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Exosomes of mesenchymal stem cells as nano-cargos for anti-SARS-CoV-2 asRNAs

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ABSTRACT

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in December 2019 and rapidly spread worldwide. Since then, scientists have searched to find an effective treatment for coronavirus disease 2019 (COVID-19). In this regard, several antiviral drugs are currently undergoing clinical trial studies to evaluate their safety and efficacy in the treatment of COVID-19. Some of these drugs have been designed based on this fact that SARS-CoV-2 is a positive-sense single-stranded RNA virus and previous studies showed the efficacy of anti-RNA virus, single strand RNA inhibiting antisense RNAs (asRNAs), for silencing virus replication, in vitro. Exosomes can be suggested as a promising candidate to transfer the anti-SARS-CoV-2 asRNAs to human respiratory epithelium. Exosomes are secreted by mesenchymal stem cells (MSCs) and can be loaded by asRNAs of an anti-RNA virus. MSCs-secreted exosomes as a nano-cargo of asRNAs of anti-SARS-CoV-2 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 not triggering an immune response. Moreover, inhalation of anti-SARS-CoV-2 asRNAs may stop SARS-CoV-2 replication. Producing specific anti-SARS-CoV-2 asRNAs by targeting the genome of virus and their delivery by MSCs exosomes are suggested and discussed. This approach will potentially shed light on gene therapy of the other human lung diseases via inhalational delivery using exosomes in future.

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Abbreviations

asRNAs, antisense RNA inhibiting RNAs; CM, Conditioned Medium; COVID-19, Coronavirus disease 2019; IL, Interleukin; iPSC, Induced Pluripotent Stem Cell; lncRNA, Long Noncoding RNA; MERS, Middle-East Respiratory Syndrome; miRNAs, microRNAs; MSCs, Mesenchymal Stem Cells; ncRNAs, Non-coding RNAs; piRNA, PIWI-interacting RNA; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; siRNA, Small Interfering RNA; ssRNA, Single Strand RNA; TNF-α, Tumor Necrosis Factor-α

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 the 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), a disease shortly led to a catastrophic worldwide epidemic health problem (2). This 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). Some therapeutics have been suggested for curing some symptoms of COVID-19 and other coronaviruses such as Remdesivir, Favipiravir and corticosteroids (6, 7). However, to the best of our knowledge, there has been few effective antiviral to treat COVID-19 (8), yet it needs more study.

Anti-inflammatory and immunomodulatory effects of MSCs as a therapeutic approach for various types of respiratory diseases have been confirmed by clinical studies (9). Different important sources of MSCs including Wharton's jelly, umbilical cord, umbilical cord blood, dental pulp, menstrual blood, were used in these clinical trials (9). Nano-size, low-toxicity and compatibility of MSCs-derived exosomes with the host immune system make them a very efficient drug carrier (10-12). Exosomes, including MSCs-derived exosomes are very complex vesicles containing almost 4400 proteins, 194 lipids, 1639 mRNAs, and 764 miRNAs (13, 14). The exosomes enriched with various proteins including tetraspanins (CD9, CD63, CD81, CD82), heat shock proteins (HSP70, HSP90) and MVB formation proteins which are used for exosomes' biological functions (14). Moreover, the exosomes contain different types of RNAs such as miRs (mostly), siRNA, ncRNAs, piRNA, and lncRNA (15). The MSCs-derived exosomes with different sources have some different biological activity. For example, the human umbilical cord stem/stromal cells (HUC)-derived exosomes showed to have IL-6 secretion inhibition effects which it lead to immunoregulation (15). In addition, the MSCs-derived exosomes play roles in immunomodulation, angiogenesis promotion, interferon γ inhibition, tissue repair stimulation, anti-inflammation, and antioxidation (15, 16).

Due to the wide biological activity of the MSCs-derived exosomes, they could be one of the best candidates as a therapeutic agent. It is also hypothesized that MSCs-derived exosomes due to their pivotal roles in the inflammatory responses suppression (17) and the regeneration of the damaged tissue may be a future treatment of SARS-CoV-2 pneumonia (18). In a murine model, hypothalamic neural stem/progenitor cells exosomes/microvesicles had antiviral immunity and could be developed to combat against SARS-CoV-2 (19). Furthermore, it is suggested that exosomes containing SARS-CoV-2 component may be capable of inducing immune cells responses (20). 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 (21), these signs may be modulated by using MSCs-derived exosomes for transportation of asRNA, as well.

