

Lessons for SARS-CoV-2 study (COVID-19 disease) from its exosome relatives

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Abstract

Our first modern global pandemic is caused by a nanosized lipid vesicle, called SARS-CoV-2. Its molecular structure and biogenesis have remarkable similarities with Extracellular Vesicles (EVs, most notably exosomes) that are constantly shed by all cells during their life. Their resemblance may not be a coincidence. Growing body of evidence has shown that EVs have significant roles in various biological processes, including viral infection, transmission and anti-viral response. Drawing comparison with the virus might shed light on how we could fight the COVID-19 disease. This may include novel EV research and diagnostics technologies as well as novel EV-based treatments.

Introduction

SARS-CoV-2 has shortly become one of the most studied viruses following the COVID-19 outbreak in Wuhan in December 2019. These nanosized lipid vesicles have put at risk the global healthcare system and the world economy. SARS-CoV-2 have striking similarities with EVs, most notably exosomes, since they share many features (diameter, density, structure, cargo type, cargo origin, cell entry mechanism and potentially exit mechanism) despite having different functions.

EVs are heterogeneous vesicles naturally released by a cell during its life and usually carry biologically active molecules that can deliver their messages to local or distant targets.

More generally, EVs and enveloped viruses have been hypothesized to be, in fact, “close relatives” ([Hoen et al., 2016](#)) and they might have common ancestry roots.

There are two possible assumptions: either enveloped viruses, such as SARS-CoV-2, developed from primitive lipid vesicles by encapsulating nucleic acids and incorporating specific membrane molecules for which cells have receptors; or EVs evolved as defective viruses that lost the machinery for nucleic acid replication and membrane molecules that mediate viral infection ([Margolis & Sadovsky, 2019](#)).

SARS-CoV-2 is part of the coronavirus family, whose birth estimates vary widely, from 10,000 years ago to 300 million years ago. We are now aware of dozens of strains ([Graham et al., 2013](#)) ([Gorbalenya et al., 2020](#)), seven of which infect humans. Among the four that cause common colds, two (OC43 and HKU1) would come from rodents, and the other two (229E and NL63) from bats. The three that cause severe disease — SARS-CoV, MERS-CoV and SARS-CoV-2 — all are believed to come from bats ([Cyranoski, 2020](#)).

SARS-CoV-2 and EV intracellular crosstalk

Cell entry & message function

SARS-CoV-2 have surface proteins on their lipid bilayer that can adhere to the membrane of specific target cells for entry ([Hoffmann et al., 2020](#)) similarly to EVs ([Hoen et al., 2016](#)). Consistently with the SARS-CoV strain ([Wang et al., 2008](#)) and MERS-CoV ([Yang & Shen, 2020](#)), SARS-CoV-2 may enter the cell via the endocytic pathway ([Shang et al., 2020](#)), which has also been described as one of the main routes for EV uptake ([van Dongen et al., 2016](#)), ([Mathieu et al., 2019](#)).

Once inside the cell, EVs and viruses are both able to deliver bioactive contents which can change cellular activity and regulate protein expression to fulfil their function: either replication & survival for SARS-CoV-2 or message delivery from releasing cells for EV.

SARS-CoV, and most likely SARS-CoV-2, release their genomic material in the cytoplasm where it is translated. It is complemented by about 14 open reading frames (ORF), each of which encodes a variety of structural and non-structural proteins. After being translated, structural and accessory proteins (including Membrane, Spike, and Envelope) are insulated in the endoplasmic reticulum and then moved to the endoplasmic reticulum-Golgi intermediate compartment (ERGIC). Meanwhile, the previously replicated genome program directly joins the nucleocapsid protein to the nucleocapsid form and move into the ERGIC. In this compartment, nucleocapsids will meet with several other structural proteins and form small vesicles ready to be exported out of the cell ([Astuti et al., 2020](#)).

EVs deliver protein-encoding mRNAs, small noncoding RNAs (involved in the regulation of gene expression) and proteins with various functional effects ([Hoen et al., 2016](#)). It is however not yet fully understood how such molecular messages are processed ([Kalluri & LeBleu, 2020](#)).

Biogenesis cross-pathing

Both enveloped viruses (including SARS-CoV-2) and EVs use the cellular vesiculation machinery which explains striking similarities ([Mathieu et al., 2019](#)). This includes the induction of membrane curvature, the inclusion of specific cargo, and membrane fission for release ([Alenquer & Amorim, 2015](#)). It has even been hypothesized that viruses exploit preexisting pathways for intracellular trafficking in a “Trojan horse” strategy ([Gould et al., 2003](#)).

