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REVIEW

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Changes and application prospects of biomolecular materials in small extracellular vesicles (sEVs) after flavivirus infection

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Abstract

Small extracellular vesicles (sEVs), also known as exosomes, are membranous vesicles filled with various proteins and nucleic acids, serving as a communication vector between cells. Recent research has highlighted their role in viral diseases. This review synthesizes current understanding of viral sEVs and includes recent findings on sEVs infected with flaviviruses. It discusses the implications of viral sEVs research for advancing arbovirus sEVs research and anticipates the potential applications of sEVs in flavivirus infections.

Keywords Exosomes, Extracellular vesicles, Flavivirus infection, MicroRNA, Virus

Introduction

Arboviruses, a taxonomically diverse group of viruses transmitted by arthropods. Among them, flaviviruses have clinical significance [1]. Flaviviruses are positive-strand RNA viruses in the family Flaviviridae. Among flaviviruses, Dengue virus (DENV), Zika Virus (ZIKV), West Nile Virus (WNV), Japanese Encephalitis Virus (JEV), and Tick-Borne Encephalitis Virus (TBEV) are widely studied. Flaviviruses can be transmitted to humans through arthropod vectors [2, 3]. Different viruses exhibit unique tissue tropisms, leading to distinct symptoms [4, 5]. The prevalence of various flaviviruses has increased globally in recent years [6–9], causing significant challenges for individuals and governments [1, 10]. Despite ongoing efforts to develop vaccines and drugs, challenges persist. Therefore, it is necessary the exploration of new approaches to prevent and treat viral infections [4, 11–13].

Small extracellular vesicles (sEVs) ranging from 30–200 nm in diameter serve as crucial mediators of intercellular communication [14, 15]. While previous studies have predominantly explored their application in tumors, tissue regeneration and drug delivery systems [14–16]. It is noteworthy that sEVs also play a significant role in viral infections. Following cellular infection by a virus, sEVs can facilitate viral spread through intercellular communication pathways, thereby enhancing viral infectivity [17, 18]. Moreover, sEVs have the capacity to modulate host cell protein expression and suppress the host immune response to the virus hereby facilitating viral dissemination [19–21]. Conversely, sEVs derived from other host cells retain the ability of inhibit viral infection and enhancing immunity against viral infections

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[21, 22]. Consequently, the detection of sEVs at various stages of viral infection is imperative. Furthermore, research on sEVs holds promise for the development of novel therapeutic interventions [23].

Numerous studies have demonstrated the biological significance of sEVs molecules released in the host following flavivirus infection with important biological significance. These molecules play a crucial role in exploring the relationship between tetraspanin protein on the surface of sEVs and virus transmission, aiding in the understanding of virus transmission mechanisms and the development of antiviral strategies. Flavivirus is difficult to prevent in some countries and regions. And the symptoms of virus infection are not obvious. sEVs secreted by individuals infected with flaviviruses can serve as biomarkers for early infection detection. In addition, the technology of engineered exosomes is advancing rapidly, opening possibilities for the development of exosome-based vaccines against viruses.

This review integrates the role of biological factors carried by sEVs secreted by arbo-flaviviruses post-infection. It also examines the potential immune mechanisms of sEVs secreted by different host cells following virus infection, aiming to enhance our understanding of the relationship between flavivirus infection and sEVs. These insights may offer novel directions for further research on flavivirus infection mechanisms and the development of antiviral strategies.

Based on the guidelines set forth by the International Society for Extracellular Vesicles (ISEV) in 2018, it is suggested to use the term "sEVs" instead of "Exosomes" due to the lack of clear and unique markers for different subtypes of extracellular vesicles [24]. Therefore, in this article, when referring to small extracellular vesicles (sEVs), we are specifically discussing those derived from cellular endosomes with a size distribution typically ranging from 30–200 nm (referred to as "exosomes" in some literature).

Biogenesis of EVs

Almost all living cells release vesicles, collectively known as "extracellular vesicles (EVs)", at various stages of their life cycle [25, 26]. Research on extracellular vesicles dates back to the 1960 s when Peter Wolf and others introduced the concept of tiny particles distinct from platelets [27], referring to them as "platelet dust". sEVs were initially documented in a 1983 article by Clifford Harding et al. [28, 29] and later confirmed by Rose M. Johnstone et al. in 1987, which was called "exosomes". Initially, cell-derived vesicles were believed to be involved in clearing outdated compounds generated by metabolism [35]. Further research has shown that EVs from various sources, such as immune cells and tumor cells, can have functional roles in biological processes. These roles include physiological effects, use as biomarkers, and applications in disease treatment, sparking increased research interest in EVs since the twenty-first century [30]. However, in practical applications, "exosomes" are often used to represent all EV-mediated processes due to challenges in isolating vesicles with different size distributions. Later, the terms sEVs and large EVs have been proposed for studies that do not clearly define the biogenesis mode of the EVs in their preparations [25, 30]. In response, the International Society for Extracellular Vesicles (ISEV) published the Minimum Information for Extracellular Vesicles Research (MISEV) Guidelines to standardize the naming and experimental requirements of EVs. These guidelines were updated in 2024 to improve clarity and consistency based on the 2018 version [24, 31, 32]. There is a relatively systematic standard for the understanding of sEVs. However, despite the discussion and standardization of many research teams, the relevant expressions of sEVs have not been unified absolutely. There is still a certain degree of flexibility in the relevant expressions of sEVs [33].

