COVID−19 hypothesis: Exosomes of mesenchymal stem cells as nano-cargos for anti-SARS-CoV-2 asRNAs

Running title: **Anti-SARS-CoV-2 asRNAs' MSC exosomes**

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Abstract

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in December 2019 is rapidly spreading worldwide. Scientists are searching to find an effective treatment for coronavirus disease 2019 (COVID-19). Several antiviral drugs are currently undergoing clinical trial studies to evaluate their safety and efficacy in the treatment of COVID-19. SARS-CoV-2 is a positive-sense single-stranded RNA virus. Previous studies showed the efficacy of anti-RNA virus, single strand RNA inhibiting antisense RNAs (asRNAs), on silencing of virus replication, *in vitro*. To transfer the anti-SARS-CoV-2 asRNAs to human respiratory epithelium, exosomes can be suggested as a promising candidate. Mesenchymal stem cells (MSCs) secret exosomes and they can be loaded by anti-RNA virus asRNAs. MSCs-secreted exosomes as a nano-cargo of anti-SARS-CoV-2 asRNAs have other therapeutic potentials such as immunomodulatory effects of their cytokine contents, affinity to respiratory epithelial attachment, anti-fibrotic activity in lung, non-toxicity for normal cells, and do not trigger an immune response. Inhalation of anti-SARS-CoV-2 asRNAs may stop SARS-CoV-2 replication. Producing a specific anti-SARS-CoV-2 asRNAs by targeting the genome of virus and their delivery by MSCs exosomes is suggested and discussed. This approach potentially sheds light on gene therapy of the other human lung diseases via inhalational delivery using exosomes in future.

Keywords: SARS-CoV-2, COVID-19, antisense RNA, exosome, mesenchymal stem cells, treatment

Introduction

Infections with the majority of respiratory viruses are a global health concern and are known as one of the main causes of death among high-risk population (1). Coronavirus disease 2019 (COVID-19) is one of the respiratory viral infections caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerging from Wuhan, China and shortly led to a catastrophic worldwide epidemic health problem (2). The virus belongs to the family of coronaviruses such as severe acute respiratory syndrome (SARS) and Middle-East respiratory syndrome (MERS) (3-5). SARS-CoV-2 is a positive-sense single-stranded RNA virus showing a high rate of pathogenesis (2). The symptoms of COVID-19 range from mild to severe and may include fever, cough, chills, shortness of breath and bilateral pneumonia in end-stage patients (4). Although, some therapeutics are suggested for curing some symptoms of COVID-19 and other coronaviruses but to the best of our knowledge, there is no effective antiviral to treat COVID-19 (6).

Anti-inflammatory and immunomodulatory effects of MSCs as a therapeutic approach for various types of respiratory diseases have been confirmed by clinical studies (7). Different important sources of MSCs including Wharton's jelly, umbilical cord, umbilical cord blood, dental pulp, menstrual blood, are used in these clinical trials (7). Compatibility of MSCs-derived exosomes with host immune system, their nano-size and low-toxicity making them a very efficient drug carrier (8-10). As in patients of COVID-19, SARS and MERS, serum concentrations of pro-inflammatory cytokines (IL-1, IL-6, and TNF-α) and chemokines (IL-8) increase (11), using MSCs-derived exosomes for transportation of asRNA may modulate these signs, as well.

As the target of this therapeutic approach is the infected epithelial cells of respiratory tract, drug administration by inhalation has been suggested. Lung cancer therapy by inhalation of MMP2/9-triggered-release micelles had appropriate effects (12). Using serum-derived exosomes as a vehicle to small RNA molecules via inhalational delivery into the lung macrophages *in vivo* has been shown in mice for lung inflammation treatment (13). In our hypothesis exosomes containing asRNAs can introduce to patients with inhalation, so the asRNA may reach to the respiratory system with more efficacy.