For SARS-CoV, viral assembly and budding occur in smooth-walled vesicles in the ERGIC ([Ashour et al., 2020](#)), while, in EVs, budding rather occurs from multivesicular bodies (MVBs), a specialized type of late endosome ([van Dongen et al., 2016](#)). Both ERGIC vesicles and MVBs then fuse with the membrane releasing the virion and the EV respectively.

SARS-Cov-2 and EV host cell surface molecules

For SARS-CoV, at least 1% of the integral membrane and 7% of membrane-associated proteins are found in the host cell, including ARF5 and COPI. They are mostly associated with the ERGIC where the virions are assembled ([Neuman et al., 2008](#)).

EVs capture selectively cell-specific proteins, lipids and RNAs from the secreting parental cell ([Margolis & Sadovsky, 2019](#)). It has been reported that several cellular membrane proteins normally associated with EVs, such as cell adhesion proteins and tetraspanins, have also been found in retroviruses, some of them having a crucial role in facilitating infectivity ([Segura et al., 2008](#)), ([Sato et al., 2008](#)), ([Hoen et al., 2016](#)).

The most documented tetraspanins found on the surface of EVs are CD9, CD63 & CD81. They have an important role in cell targeting and fusion ([van Dongen et al., 2016](#)). Some retroviruses, such as HIV-1, were mentioned to be enriched in CD63 and CD81 ([Mathieu et al., 2019](#)). It suggests that they could possess an additional “cloaking” ability that could make them more infectious: targeted cell and the immune system may confuse it with naïve EVs ([Sato et al., 2008](#)), ([Hoen et al., 2016](#)).

For coronaviruses, tetraspanins on the host cell membrane can promote entry by binding to the virus receptors and proteases contributing to the infection and pathogenesis. For instance, CD9 on the cell membrane facilitates the entry of MERS-CoV after interacting with MERS-CoV receptor dipeptidyl peptidase 4 (DPP4) ([Earnest et al., 2017](#)), ([Hantak et al., 2018](#)). Interestingly, CD81 has a role in the

entry of Hepatitis C virus (HCV) into the cell as being the first identified receptor ([Farquhar et al., 2011](#)). Having said that, it has not been confirmed yet which host cell molecules are also present on the envelope of SARS-CoV-2.

SARS-CoV-2 host cell cargo

In general, 30% of the total RNA mass in retroviruses consists of host RNA with a small amount of mRNA, rRNA and tRNA that may act during replication ([Telesnitsky & Wolin, 2016](#)).

For SARS-CoV, RNA-binding proteins were detected, among other host proteins, suggesting host RNA were also present since they are packed together ([Neuman et al., 2008](#)). Therefore, it is expected to find host cell molecules in SARS-CoV-2 as well although their identity is currently unknown.

EV cargo from SARS-CoV-2 infected cells

Given the vesicular machinery crosstalk mentioned above, it is reasonable to hypothesise that EVs secreted by SARS-CoV-2 infected cells contain viral proteins and RNA cargo.

It has been shown that EVs from infected cells can incorporate viral proteins or fragments of viral RNA ([Hoen et al., 2016](#)), especially as some retrovirus uses the EV-friendly ESCRT (endosomal sorting complexes required for transport) pathway ([Alenquer & Amorim, 2015](#)). This possibility has not been examined in relation to SARS-CoV-2 infection.

SARS-CoV-2 assembly also seems to be ESCRT-dependent. Since EVs are formed from MVBs that mature from the early endosome, they are likely to be at the proximity of SARS-CoV-2 which use this pathway to infect target cells ([Yang & Shen, 2020](#)), ([Ashour et al., 2020](#)). This can potentially have an impact on MVB formation and indirectly EVs content ([Alenquer & Amorim, 2015](#)).

It is also possible that SARS-CoV-2 might tag along with EVs whose entry is cleared by the receiving cell. HIV-1 was speculated to bind to EVs via TIM-4, CD9 and/or CD81 ([Sims et al., 2018](#)).

Potential role of EVs in promoting or blocking SARS-CoV-2 infection

Interestingly, virus-infected cells tend to produce more EVs than virions, already suggesting that they may play an important role in viral infections ([Raab-Traub & Dittmer, 2017](#)). In fact, they may have both proviral and antiviral effects.

Modified EVs secreted from infected cells can interact with other cells making them more susceptible to infection. For instance, for HIV, platelet- and megakaryocyte-derived EVs can transfer HIV co-receptors CXCR4 to CXCR4-null target cell. This mechanism renders cells ready to be infected by HIV in *in vitro* analysis ([Urbanelli et al., 2019](#)). Therefore, it might be speculated that EVs secreted by SARS-CoV-2 infected cells contain messages that force receiving cells to present more ACE2 (or other relevant receptors and binding proteins) on their surface. This would increase its infective capacity.

