis	[39] [38]
	uMSCs	miR-145, miR-199a, let-7f, and miR-221	Huh7 cells	Inhibits HCV infection *in vitro*	Targets the Ago2-HCV RNA complex	Provides a novel adjuvant for anti-HCV therapy	[40]
EV A71	HT-29	miR-146a	L919	Facilitates EV A71 infection	Suppresses TRAF6, IRAK1, and STAT1 and inhibits IFN production in recipient cells	Provides novel strategies for the treatment of EV A71 infections	[42]
	Oral epithelial cells	miR-30a	THP-1 cells	Facilitates viral replication	Suppresses the type I IFN response by targeting MyD88	Aids in understanding the pathological mechanism underlying EV A71 infections in oral epithelial cells	[44]
	RD cells	miR-155	SK-N-SH cells	Inhibits EV A71 replication	Targets PICALM in recipient cells	Provides insights into the regulatory mechanisms of viral infection	[45]
EBV	BL cells	miR-155	RPE cells	Promotes angiogenesis	Enhances transcriptional and translational levels of VEGF-A	Provides a novel approach for cancer therapy	[50]
	B lymphocytes	miR-BART13-3p	SG epithelial cells	Regulates the physiological function of salivary epithelial cells	Decreases the expression of STIM1 and AQP5	Serves as a therapeutic target for alleviating xerostomia in patients with SS	[53]
	NPC	miR-BART10-5p and miR-18a	Endothelial cells	Promotes angiogenesis	Regulates expression of VEGF and HIF1-α in a Spry3-dependent manner	Offers novel strategies for the treatment of NPC and other virus-associated tumors	[55]

Table 1 (continued)

Virus	Donor cells	Exo-miRNA	Recipient cells	Function	Mechanism of action	Clinical significance	Reference
HIV	HIV-1-infected patients	miR-378g and miR-630	-	miR-378g and miR-630 exhibit an accuracy rate of 86% in predicting HIV-1 infections	miR-378g can suppress the expression of TARBP2 by targeting the 3′-UTR of TARBP2 and thus aid in predicting HIV-1 infections	Act as biomarkers of HIV infection	[57]
	Jurkat T-cells	miR-155-5p	CaSkicells	Accelerates the migration and invasion of cervical cancer cells	Promotes invasion by inhibiting the expression of ARID2 through ERCC5-NF-κB signaling. Promotes migration of cancer cells by increasing the expression of proinflammatory cytokines	Serves as a potential target for the treatment and prevention of HIV-associated cervical cancer	[59]
	A172 cells	miR-9	BV-2 cells	Facilitates migration of microglia	Targets PTEN	Provides new approaches for the development of novel therapeutic targets for inhibiting neuropathogenesis	[65]
	J1.1 cells	TAR miRNA	293T cells	Downregulates apoptosis to protect cells from death	Downregulates the proapoptotic proteins Bim and Cdk9	Improves the understanding of host-virus interactions and disease treatment	[62]
SARS-CoV-2	HEK-293T	miR-148a and miR-590	Human microglia	Causes damage to the CNS	Regulates the expression of the neuroinflammatory gene *USP33*	Allows an initial exploration into the treatment of neuropathogenesis caused by SARS-CoV-2 infections	[68]
	MSC cells	miR-125a-3p, miR-125b-3p miR-202-3p and miR-769-3p	-	Decreases the production of inflammatory cytokines, minimizes cell death	Targets the corresponding 3′-UTRs of multiple mRNAs	Offers a potential approach for improving the survival rates of patients with severe COVID-19	[70]
	Ginger cells	aly-miR396a-5p	-	Inhibits the cytopathic effect (CPE) caused by SARS-CoV-2	Suppresses the expression levels of genes encoding NSP12 and spike proteins	Serves as a potential therapeutic agent for treating pulmonary inflammation caused by COVID-19	[71]
	Edible plants	ELN miRNAs	-	Combats SARS-CoV-2 infections	Targets SARS-CoV-2 at the corresponding sites	Serves as a non-toxic therapeutic agent for the treatment of COVID-19	[74]

-: not mentioned

Because of the similarities in size, shape, and molecular characteristics between exosomes and viruses, the lack of efficient methods for isolation of pure exosome preparations remains a major challenge.

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Declarations

Conflict of interest The authors have no potential conflicts of interest to declare.

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