As the target of this therapeutic approach is the infected epithelial cells of the respiratory tract, drug administration by inhalation has been suggested. Inhalation therapy by MMP2/9-triggered-release micelles had appropriate effects on lung cancer (22). The serum-derived exosomes were used as a vehicle to deliver small RNA molecules via inhalation system into the lung macrophages in vivo in mice for lung inflammation treatment (23). In our hypothesis exosomes containing asRNAs can introduce to patients through inhalation, so the asRNA may reach to the respiratory system more effectively.

We hypostatized MSCs-derived exosomes can perform as a carrier for delivering anti-SARS-CoV-2 miR-5197-3p to infected cells to decrease the viruses' replication and transcription in vivo (24). Table 1 showed locations and products of single strand RNA of SARS-CoV-2 that can be inhibited by asRNAs detected by Ivashchenko et al. (24). It may be useful to exploit the MSCs-derived exosomes to efficiently deliver anti-SARS-CoV-2 asRNAs for targeted drug delivery (10). In addition, previous studies have demonstrated that exosome-based drug delivery can protect exosome-encapsulating RNAs from RNase enzymes (25). Different exosomes with cell-specific surface proteins may have distinct routes and circulation pattern all over the body (26). Accordingly, it is possible to predict 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 (27). The purity of MSCs isolated is confirmed by flow cytometry analysis of CD markers of MSCs and hematopoietic stem cells.

In order to appraise the differentiation capabilities of MSCs, the cells are tested in terms of their ability for osteogenic, adipogenic, and chondrogenic differentiation. The conditioned medium (CM) is prepared using MSCs (28). The exosomes are isolated from CM by a commercial kit (29). Transmission electron microscopy test is used for morphological assessment of the isolated exosomes (30).

Table 1. Locations and products of single strand RNA (ssRNA) of SARS-CoV-2, MERS, and SARS can be inhibited by antisense RNAs (asRNA) (24)

	SARS-CoV-2	MERS	SARS
asRNA name	miR-5197-3p	miR-6864-5p	miR-4778-3p
asRNA sequence	UAAGCUAC UGAGUCAG AGAAGAA	CCGUAUAGA CUGAACAGG GAAGUU	AGUUGAG ACGUUUCC UUC-UUCU
Matched sequence of ssRNA	AUUCGAAG ACCCAGUC CCUACUU	GGCGUUUCU GACUUGUCC CUCAAA	UCGACUCC GCAAGGGA GGUAGGA
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

Transportation of antisense RNA inhibiting RNAs (asRNAs)

RNA viruses use intracellular host cell machinery for DNA replication, RNA transcription and protein synthesis (31). The pathogenesis of such viruses mainly depends on the rate of genome replication and cell destruction following host immune responses (32). Thus, blocking the viral genome replication and transcription can be a promising approach to combat the infection (33). Although, there are many targets to selective inhibition of RNA virus replication (33), anti-replication effect of antisense RNA inhibiting RNAs (asRNAs) as a non-coding RNAs (ncRNAs) has not been investigated for coronaviruses. Despite significant improvements in the field of nucleic acid-based therapies and the use of numerous carriers to transport molecules such as asRNA, studies on finding suitable carriers for these kinds of molecules are still ongoing (34). The other challenge of designing an anti-SARS-CoV-2 asRNA is the transportation of them into the replication site, host respiratory epithelial cells. The nanometer size of asRNAs allows them to go through the vessels and affect other parts of the host's body (35), but, one of the main problems in the asRNA transfer, is related to their instability and negative charge so that even in the presence of a suitable transfection reagent, they cannot effectively penetrate the hydro¬phobic cell membranes (10, 36). To minimize the side effects of asRNAs on other tissues, a targeted drug delivery system can be used. Exosome-based drug delivery approach can be suggested as one of the best candidates (37).