EVs can be broadly categorized into ectosomes and sEVs based on their size. Ectosomes are vesicles that bud directly from the plasma membrane, ranging in size from 50 nm to 1 μm, while sEVs are derived from endosomes and typically have a size of less than 200 nm [34]. Ectosomes also have different contents from sEVs and different extracellular durations [35]. Further classification of EVs includes apoptotic bodies (1–10 μm), microvesicles (200–2000 nm), and sEVs (30–200 nm) [36, 37]. Apoptotic bodies are also termed as oncosomes, which can bleb off the cell membrane. They are tumor-specific vesicles that can carry carcinogens [38]. For the same, microvesicles are type of smaller vesicle, which can discharged directly from the plasma membrane [39]. These membrane vesicles, such as sEVs, are enclosed by a lipid bilayer membrane [40, 41]. During secretion, EVs selectively encapsulate signaling molecules like DNA, RNA, proteins, and lipids, which are then transported to recipient cells to facilitate intercellular communication or cellular waste removal [36, 42].

The production and release of sEVs involves several intracellular steps: Cells form vesicles through endocytosis, specifically by plasma membrane invagination and membrane fission. These vesicles, known as *Early Endosomes (EEs)*, play a crucial role as sorting stations within cells. Some EEs are recycled to the plasma membrane, while others undergo protein and lipid remodeling and acidification to become *Late Endosomes (LEs)* in the periphery. Subsequently, the LEs migrate to the perinuclear region of the cell, where they undergo screening. Some LEs fuse with lysosomes

for degradation [34, 43–45]. During the maturation process from EEs to late endosomes LEs, the endosomal membrane undergoes further invagination facilitated by *Endosomal Sorting Complexes Required for Transport (ESCRT)*, leading to the creation of *Intraluminal Vesicles (ILVs)*. This process is mainly driven by ESCRT and associated proteins ALG2-interacting protein X (ALIX) and tumor susceptibility gene 101 (TSG101) [46] (Fig. 1). These ILVs accumulate within the endosomal structure, forming *Multivesicular Bodies (MVBs)*. Before these processes of inward membrane budding on endosomes can take place, the appropriate cargo needs to be recruited to the vesicles. Different cargoes have different sorting mechanisms. Among them, proteins are added to endosomes through monoubiquitination. ESCRT-I and -II contain ubiquitination recognition motifs to guide the spatial organization of cargoes [47]. MiRNAs can be enveloped into sEVs via binding to the heterogeneous nuclear ribonucleoprotein A2B1 [48]. Following a sorting process, some MVBs are targeted for degradation by lysosomes, while the remaining MVBs fuse with the plasma membrane to release exosomes, also known as sEVs [41, 49] (Fig. 1).

The relationship between viral infection and sEVs

Healthy cells can continuously secrete sEVs. Following viral infection, the composition of sEVs secreted by cells undergoes changes. These viral sEVs exhibit a dual role in infection: they can either impede viral infection by triggering the body's immune response or restricting viral dissemination, or they can facilitate viral infection by aiding in replication and spread (Fig. 2).

sEVs mediate immune effects during viral infection

The host can exhibit various responses to viral infection, among which resistance is a key factor. Apart from the virus particles, sEVs released by host cells post-infection

