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Review Paper

Investigating Exosomes in SARS-CoV-2 Infection: A Potential Partner for Coronavirus Reinfection/ Reactivation

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ABSTRACT

Virus-infected cells secrete exosomes and other extracellular vesicles which could deliver viral components, including structural proteins and viral derived-ribonucleic acids to other cells.

Released extracellular vesicles carry virus-specific receptors that increase the sensitivity of the target cells to viral infection. Exosomes may contribute to the systemic spreading of SARS-CoV-2 virus by transferring essential receptors, such as angiotensin-converting enzyme 2 and CD9 that promote the ability of the virus to dock into the target cells. Subsequently, the SARS-CoV-2 virus might also enter into the exosomal pathway to use this system for packaging their components into exosomes for secretion.

This study suggests that one of the potential explanations for the relapse and persistence of the SARS-CoV-2 virus infection could be an endocytic transport pathway related to the secretion of COVID-19-loaded extracellular vesicles.

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Introduction

Cytokine storm is a hyperinflammatory state that can occur in response to COVID-19 infection, leading to rapid spread of the virus throughout the body [1]. The cytokine storm results from a sudden acute increase in circulating levels of different pro-inflammatory cytokines, including interleukin 6, interleukin-1 and tumor necrosis factor-alpha [2]. The cytokine storm can help COVID-19 to spread rapidly throughout the body by causing endothelial dysfunction and systemic inflammation, which can lead to multi-organ failure and death [3]. Various drugs, such as tocilizumab and sarilumab, which are monoclonal antibodies targeting interleukin 6 activity, are being used to treat patients with COVID-19, and trials of these agents typically cite the cytokine storm as their rationale [4].

In the early phase of infection, COVID-19 targets cells (pneumocytes, bronchial and nasal epithelial cells) via the spike glycoprotein (S) that interacts with the angiotensin-converting enzyme 2 (ACE2) receptor [5]. In addition, the type 2 transmembrane serine protease (TMPRSS2), expressed in the host cell surface, encourages viral uptake by excising ACE2 and activating the COVID-19 S glycoprotein, which facilitates virus entry into host cells. TMPRSS2 and ACE2 are presented in host target cells, mainly pneumocytes, bronchial and nasal epithelial II cells [6]. However, coronavirus can spread rapidly throughout the body and approximately infect all types of cells in different organs, which leads to multi-organ failure in some patients [7]. Therefore, other factors in the pathophysiology of SARS-CoV-2 infection should be considered. Accordingly, we suggest a model for the persistence and reactivation of the COVID-19 virus. In this model, one of the potential mechanisms for the relapse and persistence of the SARS-CoV-2 virus infection could be an endocytic transport pathway related to the secretion of COVID-19-loaded extracellular vesicles and exosomes.

Extracellular vesicles: Key players in the COVID-19 transmission

The attachment, fusion and entrance of SARS-CoV-2 are exerted by a trimeric spike glycoprotein (S protein) which is expressed on the surface of the virus (Figure 1). Analogous to the envelope of hemagglutinin of influenza species or HIV, SARS-CoV-2 S protein also belongs to class I fusion proteins [8]. Moreover, the presence of receptor-binding domain and intracellular proteolytic cleavage is necessary for the fusion of SARS-CoV-2 into the target cells [9]. As coronaviruses use transmembrane glycoprotein receptors for their entrance into the target cells, those cells with more expressed receptors are more vulnerable to viral infection [10]. The interaction between the SARS-CoV-2 spike protein with the host cell protein named dipeptidyl peptidase 4 is essential for the fusion of the virus into the host cells to the degree that induction of any polymorphism in this protein could influence the entry of the virus [11]. Furthermore, aminopeptidase N (APN) acts as a receptor for various coronaviruses, such as transmissible gastroenteritis virus, canine coronavirus, feline coronavirus, and human coronavirus [12] and regarded as a target for the treatment of cancer [13]. APN exists within exosomes and extracellular vesicles (EVs) from glial cells (Potolicchio et al. 2005) [14] and mast cells [15]. The protease system of SARS-CoV-2 is transmembrane-anchored and intracellular linked with the type II transmembrane serine protease family which is critical for virus infection [16]. Previous studies have demonstrated that TMPRSS2 and type II transmembrane serine protease family cleaves SARS-CoV-2 spike glycoproteins to organize fusion-catalyzing and unlocked structure at the cell surface and facilitate virus entrance [16, 17]. Several glycoproteins have been reported to be necessary to complete the fusion process [18]. Various tetraspanins that are anchored in extracellular vesicle membranes may contribute to the SARS-CoV-2 fusion event [19]. EVs elucidate a new frontline in the viral infections field. Initial studies reported that during viral infection the number of exosomes released by infected cells increased significantly and that exosomes participate in the spread of viral components in different host cells, an event that leads to disease progression [20].