For this purpose, the following steps can be suggested: 1) production of the target asRNAs using a cost-effective DGCR8-independent stable asRNA expression (DISME) strategy (38). 2) Increasing the expression level of asRNAs by inserting units of a U6 promoter-driven expression cassette in the vector (38). 3) Loading anti-SARS-CoV-2 miR-4778-3p in the exosomes using electroporation method (10). 4) Re-isolating the loaded exosomes using a commercial kit (29). 5) Estimating the amount of anti-SARS-CoV-2 asRNAs oligonucleotides in mesenchymal stem cells (MSCs)-derived exosomes (10). 6) Exposing the asRNA loaded exosomes to SARS-CoV-2 culture in transmembrane protease. 7) Analyzing serine 2 (TMPRSS2)-expressing VeroE6 cell line and virus replication (39). 8) In vivo analysis of the exosomes supplemented with anti-SARS-CoV-2 miR-4778-3p in animal model of the SARS-CoV-2 disease including the amount, efficacy and biosafety of the new drug (40). 9) In vivo analysis of the cargo in the human body and analyze its effect on serum concentrations of pro-inflammatory cytokines and chemokines (41). All the steps should be done in the biology laboratory with BSL-3 or 4 level (42).

To test the therapeutic method in animal model, two approved models of rhesus macaques and ferrets can be used (43). These models can be infected with SARS-CoV-2 and show virus replication and shed virus (43). After confirming the treatment in an animal model and analyzing asRNAs efficiency, the asRNAs encapsulated with exosomes can be introduced to the patient with inhalation (24). Figures 1 and 2 show the schematic of this therapeutic approach.

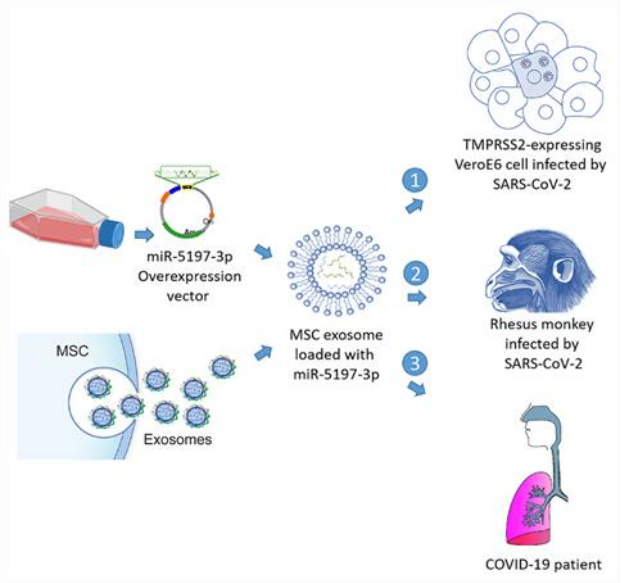

Figure 1. Schematic diagram of developing a therapeutic method for COVID-19 using exosomes of mesenchymal stem cells as nano-cargos for antisense RNAs for inhibiting single strand RNA (ssRNA) of SARS-CoV-2.

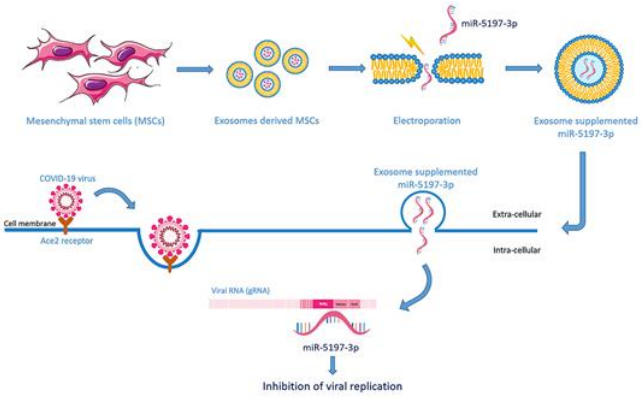

Figure 2. Schematic diagram of a methodology of a therapeutic method for COVID-19 using exosomes of mesenchymal stem cells as nano-cargos for antisense RNAs for inhibiting single strand RNA (ssRNA) of SARS-CoV-2

Discussion

Exosomes have been introduced as a new alternative to the transmission system of therapeutic molecules (37). Exosomes are nano-sized lipid package and those with 30–150 nm size are the smallest particles between entire exosomes (44). Exosomes are produced in the most eukaryotic cells (45) and are capable of being drug carriers since they are composed of cell membranes, rather than synthetic polymers (37). Exosomes have many functions in human bodies including cell to cell communication, wound healing, tissue regeneration and even cell death (46). They have some information from their primary parental cells (47). They can interact with macromolecules and serve as distributors for proteins, lipids, mRNA, miRNA, and DNA (48). Exosomes may interact with the host's immune system, so the selection of the parent cell for exosome production needs to be performed carefully (49). Application of human exosomes for treatment of diseases was tested in clinical trials (50) and has a developing market (51).