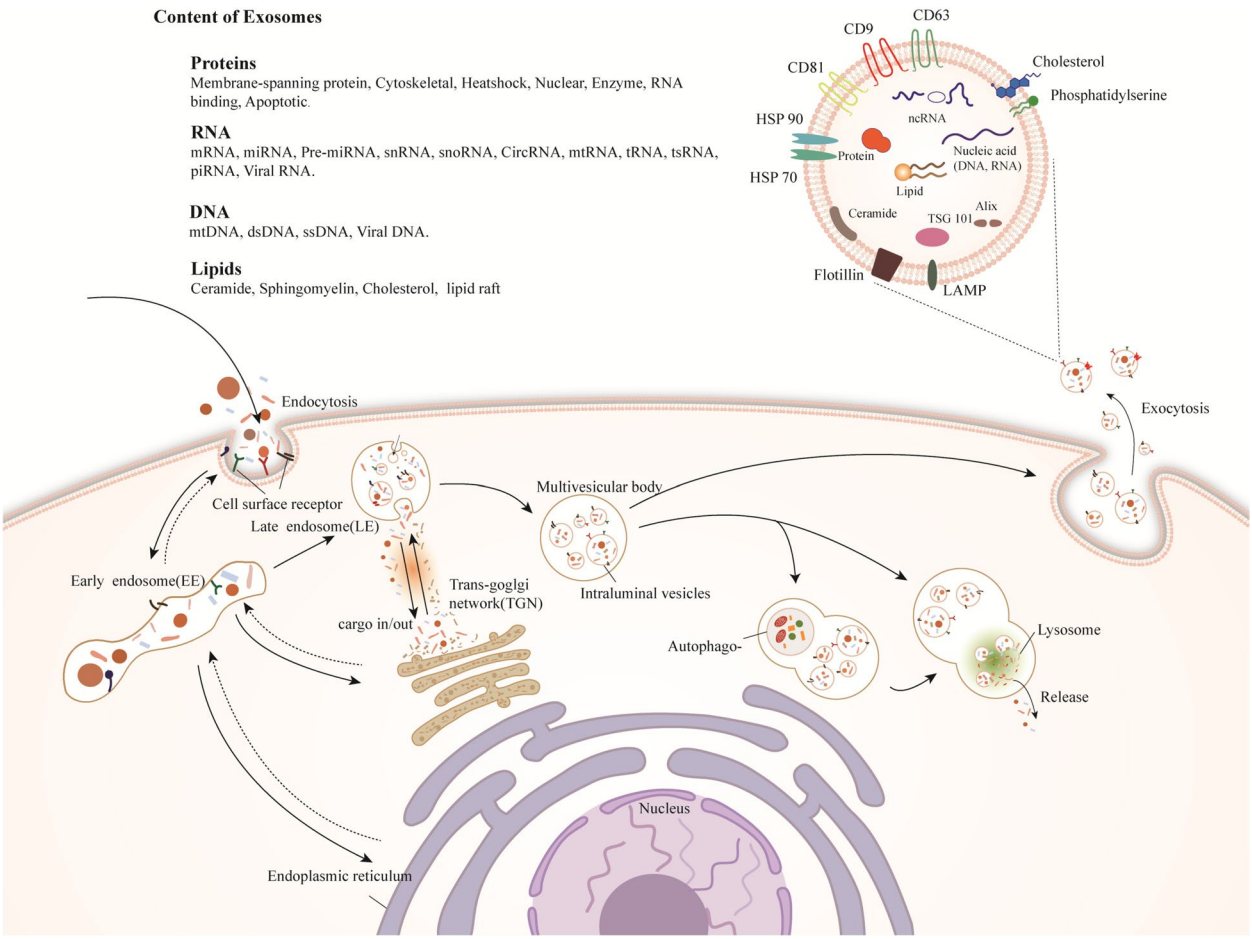

Fig. 1 Production of sEVs and their possible contents. Exosome protein markers: CD63, CD9, CD81, TSG101, HSP70, Alix. Proteases: acetylcholinesterase (Ache), neutral sphingomyelinase (nSMase). Immune-related proteins: complement C3, interleukin- 6 (IL- 6); Nucleic acids: mRNA, miRNA, sncRNA, etc

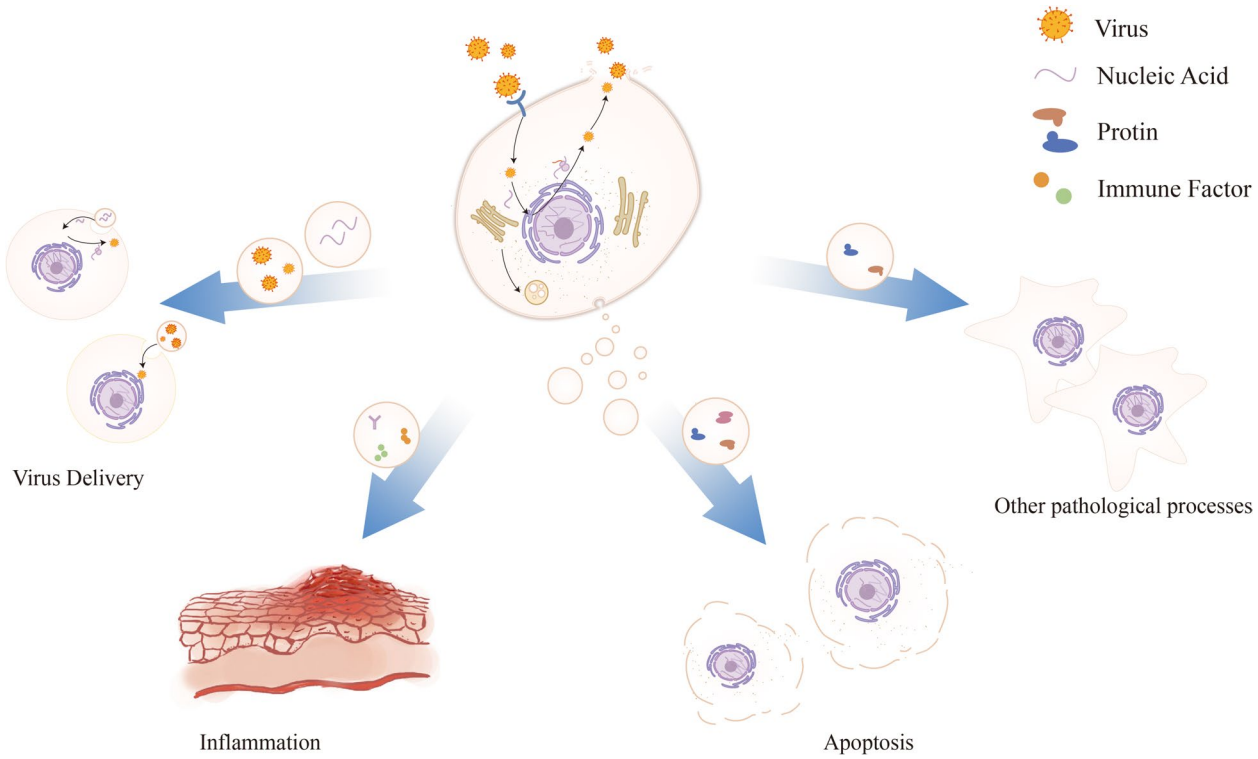

Fig. 2 The function of viral sEVs. As a vector for virus transmission. Causes local tissue inflammation. Leading to peripheral cell apoptosis and pathological changes in other cells