It has also been observed that EVs could increase virus binding, induce death of antiviral immune cells or even act as decoys for anti-viral antibodies ([Urbanelli et al., 2019](#)).

Inversely, modified EVs secreted from infected cells may mediate antiviral effects by presenting the viral antigen to immune cells stimulating the immune response. Additionally, EVs from healthy cells may transport antiviral proteins to other cells more susceptible to infection. ([Hoen et al., 2016](#)), ([Raab-](#)

[Traub & Dittmer, 2017](#)). It is tempting to speculate that engineered EVs might be used to compete or interfere with virus entry and infection but such approaches have not been tested.

Discussion

Helping the global research & diagnostics effort on SARS-CoV-2 with novel EV tools

There are two main challenges for SARS-CoV-2 research: low-contaminant isolation & functional virion quantification in various biospecimens.

Regarding virus isolation in infected patients or cell media culture, they both contain a high amount of small EVs whose biochemical similarities (size, density) ([Renner et al., 2018](#)) make them indistinguishable from most of viruses for standard techniques (e.g. ultracentrifugation or size exclusion chromatography). They are therefore co-isolated together -along with other contaminants-, creating problems for downstream research applications such as mass spectrometry or X-ray crystallography ([Mateu, 2013](#)), ([Greco et al., 2014](#)). Besides, hybrid virion-EVs may complicate things further ([Hoen et al., 2016](#)). Novel EV immuno-isolation techniques (with one or two surface markers) could eliminate unwanted EVs or simply be repurposed to isolate the virus using its unique surface markers (e.g. Spike protein, Envelope protein and/or Membrane protein). It is interesting to note that SARS-CoV-2 may also present tetraspanins and other host molecules on its surface, therefore standard, single marker isolation approaches might not be sufficient.

Once separated, the virions could be lysed to study more stable virion proteins than fast-mutating Spike protein. This could be very valuable ([Schoeman & Fielding, 2019](#)) in designing more sustainable SARS-CoV-2 vaccines.

Regarding functional virion quantification, it may be useful to better understand virulence factors, such as disease progression, by identifying viable versus non-viable particles. Accurate isolation and quantification approaches using novel EV capture and quantification tools modified to handle viruses, could facilitate patient stratification and prognosis efforts.

There is one remaining clinical challenge for COVID-19 diagnostics which could be dealt with by using EVs: assess disease severity for effective triage of patients. EVs can be good indicators of lung injury and inflammation. For instance, there are substantial EVs secreted by various cell types in bronchoalveolar lavage fluid (BALF) after pulmonary infection or injury ([Lee et al., 2018](#)) ([Lanyu & Feilong, 2019](#)). Careful characterization of EV subpopulation from COVID-19 patients has not been performed thus far but might assist efforts to better understand and manage lung (and other organs) complications related to COVID-19.

Helping the prevention or treatment of COVID-19 disease with novel EV-based therapies

Considering the proviral effect that EVs from infected cell might have, the use of engineered EVs with non-pathogenic viral proteins or molecules could be considered as a vaccine strategy ([Hoen et al., 2016](#)), ([Urbanelli et al., 2019](#)). One of these strategies was already studied for SARS-CoV where an EV vaccine containing Spike protein of the virus could induce high levels of neutralizing antibodies in mouse models ([Kuate et al., 2007](#)).

In terms of antiviral, engineered EVs can also be used for targeted drug delivery by using the same viral protein that binds cells susceptible to being infected. Alternatively, some EVs have natural affinity to inflammation sites. Platelet-derived EVs loaded with anti-inflammatory TPCA-1 were shown to calm cytokine storm, often associated with severe COVID-19 cases, in mice model with acute lung injury

([Ma et al., 2020](#)). EVs expressing specific proteins, such as ACE2, could act as virus binders and thereby reduce infectivity.