In recent years, an increasing number of studies have demonstrated the intrinsic role of EVs in injury [21], inflammation [22] and viral infection of the respiratory tract and lung [23]. EVs are membrane-enclosed structures secreted by cells and participate in cell-to-cell connection through the horizontal delivery of various molecules at short and long distances. Viruses and EVs have similar physicochemical characteristics, such as heterogeneity in size distribution and small size [24], and applying similar mechanisms for cell entry and biogenesis [24]. Viruses enter the uninfected or healthy cells through the endocytic pathway and egress the infectious cell by direct budding via the membrane. Commonly, in viral infections, EVs surround pathogen-derived lipids, proteins, and nucleic acids and become delivery carriers for viral elements [24].

Vulnerable cells for coronavirus likely express two agents for fusion, proteases, and receptors, with a close

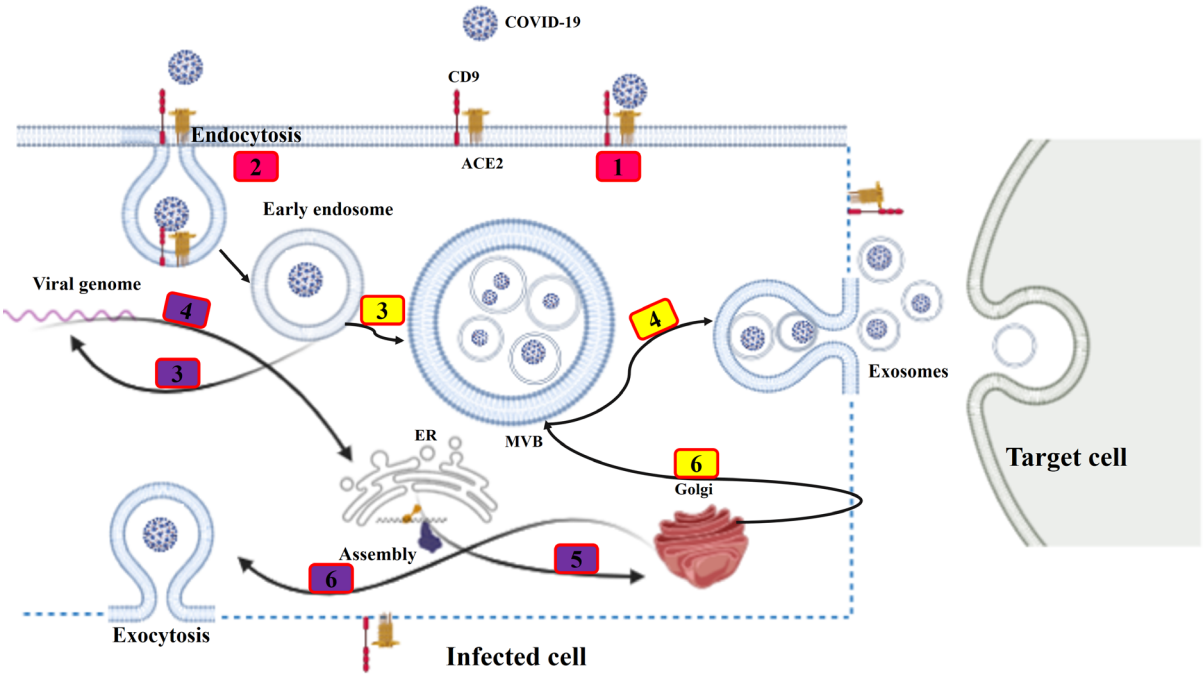

Figure 1. A proposed model for SARS-CoV-2 life-cycle in human lung cells

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Notes: 1) Through interaction with the angiotensin-converting enzyme 2 receptor, SARS-CoV-2 uses its Spike protein to enter into the target epithelial cells. Upon binding to the angiotensin-converting enzyme 2 receptor, the conformation of spike protein changes in the way that it could recruit endosomal pathways, an event that augments the fusion ability of the virus; 2) Subsequently, the entered SARS-CoV-2 distributes viral RNA into the cytoplasmic space; 3) Alternatively, intact viruses or components of COVID-19 may find a way into the exosomal pathway via multivesicular bodies; 4) In response to viral proteinases, the replica polyproteins, which were produced by the released RNA, degrade into viral particles; 5 & 6) After assembling as virions in host cells' endoplasmic reticulum and Golgi, viral components exit the host cells as extracellular vesicles and exosomes. The exosomal pathway contributes to the spread and persistence of the viral particles by delivering CD9 and angiotensin-converting enzyme 2 to other target cells.