can also activate immune cells and prompt immune reactions. Specifically, sEVs released by lung epithelial cells following Influenza A Virus (IAV) infection and hepatocytes after Hepatitis B Virus (HBV) infection can promote the M1 polarization of macrophages. This leads to local inflammation and hampers viral replication and spread in turn [50, 51]. After influenza virus infection, sEVs released into the airways can transport viral proteins, leading to the activation of cellular immune responses [52]. Following this immune activation, immune cells release specific sEVs in response to the viral infection. Notably, sEVs released by mast cells infected with influenza viruses H1 N1 and H7 N2 contribute to innate immunity against the infection [53]. These sEVs, derived from virus-infected cells, contain distinct components compared to regular extracellular vesicles and are capable of triggering immune responses. Consequently, sEVs released by cells post-viral infection exhibit functions akin to the virus particles themselves, suggesting that sEVs associated with viral infection may facilitate virus spread by evading the immune system or through cell-to-cell transmission.

sEVs derived from virus-infected cells promote viral immune evasion

The use of sEVs by viruses for immune evasion is exemplified by the'Trojan exosomes hypothesis', which was proposed at the start of the century. Essentially, retroviruses utilize vesicles carrying lipids and proteins that mimic those of their hosts to evade cellular immunity and facilitate intercellular transport [54]. Not only retroviruses, but other types of viruses can also utilize vesicles within the host to enhance their spread. sEVs are capable of transporting hepatitis B virus (HBV) into uninfected hepatocytes, resulting in the same infection effect as free viruses [52]. Additionally, enterovirus 71 (EV71) can induce cells to release sEVs and encapsulate itself within these vesicles in a non-enveloped form, allowing for spread without cell lysis [53, 54]. These vesicles closely resemble the host's own protein composition, enabling them to evade detection by the host's immune system and facilitate the dissemination of viral particles.

The immune evasion strategies employed by viruses utilizing sEVs extend beyond mere avoidance. sEVs originating from virus-infected cells can transport diverse biological factors that disrupt the immune system. For instance, hepatocytes infected with HCV release sEVs

containing TGF-β, which can stimulate the proliferation of T follicular regulatory (Tfr) cells. This process hinders the protective Tfh response, ultimately facilitating virus persistence [55]. The impact of microRNAs carried in exosomes, encoded by various viruses, is significant. These microRNAs have the ability to suppress the host's immune response and trigger cellular pathological processes. For instance, miR-aU14 encoded by the herpes virus (HHV) can lead to mitochondrial damage and hinder the activation of IFN-I. sEVs play a role in transporting these microRNAs, influencing neighboring cells [56]. The biosynthesis of sEVs is closely linked to the spread of viruses within the host, as viruses can package their components or biological factors into sEVs. Therefore, studying the contents of sEVs released by cells post-viral infection may unveil novel mechanisms of viral infection and transmission (Fig. 2).

The role of biomolecules carried by sEVs secreted by flaviviruses in human bodies and virus transmission

There have been many studies on the role of sEVs in viruses. These results can also bring a lot of inspiration to the study of flavivirus sEVs. In recent years, flaviviruses have emerged as a significant public health concern. These zoonotic pathogens, belonging to the genus Flavivirus, including DENV, ZIKV, and WNV, are primarily spread through arthropod vectors. Unfortunately, there are no widely accessible drugs or vaccines specifically targeting flaviviruses at present [57]. Research on flaviviruses has delved deeper into viral transmission and assembly post-infection in the human body, leading to the emergence of new insights. It has been noted that sEVs also play a crucial role in the viral infection process. Arthropod-derived sEVs have been identified as vectors facilitating the transmission of viral RNA and proteins from arthropods to humans since the early stages of spread from nature to human populations [58, 59]. Following the entry of viral particles into the human host, sEVs have been observed to aid in the dissemination of different viruses belonging to the Flavivirus genus. The impact of EVs on flaviviruses will be discussed in the subsequent sections from multiple viewpoints.