We hypostatized MSCs-derived exosomes can perform as a carrier to deliver anti-SARS-CoV-2 miR-5197-3p to infected cells to decrease the viruses' replication and transcription *in vivo* (14). Table 1 summarized locations and products of single strand RNA of SARS-CoV-2 can be inhibited by asRNAs. It may be useful to exploit the MSCs-derived exosomes to efficiently deliver anti-SARS-CoV-2 asRNAs for targeted drug delivery (8). Also, previous studies have demonstrated that exosome based drug delivery can protect exosome-encapsulating RNAs from RNase enzymes (15). Different exosomes with cell-specific surface proteins may have distinct routes and circulation pattern all over the body (16). Accordingly, it can be predicted that MSCs-derived exosomes contain the targeted asRNA for those viruses. To our knowledge, the function of these asRNA filled exosomes derived from MSCs has not been investigated.

Human Wharton jelly-derived MSCs is one of the most important sources of primary MSCs that can be obtained from umbilical cord with standard methods (17). MSCs and hematopoietic stem cells CD markers can be analyzed by flow cytometry to confirm purity of MSCs isolation. In order to appraise the differentiation capabilities of MSCs, the cells will be test for osteogenic, adipogenic, and chondrogenic differentiation. The conditioned medium (CM) will be prepared using MSCs (18). The exosomes will be isolated from CM by commercial kit

(19). Transmission electron microscopy test will be used for morphological assessment of the isolated exosomes (20).

Transportation of antisense RNA inhibiting RNAs (asRNAs)

RNA viruses use intracellular host cell machinery for DNA replication, RNA transcription and protein synthesis (21). The pathogenesis of such viruses is mainly depends on the rate of genome replication and cell destruction following host immune responses (22). Thus, blocking the viral genome replication and transcription can be a promising approach to combat the infection (23). To selective inhibition of RNA virus replication, many targets exist (23). However, anti-replication effect of antisense RNA inhibiting RNAs (asRNAs) as a non-coding RNAs (ncRNAs) has not been investigated for coronaviruses. Although, significant improvements have been made in the field of nucleic acid-based therapies and numerous carriers have been used to transport molecules such as asRNA, studies on finding suitable carriers for these kinds of molecules are still ongoing (24). The other challenge of designing an anti-SARS-CoV-2 asRNA is transportation of them into the replication site, host respiratory epithelial cells. As a consequence of the nanometer size of asRNAs, they can go through the vessels and affect other parts of the host's body (25). One of the main problems in the asRNA transfer, related to their instability and negative charge, and hence, even in the presence of a suitable transfection reagent, it cannot effectively penetrate the hydrophobic cell membranes (8, 26).

To minimize the side effects of asRNAs on other tissues, we can use targeted drug delivery system. For this purpose, exosome-based drug delivery approach can be one of the best candidates (27). On the other hand, the mesenchymal stem cells (MSCs) have been extensively studied for their immunosuppressive effects.

The target asRNAs will be produced using a cost-effective DGCR8-independent stable asRNA expression (DISME) strategy (28). To increase the expression level of asRNAs, units of a U6 promoter-driven expression cassette will be inserted in the vector (28). The exosomes will be loaded with anti-SARS-CoV-2 miR-4778-3p, using electroporation method (8). The loaded exosomes were reisolated using commercial kit (19). The amount of anti-SARS-CoV-2 asRNAs oligonucleotides in MSCs-derived exosomes will be estimated as previously described (8). Then, the asRNA loaded exosomes will be exposed to SARS-CoV-2 culture in transmembrane protease, serine 2 (TMPRSS2)-expressing VeroE6 cell line and virus replication will be analyzed (29).

To test the therapeutic method in animal model, two approved model of rhesus macaques and ferrets can be used (30). These models can be infected with SARS-CoV-2 and they show virus replication and shed virus (30). After confirming of the treatment in animal model, asRNAs efficiency analyzing, the asRNAs encapsulated with exosomes will be introduced to the patient with inhalation (14). Figure 1 summarized this therapeutic approach.