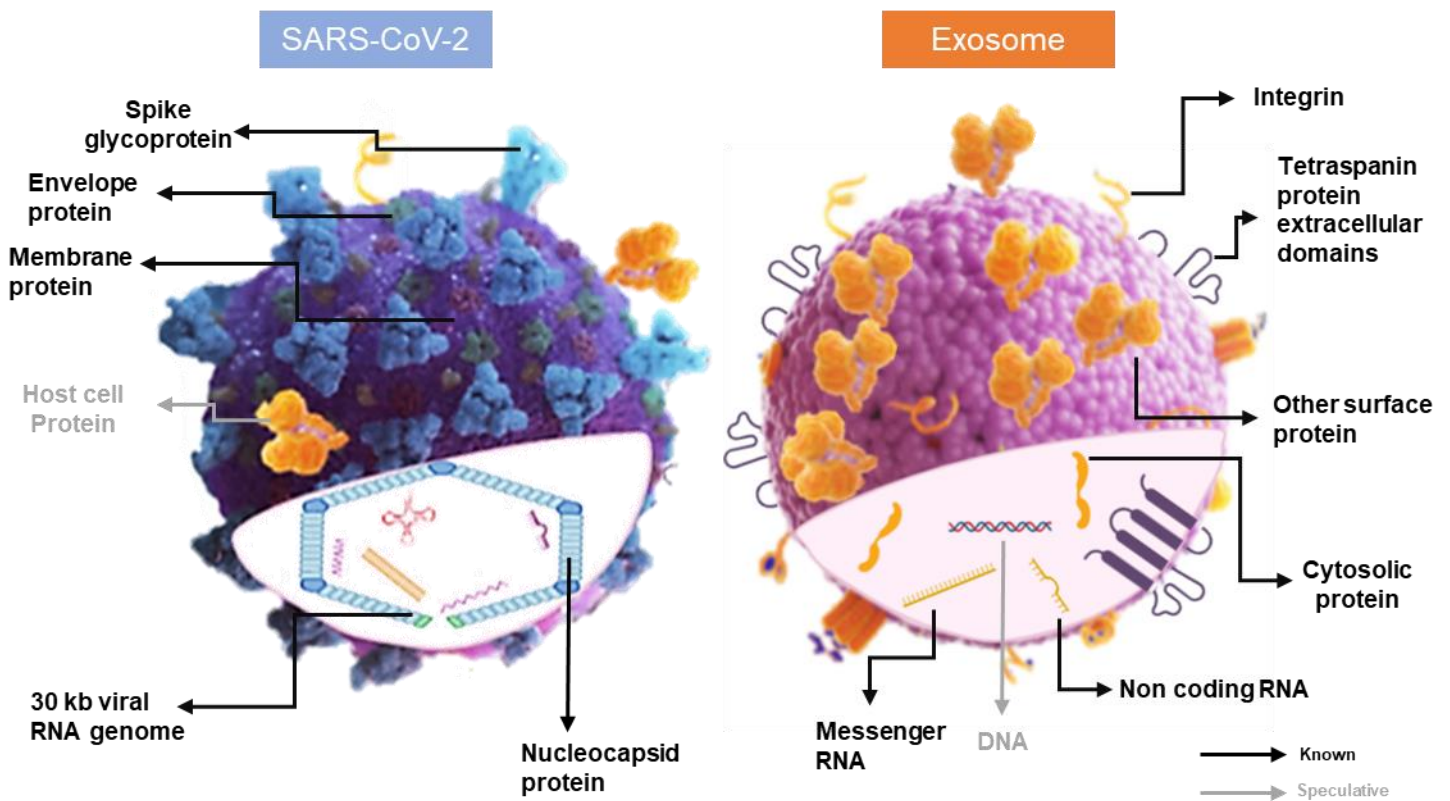
Another promising prospect of EV-based therapeutics is the administration of mesenchymal stem cells (MSCs)-derived EVs to reduce inflammation and injury in respiratory diseases. This is currently being tested in clinical trials in China for to the treatment of COVID-19 related severe pneumonia ([Shah et al., 2019](#)), ([NCT04276987](#)) ([ChiCTR2000030484](#)) ([ChiCTR2000030261](#)). International Society for Extracellular Vesicles (ISEV) and International Society for Cell and Gene Therapy (ISCT) have recently made a joint statement about its potential under the right conditions ([Borger et al., 2020](#)).

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Key similarities		
80-120 nm	Diameter	50-150 nm
1.16–1.18 g/L	Density	1.13–1.18 g/L
Lipid bilayer envelope	Structure	Lipid bilayer envelope
Protein, RNA, others	Cargo type	Protein, RNA, others
Releasing cell	Cargo origin	Releasing cell
Endocytic pathway, others	Cell entry mechanism	Endocytic pathway, others
Key differences		
Replication & survival	Function	Message delivery
Endoplasmic reticulum–Golgi intermediate compartment (ERGIC)	Intracellular biogenesis	Multivesicular body (MVB)
Host cell budding	Cell exit mechanism	MVB-Plasma Membrane (PM) fusion or surface shedding