sEVs assist flavivirus spread in the host

sEVs have long been recognized as playing a role in aiding the spread of viruses within a host. Many proteins required for flavivirus infection of cells overlap with the secretion pathway of sEVs. For example, heat shock protein 70, as an important membrane protein of sEVs, has also been shown to assist DENV1, DENV2 and infected cells [60]. When cells infected with a virus secrete sEVs, the composition of these vesicles is altered due to the synthesis and packaging of viral proteins. Typically, sEVs released by cells infected with flaviviruses contain viral envelope glycoprotein (E protein) or viral non-structural proteins (NS1, etc.). These viral proteins can induce a series of immune responses in the host. The NS1 protein of ZIKV can induce the formation of neutrophil extracellular traps (a protein-associated DNA scaffold) and lead to neural tissue damage [61]. The E protein is important structure protein and is the main protein involved in receptor binding and fusion [62]. Besides, viral EVs can temporally disturb the monolayer integrity of blood–brain barrier-mimicking cells, possibly by inducing structural rearrangements of the adherent protein VE-cadherin. This makes it easier for the ZIKV to infect target cells [63, 64]. These sEVs carrying viral structural proteins can act as vehicles for transporting viral materials and facilitate the virus's ability to infect other cells. However, there is still limited research on other viral non-structural proteins. How these proteins are related to the biogenesis pathways of sEVs in cells. Some researchers suggest that these proteins may induce lipid remodeling in the endoplasmic reticulum of infected cells, potentially aiding viral replication or impacting intercellular communication. But strong evidence is still lacking.

In addition to delivering viral proteins through sEVs, flaviviruses may also induce changes in cellular components to facilitate their spread. For example, during ZIKV infection, the virus can stimulate the activity of neutral sphingomyelinase (nSMase), leading to an increase in sEVs within virus-infected cells like neurons and microglia. Moreover, ZIKV can exploit the sEVs synthesis pathway to encapsulate viral components, thereby augmenting the secretion of sEVs containing specific viral protein profiles and infectious genomes [65, 66]. In addition, sEVs released from DENV-infected cells contain the autophagy marker LC3-II, which protects the virus from anti-dengue neutralizing antibodies [67]. Flaviviruses are like other viruses in the host body. In addition to killing host cells and releasing virus particles or their genomic RNA for spread, flaviviruses may also use the cell vesicle secretion pathway in the host body to spread. We can also think that it can be spread in the "Trojan horse" mode [63].

sEVs mediate inflammatory and antiviral responses in vivo after flavivirus infection

Since the vesicles secreted by flaviviruses after infecting cells can serve as another route for virus transmission, it is conceivable that, like other viruses, these sEVs can also be recognized by host immune cells and induce immune responses or cause damage to other tissue cells.

Cell signaling pathways are activated and transmitted to surrounding cells to resist viral infection. A research team conducted a gene ontology (GO) enrichment analysis on the mRNA contained in sEVs secreted by WNV-infected A549 cells. The results showed that the EV mRNA secreted by cells after virus infection is related to the defense response to the virus, Wnt signaling pathway, and I-IFN signal conduction pathway and many other antiviral cells signaling pathways are significantly related to [68]. This can be considered as EVs playing a role in the induction and triggering of antiviral responses, reflecting that sEVs contribute to resisting viral infection. In addition to preventing the virus from infecting itself, sEVs secreted by DENV-infected cells can also deliver IFITM3 molecules to susceptible cells to deliver their antiviral activity, thereby establishing the host's antiviral state (Fig. 3) [69]. Although it seems that the antiviral response in the host can be established through the transfer of sEVs between cells, sometimes this process is not satisfactory. The sEVs secreted by A549 cells after being infected with ZIKV contain the DEFA1B molecule. As a means for the body to inhibit viral infection, this molecule can cause the cell cycle to slow down. Such behavior of slowing down the cell replication cycle can reduce the synthesis of viral genomic RNA and viral particles to a certain extent, but the developmental delay caused by this has adverse consequences. Therefore, this is also considered to be a possible cause of microcephaly in newborns caused by ZIKV [70]. Such a mechanism can be considered a "side effect" of the body's resistance, but it should be seen more as a histopathological process caused by viral infection as well, and sEVs are not exempt from the blame.

Because of their ability to easily carry various biological molecules, sEVs have also been proven to be deeply involved in the inflammatory response after