Discussion

Exosomes have been introduced as a new alternative to the transmission of therapeutic molecules (27). Exosomes are nano-sized lipid package that are produced in the most eukaryotic cells (31). They are capable of being drug carriers since they are composed of cell membranes, rather than synthetic polymers (27). Exosomes have many functions in human bodies including cell to cell communication, wound healing, tissue regeneration and even cell death (32). They have some information from their primary parental cells (33). Exosomes with 60-80 nm size are the smallest particles between entire exosomes (34). They can interact with macromolecules and that they can

serve as distributors for proteins, lipids, mRNA, miRNA, and DNA (35). Exosomes may interact with the host's immune system, so selection of the parent cell for exosome production need for careful (36). Application of human exosomes for treatment of diseases are tested in clinical trials (37) and have a developing market (38).

MSCs have been used in many clinical trials of transplant rejection, autoimmune disorders, and inflammation- associated diseases (39). It has been shown that MSC-secreted factors suppress T-cell proliferation (40). Therefore, MSCs-derived exosomes have the same properties as their parent cell (41). With immunomodulatory characteristics, MSCs-derived exosomes suppress the secretion of tumor necrosis factor-α (TNF-α) and interlukin-1 (IL-1) in peripheral blood mononuclear cells (41). Some of the earliest exosome research indicated that they can carry the MHC-peptide complexes that are recognized by T lymphocytes (42) in order to more activate cell immune system and destroy the tumor and other cell contaminant (for example viruses) (43).

The asRNAs therapy is suggested as a new method for controlling viral infections (44). Here, we hypothesized MSC derived exosomes containing anti- SARS-CoV-2 asRNA to help finding a specific treatment for COVID-19. Since the SARS-CoV-2 genome replicates in respiratory epithelium of host cells, the developed asRNAs should have characters in order to not damage genome of host cells. Over the past decade, discovery of ncRNAs including microRNA, small interfering RNA (siRNA), PIWI-interacting RNA (piRNA) and long noncoding RNA (lncRNA) is a well-defined research topic. Application of asRNAs considering the required purpose undergoes two challenges: 1) The asRNA binding to the exact site of the RNA virus genome. 2) Side effects of the randomly binding of these asRNAs to human mRNAs due to the subjective set of nucleotides in synthetic asRNAs those bind with complete complementarity

(45-48). It is necessary to select asRNAs that can most effectively bind to the nucleotide sequence of the RNA genomes of these coronaviruses. Therefore, constructing specific asRNAs for coronaviruses of COVID-19, SARS, and MERS that will not affect the expression of human or animal genes is crucial (14).

In this case, asRNA with the ability of specific binding to viral genome, must be selected (14, 49). *In-silico* analysis showed that miR-5197-3p (cc-miR, fully complementary miRNA) has affinity to ssRNA of SARS-CoV-2 and also two miR-6864-5p and miR-4778-5p can strongly bind to MERS (cc-miRm) and SARS (cc-miRs) genomes, respectively (14) (Table 1). The miR-5197-3p can bind to a few human genes which they have similar characteristic (14). In order to avoid the potential side effects, this asRNA has sustained some changes in the length and nucleotide sequences and called cc-miR2 (fully complementary miRNA). This new asRNA is more efficient to bind with ssRNA of SARS-CoV-2 without reacting with human protein- coding genes and presenting related side effects (14). The next concern of this therapeutic approach is related to the possibility of SARS-CoV-2 genomic mutations during worldwide spreading of the virus. Fortunately, comparative sequence analyses of SAR-CoV2 genomes isolated from different geographical locations have unique features (50). Therefore, designing a conserved cc-miRNA is possible.

The SARS-CoV-2 binds to epithelial cells of the nasal cavity after inhalation of virus and replicates there (51). Then along the conducting airways, SARS-CoV-2 migrates down the respiratory tract (52). Studies utilizing exosomes for treatment of different kind of lung diseases are mainly administer by systemically into a vein or direct tissue injection. But treatment of the lungs by inhalation is the minimally invasive and most direct route of delivery (53). Nonetheless, innovative methods must be developed for the scale up of exosome production and isolation.