MSCs have been used in many clinical trials of transplant rejection, autoimmune disorders, and inflammation- associated diseases (52). Currently, after COVID-19 endemic situation, clinical trials studied MSC therapy for the treatment of COVID-19 (Table 2). It has been shown that MSC-secreted factors suppress T-cell proliferation (53). Therefore, MSCs-derived exosomes have the same properties as their parent cell (54) and suppress the secretion of tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) in peripheral blood mononuclear cells because of their immunomodulatory characteristics (54). Some of the earliest exosome research indicated that they can carry the MHC-peptide complexes, which are recognized by T lymphocytes (55), to activate more immune system cells and destroy the tumor and other cell contaminants (for example viruses) (56). However, there are some challenges in face of the exosome therapy. One of the biggest challenges is the exosome isolation at the GMP grade. However, it can be produced at small scale yet (57). This could be facilitating by exosome isolation kit with GMP grade. Moreover, the storage and stability of exosomes needs more study (58).

The asRNAs therapy is suggested as a new method for controlling viral infections (59). Here, we hypothesized MSC derived exosomes containing anti- SARS-CoV-2 asRNA to be helpful for finding a specific treatment for COVID-19. Since the SARS-CoV-2 genome replicates in the respiratory epithelium of host cells, the developed asRNAs should not damage the genome of host cells. Over the past decade, the discovery of ncRNAs including microRNA, small interfering RNA (siRNA), PIWI-interacting RNA (piRNA) and long noncoding RNA (lncRNA) has been a well-defined research topic. Application of asRNAs considering the required purpose undergoes two challenges: 1) Binding the asRNA to the exact site of the RNA virus genome. 2) Side effects of the random binding of these asRNAs to human mRNAs due to the subjective set of nucleotides in synthetic asRNAs, which bind with complete complementarity (60-63). 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 (24).In this case, asRNA with the ability of specific binding to the viral genome, must be selected (24, 64). In-silico analysis showed that miR-5197-3p (cc-miR, fully complementary miRNA) has an affinity to ssRNA of SARS-CoV-2 and miR-6864-5p and miR-4778-5p can strongly bind to MERS (cc-miRm) and SARS (cc-miRs) genomes, respectively (24) (Table 1).

The miR-5197-3p can bind to a few human genes with similar characteristic (24). 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 (24). The next concern of this therapeutic approach is related to the possibility of SARS-CoV-2 genomic mutations during the worldwide spreading of the virus. Fortunately, comparative sequence analyses of SAR-CoV2 genomes isolated from different geographical locations have unique features (65). Therefore, designing a conserved cc-miRNA is possible.

The SARS-CoV-2 binds to epithelial cells of the nasal cavity after its inhalation and replicates there (66). Then, along the conducting airways, SARS-CoV-2 migrates down the respiratory tract (67). Although, the treatment of the lungs by inhalation is the minimally invasive and most direct delivery route (68), studies conducting on the utilize of exosomes for treatment of different kind of lung diseases, mainly administered exosomes systemically into a vein or through direct tissue injection. Nonetheless, innovative methods must be developed for the scale up of exosome production and isolation.