affiliation [25]. The TMPRSS2 and tetraspanin CD9 promote coronavirus entrance and severe pulmonary infection in the murine model [26]. In a similar investigation, Böker et al. demonstrated that increased CD9 expression had the potential to enhance the speed and efficiency of lentiviral transduction in different cell lines, including SH-SY5Y, HEK293, T lymphocytes, HeLa and B cell [27]. CD9 molecules are expressed in the surface of EVs and exosomes and have an essential function in exosomes loading cargo and biogenesis [28]. Accordingly, EVs and exosomes released from infectious cells hold a respectable share in the infection of other cells through delivering CD9 [26]. The uptake of exosomes by recipient cells leads to the delivery of exosomal cargo [29] and increases susceptibility to viral infection. Additionally, CD9 molecules load exosome cargo by recruiting the protein-protein communication network in the membrane of multivesicular bodies [30] (Figure 1). Meanwhile, COVID-19 infection enhanced circulating exosomes enclosing viral antigens, lung-related self-antigens and 20S proteasome [31]. This finding reinforces the idea that SARS-CoV-2 virus-infected cells release exosomes comprising virus particles (Figure 1) or viral components (Figure 2). Exosomes further contribute to the systemic spreading of COVID-19 by two main mechanisms as follows. Firstly, inhibiting induced immune response due to the presence of self-antigens [32]. Secondly, facilitates coronavirus infection through receptors that are separate from viral receptors [33]. SARS-CoV-2 uses EVs, specifically exosomes, for host cell entrance, viral spreading and evasion from the immune response.

Conclusion

By bringing together the findings from these researches, we suggest that one of the potential explanations for the relapse and persistence of the SARS-CoV-2 virus infection could be an endocytic transport pathway related to the secretion of COVID-19-loaded EVs and exosomes (Figure 1). This "Trojan Horse" mechanism may bring a possible description for the re-emergence of the viral RNA in the recovered SARS-CoV-2 individuals 1-2 weeks post-discharge, signifying that viral elements

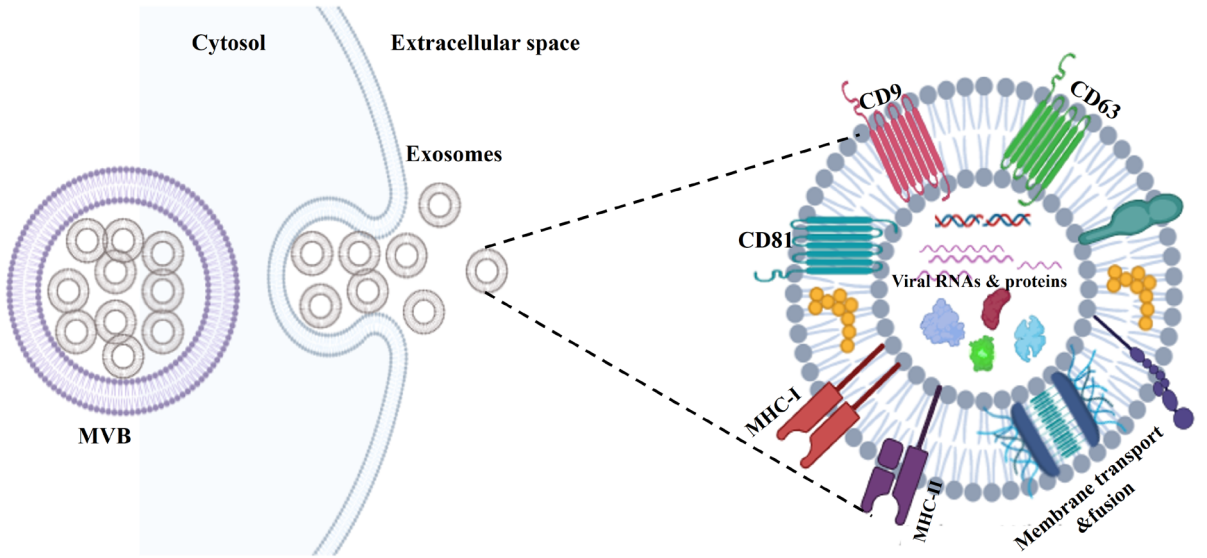

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Figure 2. Model of the biogenesis and transport mechanisms of SARS-CoV-2 by exosomes

Notes: The early endosome membrane bulges inward to form multivesicular bodies (multivesicular bodies) that deliver exosomes carrying viral components via membrane fusion. Tetraspanin molecules (CD9, CD63 and CD81) are expressed on the surface of the exosomes and have an essential function in exosomes loading cargo and delivery of exosomes to target cells.

were concealed within such exosomes or EVs during this latency period and then initiated to re-spread again.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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