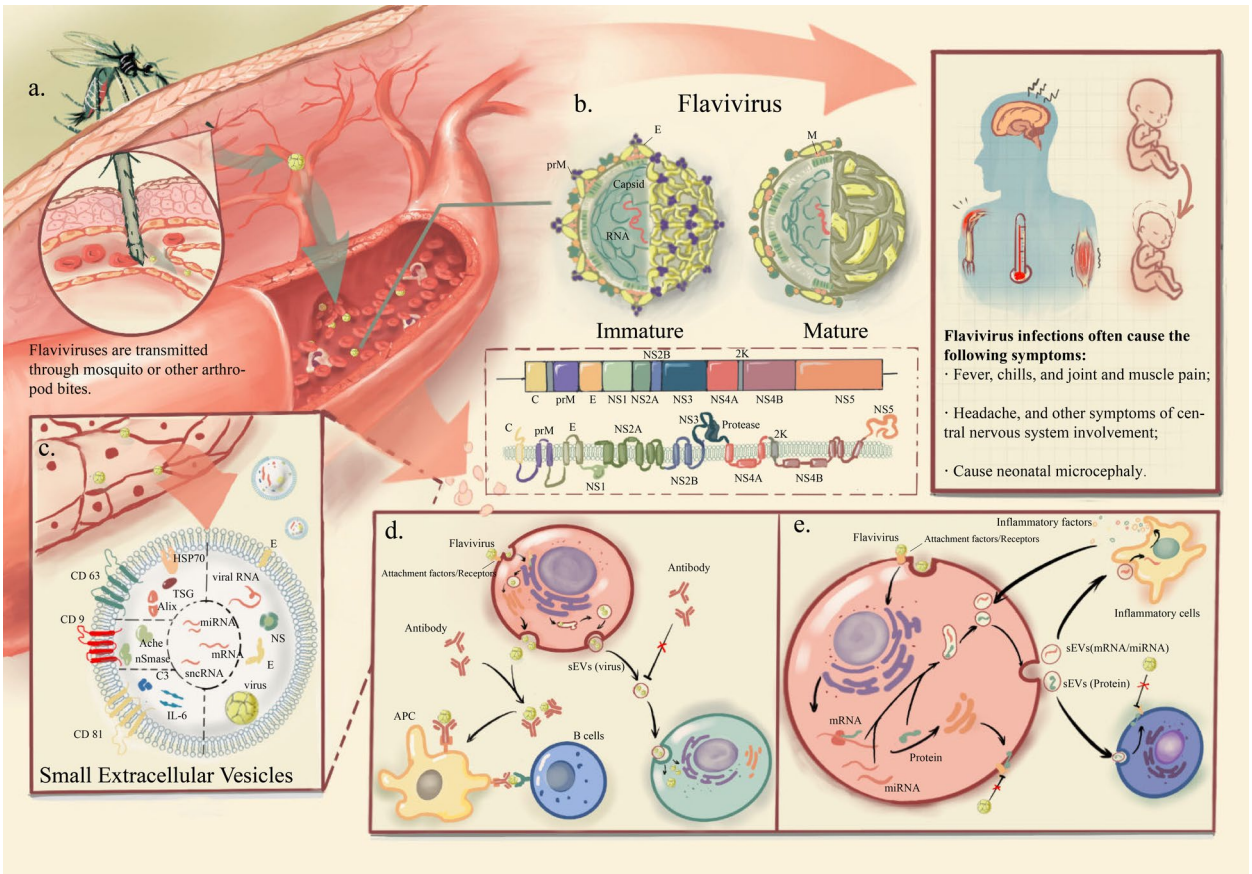

Fig. 3 Flavivirus infection and flavivirus sEVs. **a** Transmission and symptoms of flaviviruses; **b** structural representation of immature and mature states of flavivirus particles and membrane topology of mature viral proteins [10, 77]; **c** substances that may be contained in sEVs derived from viral infection of cells. Mainly viral components and exosome markers; **d**, **e** viral sEVs can mainly assist the cell-to-cell spread of viruses and reduce interference from the host immune system; at the same time, the antiviral effect of cells due to viral infection can also be transmitted with sEVs, and the mRNA and ncRNA carried in sEVs can promote inflammatory responses. Correspondingly, the inflammatory response will in turn promote changes in the composition of sEVs

viral infection. As an important tool for intercellular communication, sEVs change the molecules they carry under viral stimulation, thereby inducing inflammatory effects between cells. For example, DENV can activate platelets through CLEC2 to release vesicles including sEVs and further mediate inflammatory responses [71]. Similarly, in the study of the mRNA carried by sEVs secreted after WNV-infected cells, it was found that the inflammatory effect of cells can in turn act on sEVs, causing changes in the molecules carried in them, thereby changing the signals transmitted between cells [68]. Therefore, after flavivirus infects cells, on the one hand, the sEVs secreted by the cells can promote the occurrence and development of inflammation, and on the other hand, inflammation can cause changes in the components carried in the sEVs secreted by the cells.

In summary, sEVs derived from flavivirus-infected cells are deeply involved in the "battlefield" between cells and viruses in the host body and may serve as both promoters of pathological processes caused by viruses and as transmitters of antiviral substances.

Non-coding RNAs contained in EVs following flavivirus infection

Recent research by scholars focused on non-coding RNA associated with flaviviruses transmitted by arthropods has identified a specific non-coding RNA, named small flavivirus RNA (sfRNA), that could induce cell pathology and pathogenesis [69]. This type of RNA can lead to cellular pathology and pathogenesis.

Additionally, during viral infections, small extracellular vesicles (sEVs) originating from virus-infected cells may contain various non-coding RNAs, such as small RNAs and MicroRNAs (miRNAs). These non-coding RNAs can be transferred to recipient cells through the spread of sEVs from cell to cell, thereby exerting a certain biological function [72]. Next-generation sequencing of sEVs secreted by WNV-infected cells by Andrii Slonchak et al. revealed that virus-infected cells can lead to increased incorporation of miRNA into sEVs [68]. Exploring the specific functions of these miRNAs may uncover novel insights into virus transmission and cellular pathology. For instance, JEV has been shown to stimulate microglia to express let- 7a/b, package it into sEVs, and transport it to peripheral neurons, resulting in cell death through the activation of the Caspase pathway and subsequent nerve damage [73]. Furthermore, studies on certain serotypes of DENV have demonstrated that miRNAs linked to DENV infection can also be released into sEVs, enhancing their potential as circulating biomarkers [74]. In the study of tumor sEVs, miRNA of circulating sEVs secreted by tumor cells into plasma can be used as detection targets [75]. These circulating sEVs are also present during viral infections. Such as the circulating sEVs of the novel coronavirus can carry a variety of biomarkers and cause inflammation and tissue damage [17]. Flavivirus infection has a long incubation period [76]. The miRNA or other non-coding RNA carried by flavivirus sEVs that are different from normal cells can also be used as detection targets. The detection of these RNA of circulating sEVs is expected to achieve early detection of viral infection.