Table 2. Completed, active, and recruiting clinical trials on mesenchymal stromal/stem cell-based therapy of COVID-19 (U. S. National Library of Medicine)

Organ system	Phase	Country	Transplantation	S	CT code
Adipose tissue	1&2	Spain	Allotransplant	A	NCT04366323
Adipose tissue	2	US	Allotransplant	A	NCT04362189
Adipose tissue	2	US	Autotransplant	A	NCT04349631
Adipose tissue	1	Mexico	Allotransplant	R	NCT04611256
Adipose tissue Exosome	1	China	Allotransplant	C	NCT04276987
Bone marrow	1&2	Belgium	Allotransplant	R	NCT04445454
Bone marrow	1	Canada	Allotransplant	R	NCT04400032
Bone marrow	2	Pakistan	Allotransplant	R	NCT04444271
Bone marrow	2	Spain	Allotransplant	R	NCT04361942
Bone marrow	1	Sweden	Allotransplant	R	NCT04447833
Bone marrow	1	US	Allotransplant	R	NCT04397796
Bone marrow	3	US	Allotransplant	R	NCT04371393
Bone marrow	1	US	Allotransplant	R	NCT04629105
Cord blood	1	US	Allotransplant	R	NCT04565665
Cord tissue	1&2	US	Allotransplant	R	NCT04399889
Dental pulp	1&2	China	Allotransplant	R	NCT04336254
Extracorporeal	1&2	US	Allotransplant	R	NCT04445220
iPSC	1&2	Australia	Allotransplant	R	NCT04537351
ND	1	Indonesia	Allotransplant	R	NCT04535856
ND	2	Mexico	Allotransplant	R	NCT04416139
ND	2	Spain	Allotransplant	R	NCT04615429
ND	2	US	Allotransplant	R	NCT04466098
ND	1	Brazil	Allotransplant	R	NCT04525378
ND Exosome	2&3	Iran	ND	R	NCT04366063
ND	1&2	US	Allotransplant	R	NCT04524962
UC	1&2	US	Allotransplant	C	NCT04355728
UC	1	US	Allotransplant	C	NCT04573270
UC	2	China	Allotransplant	C	NCT04288102
UC Wharton's jelly	1&2	France	Allotransplant	A	NCT04333368
UC	1&2	Ukraine	Allotransplant	R	NCT04461925
UC	2	Germany Israel	Allotransplant	R	NCT04614025
UC	2	US	Allotransplant	R	NCT04389450
UC	1&2	China	Allotransplant	R	NCT04339660
UC	1	Indonesia	Allotransplant	R	NCT04457609
UC	2	Spain	Allotransplant	R	NCT04366271
UC	1&2	US	Allotransplant	R	NCT04494386
UC	2	Pakistan	Allotransplant	R	NCT04437823
UC	1	China	Allotransplant	R	NCT04252118
UC	1&2	Turkey	Allotransplant	R	NCT04392778
Wharton's jelly	1	Jordan	Allotransplant	R	NCT04313322
Wharton's jelly	1&2	Spain	Allotransplant	R	NCT04390139

ND, no data; iPSC, induced pluripotent stem cells; UC, umbilical cord; S, stage; A, active; R, Recruiting; C, Completed

Opinion and perspective

Since emerging COVID-19 in 2019, scientists and companies have tried to make effective drugs and vaccines for this pandemic disease. Nowadays, some companies have produced vaccines such as Pfizer, Sputnik V, Moderna, Johnson & Johnson, Sinopharm, and etc. which reduced mortality (69), transmission (70) and symptoms of COVID-19 and increase immune protection against it (69). However, the disease still remained in most of the countries and besides of COVID-19 symptoms which it could be life disturbance and threatening, it still is reason of people death worldwide. In order to reduce symptoms and issues of COVID-19, an accessible and effective treatment will be needed. An inhalator anti-COVID-19 device containing MSCs-derived exosomes supplemented with asRNA anti-viral RNA could be effective. Besides, the anti-inflammatory and immunomodulatory activity of MSCs-derived exosomes and their potential for triggering tissue regeneration make them a good candidate for treatment of COVID-19.

Conclusions

In one hand, the asRNAs with ability to inhibit ssRNA of SARS-CoV-2 can be effective in silencing this virus replication. In other hand, MSCs secret exosomes, which can be loaded by asRNA of an anti-RNA virus. Consequently, production of a specific anti-SARS-CoV-2 by targeting the viral genome is suggested for developing a novel therapeutic candidate for COVID-19.

Declarations

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Conflicts of interest
Author Mohammad Amin Behzadi was employed by the company Auro Vaccines LLC. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' Contributions
A.A., M.Z., Z.F., A.H., N.B., A.K., and H.H. collected information and co-wrote the draft. I.N., R.S., M.A.B., and A.T. designed the idea, and critically revised the paper.

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