Conclusions

The asRNAs can inhibit ssRNA of SARS-CoV-2 will be effective on silencing of virus replication. MSCs secret exosomes and they can be loaded by anti-RNA virus asRNA. Producing a specific anti-SARS-CoV-2 by targeting the viral genome can be suggested for developing a novel therapeutic candidate tor the disease.

Authors' contributions

A.A., M.Z., Z.F., A.H., A.K., and H.H. collected information and co-wrote the draft. K.V., I.N., M.A.B., and A.T. designed the idea, and critically revised the paper.

Competing interests

There is no conflict of interest.

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Table 1

Table 1. Locations and products of single strand RNA (ssRNA) of SARS-CoV-2, MERS, and SARS can be inhibited by antisense RNAs (asRNA) (14)

	SARS-CoV-2	MERS	SARS
asRNA name	miR-5197-3p	miR-6864-5p	miR-4778-3p
asRNA sequence	UAAGCUACUGAGUCAGAGAAGAA	CCGUAUAGACUGAACAGGGAAGUU	AGUUGAGACGUUUCCUUC-UUCU
Matched sequence of ssRNA	AUUCGAAGACCCAGUCCCUACUU	GGCGUUUCUGACUUGUCCCUCAAA	UCGACUCCGCAAGGGAGGUAGGA
Matching (%)	89	88	91
asRNA length	23	24	22
Gene name	spike glycoprotein	orf1ab	orf1ab
Location	21874-21896	1188-1211	1450-1472
Product of complete gene	Surface glycoprotein	1AB polyprotein	Counterpart of MHV p65

Figure 1

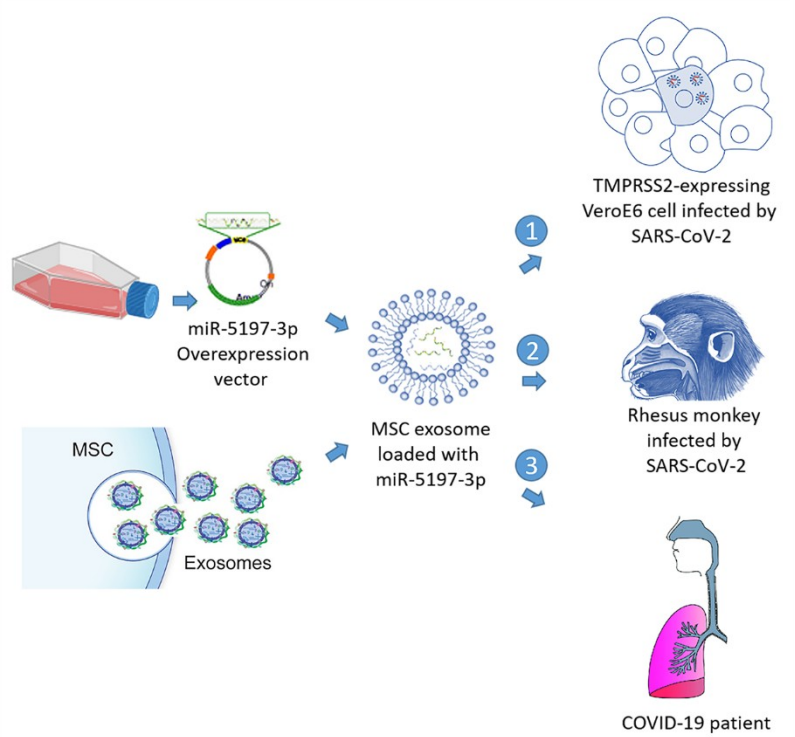

Figure 1. Schematic diagram of developing a therapeutic method for COVID-19 using exosomes of mesenchymal stem cells as nano-cargos for antisense RNAs for inhibiting single strand RNA (ssRNA) of SARS-CoV-2