Flaviviruses generally result in the upregulation of sEVs miRNA expression in cells. Exploring the function and specificity of these miRNAs in sEVs released by virus-infected cells can offer valuable insights into the virus transmission mechanism and contribute to the advancement of detection technologies.

Discussion

Flaviviruses have been increasingly prevalent worldwide in recent years, with notable public health events such as the ZIKV outbreak and the resurgence of dengue and chikungunya causing concern for health systems in multiple countries [78]. Therefore, research on arboviruses holds great significance in protecting the well-being of people globally. sEVs have evolved from being considered mere metabolic waste products of cells to becoming vital materials in various disease research fields today. Recent studies have revealed a close connection between sEVs and viral infections, highlighting the promising research potential of flavivirus associated with sEVs.

EVs have been confirmed as mediators for virus transmission and host defense against viral infection. Recent studies have shown that sEVs are also involved in flavivirus infection. sEVs play a crucial role in various stages of flavivirus infection. In Aedes mosquitoes, sEVs were found to prevent DENV fusion with mosquito cell membranes, thus hindering viral infection [79]. While this defense mechanism was not effective in vertebrate cells, it did highlight the early interaction between viruses and extracellular vesicles in mosquitoes. Furthermore, when arboviruses infect human cells, there is a noticeable change in the type of sEVs secreted compared to healthy cells. Virus infection also leads to alterations in cell protein expression, which is reflected in the composition of sEVs, serving as a'snapshot'of the infected cells.

sEVs from different cell sources have varying effects in the field of viral sEVs. Their function can be categorized into two aspects: assisting infection and inhibiting transmission. First, sEVs could diminish immunogenicity, attributed to biocompatibility and a protective bi-layered

lipid structure safeguarding genetic cargo from detection or degradation [39]. Flaviviruses happen to exploit the vesicle secretion pathway of host cells for cell-to-cell transmission. This allows viral components, like viral proteins and RNA, to be transferred between cells in a safer way. On the other hand, vesicles secreted by host cells can hinder viral infection through biological pathways induced by proteins or non-coding RNA molecules they carry. For example, the expression of defensin α1B (DEFA1B), which has antiviral activity, was significantly increased in exosomes isolated from A549 cells infected with Zika virus [58, 63, 80]. Some flaviviruses also indirectly promote the spread of viruses between cells by affecting cell signaling pathways and altering their characterization (Table 1). Moreover, the viral non-coding RNA or protein carried in sEVs play a significant role in various cell signaling pathways, triggering a cascade of pathophysiological processes. Upon activation of these signals, they can lead to increased vesicle secretion, cell cycle deceleration, suppression of the body's immune response, inflammation induction, and enhanced viral packaging. The mechanism of viral infection is still unclear. It is worth noting that many research results have pointed out that the secretion of sEVs highly overlap with the replication and transmission pathways of flaviviruses. Therefore, the secretion of sEVs seems to be strongly correlated with the life cycle of flaviviruses. There is still a lack of relevant research in this area [10, 60, 81]. Finally, although many research teams agree that sEVs can assist in the spread of viruses in the field of viral sEVs research. Because sEVs are an important communication pathway between cells, it is difficult to prevent sEVs-mediated immune escape without disrupting cell communication.

Circulating sEVs have emerged as a focal point in the exploration of diagnostic biomarkers for cancer. As a promising target for cancer biopsy due to their stability, rich information content, and extensive repertoire [82]. Additionally, miRNAs encapsulated within sEVs have displayed potential as markers for diagnosing autoimmune diseases [83]. The advancement of sequencing technology has facilitated comprehensive analysis of molecules carried by sEVs, leading to the discovery of numerous nucleic acid or protein molecules exhibiting altered expression patterns compared to normal cells following high-throughput sequencing of sEVs post-viral infection [58, 68]. In addition to investigating the specific role of molecules in infection, these molecules can also serve as potential biological markers for detecting viral infections. For example, in flavivirus infection, specific populations secreted by flavivirus-infected cells in sEVs carrying molecules could be identified to enhance the efficiency of diagnosing viral infections. Currently, virus detection primarily relies on viral genetic tests, human antibody tests, and viral antigen tests. However, serological assay techniques may impact results due to antigen–antibody cross-reactions, the sensitivity of RT-PCR assay is dependent on the extracted RNA content, and false-negative results may occur early in infection due to low viral loads [84]. EVs derived from virus-infected cells contain unique miRNAs and proteins, distinguishing them from normal cell tissues. These distinct characteristics make them promising targets for potential applications. At the same time, the tracing technology of exosomes has also been widely studied. For example, PKH- 26 and gold nanoparticles were used as labeling agents for in vitro imaging and in vivo CT imaging of sEVs [85]. By effectively combining these EVs with existing technologies, we can maximize the advantages of both and develop diagnostic methods with high sensitivity and specificity, thus overcoming the limitations of conventional approaches.

Currently, the most widely used treatment method for sEVs is to engineer sEVs. sEVs are made to carry nucleic acids or other molecules for treatment. This method also has certain applications in viral therapy. For example, sEVs carrying siRNA can inhibit the replication of viral nucleic acids [86]. Moreover, the production of these engineered sEVs can be increased through a variety of methods, such as genetic engineering, creating hypoxic conditions, and adding cytokines [87]. In addition, ZIKV has been shown to be useful in treating nervous system tumors. If the active ingredients are added into engineered sEVs, it is expected that a more efficient biomolecular therapy will be constructed. There are also some new technologies for single sEVs characterization (Exoview). They mainly use immune recognition to capture and separate specific exosomes and analyze the surface markers and contents of exosomes. They can be detected without separation from biological samples [88]. However, sEVs are still some distance away from being used in actual applications. There are still many problems to be solved. Because the particle size of sEVs and viral particles is relatively close, it is difficult to separate the two by ultracentrifugation. Other commonly used methods are ultrafiltration, immunoaffinity capture, size exclusion chromatography and microfluidics [89]. Despite this, these technologies all face the problems of low yield and high cost. At the same time, sEVs treatment also requires a way to industrialize mass production. However, there is currently a lack of unified standards and processes for the transportation, storage, and treatment of exosomes [90]. These all require further efforts. While there has been extensive research on viral sEVs, the study of flavivirus sEVs

Table 1 Summary of the role of sEVs in mediating flavivirus infection and pathogenesis

Virus	Cells secreting sEVs	Viral sEVs carry associated proteins or non-coding RNAs	Functions	Results	References
ZIKV	HuMEC/C3, A549, Monocyte, etc	Viral proteins (E, NS1)	Infect cells and induce other pathological processes to synthesize and assemble virus particles	Delivers the virus to susceptible cells and promotes viral replication	[63, 80, 91]
	Neurons, astrocytes	Neutral sphingomyelinase (nSMase)	Hydrolyzed sphingomyelin consists of choline phosphate and ceramide	Enhanced sEVs release in cortical neurons	[65, 66]
	A549	Defensin α 1B (DEFA1B)	Interacted with the origin recognition complex 1	Anti-ZIKV infection, delay cell cycle	[70]
	Rhesus macaque trophoblast stem cells (TSC)	AP3S1, PLS1, etc	/	Involved in protein transport	[92]
		SCFD1	/	Involved in vesicles transport	
		LLGL2	/	Involved in exocytosis	
		CYFIP2	/	Involved in apoptosis	
		SMARCA2	/	Negatively regulates cell growth	
DENV	A549, etc	Viral proteins (NS1)	Infect cells and induce pathological processes to synthesize and assemble virus particles	Delivering the virus to susceptible cells	[80]
	U937 Macrophage cell line	Viral proteins (NS3)	Involved in the regulation of cellular responses during infections	Cause cytopathic processes	[93]
		/	Denv- 2-infected U937 macrophage-derived EVs can cause an increase in the transendothelial electrical resistance (TEER)	Regulate endothelial cell response and change vascular permeability	
	Platelet	/	Dengue virus activates platelets via CLEC2 to release extracellular vesicles (EVs) further activates CLEC5 A and TLR2 on neutrophils and macrophages	Induce neutrophil extracellular trap (NET) formation and proinflammatory cytokine release	[71]
	HuVEC, 293 T, etc	IFITM3	Blocking the virus from fusing with the cell membrane	Prevents the virus from entering the cell and inhibits viral infection	[69]
	Huh 7	LC 3-II	The virus uses the LC3-II process to create a huge secretory structure	Help spread virus particles between neighboring cells	[67]
JEV	Aag2, C6/36	*Let- 7a/b*	Interacts with TLR7 and NOTCH signaling pathways to enhance the release of TNF-α by microglia and the activation of Caspase in uninfected cells	Induced local inflammatory response and bystander cell death	[73]
WNV	A549, BVDV-NPro	miR- 26a, miR- 29, etc	May transmit antiviral or proinflammatory signals	Induces cellular inflammatory response or antiviral effect	[68]
Langet Virus (LGTV)	Neuros, tick cells	Viral infectious proteins (E, NS1)	Infect cells, synthesize, and assemble virus particles	Delivering the virus to susceptible cells	[58]

remains limited. Most of them lack of effective drugs and vaccines. The global spread of arboviruses due to economic globalization and climate change poses challenges for all nations. Therefore, leveraging the knowledge gained from viral sEVs research to explore the sEVs originating from arbovirus-infected cells and developing novel diagnostic and therapeutic approaches can have widespread practical benefits and improve global well-being.

Author contributions
W.T. obtained funding support. W.T. was responsible for project administration. G.D. manuscript. L.S., Z.C., Y.Z., L.C. data collection. J.H., C.Z. help cure the data. Y.X., H.H., F.M., D.H. visualized the content. J.Q., M.Y., Y.H., G.D., Y.Q. help to revise the latest version of the manuscript. All authors reviewed the manuscript. All authors approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Competing interests
The authors declare no competing interests.

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