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Exosomal mediated signal transduction through artificial microRNA (amiRNA): A potential target for inhibition of SARS-CoV-2

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ABSTRACT

Exosome trans-membrane signals provide cellular communication between the cells through transport and/or receiving the signal by molecule, change the functional metabolism, and stimulate and/or inhibit receptor signal complexes. COVID19 genetic transformations are varied in different geographic positions, and single nucleotide polymorphic lineages were reported in the second waves due to the fast mutational rate and adaptation. Several vaccines were developed and in treatment practice, but effective control has yet to reach in cent presence. It was initially a narrow immune-modulating protein target. Controlling these diverse viral strains may inhibit their transducing mechanisms primarily to target RNA genes responsible for COVID19 transcription. Exosomal miRNAs are the main sources of transmembrane signals, and trans-located miRNAs can directly target COVID19 mRNA transcription. This review discussed targeted viral transcription by delivering the artificial miRNA (amiRNA) mediated exosomes in the infected cells and significant resources of exosome and their efficacy.

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1. Introduction

The COVID19 variants are the most important issue for vaccine development; perhaps different mutants have been reposted in recent days and countries representing new mutants, especially the UK, USA, Nigeria, South Africa, and India. Currently, several vaccines are being affected against COVID19, but strains variations are intense to less effective and need alternatives [1]. Exosomes act as vesicular cargo which carries signaling molecules, enzymes for a metabolic task, nanomolecular disposal, and they can provide mono and bidirectional functions by cellular conversations [2]. miRNAs are being used as a platform for live attenuated DNA vaccines, and it also manipulates the host endogenous RNA viruses by providing both positive and negative senses [3]. Exosomal miRNAs utilize therapeutic approaches [4] and are considered unique biomarkers for targeted gene therapy [5]. Based on characteristic approaches, inactivation and inhibition of miRNA-based therapeutics tactics are reported, and specific miRNAs are accountable for the immune response against COVID19 and viral strains [6,7]. According to Bunggulawa et al. 2018 [8], exosomes can provide cellular conversation, and modified exosomes are confident future drug delivery research. RNAi techniques are used in different systems to persuade antiviral resistance by targeting the exact dilapidation of RNAs. However, the RNAi-based tool containing artificial miRNAs is a favourable antiviral tool targeting viral RNAs with concurrent expression in several synthetic trans-acting small interfering RNAs that transact synthetically from a single precursor [9]. To overcome current issues in vaccine efficacy against mutant COVID19, we propose to utilize the artificial miRNA-mediated exosome delivery to target gene therapy for rapid recovery of pandemic disease.

2. SARS-CoV-2 transformation and reported variants

Coronavirus can infect humans, bats, birds, mice, and other wild animals [10,11]. The evaluation and transformation of CoV-2 led to a sudden outbreak in the whole part of the world, and it became pandemic. Today, ecological changing parameters and human social interaction may increase the high risk of an outbreak. Several proofs intimate the zoonotic spill over transmission of the pathogen from an animal to a human host, this concept is unclear, but certain factors determine the zoonotic transmission [12]. The spike protein plays a major role in coronavirus attachment to the ACE2 on the host cells. The initiation of infection involves getting the RNA genome of the host cell via fusion of the virus and host cell membranes [13]. Similar conformational transformations have been observed for the SARS-CoV spike protein [14] and mouse hepatitis virus [15]. The spike protein study is helpful for SARS-CoV-2 infection. CoV-2 variant while to stop the spread of the virus to prevent the mutation. It can help the effectiveness of the existing vaccine. They are beginning of 2019, and the CoV-2 virus spread over from animal to human being. Los Angeles Times reported (02/04/2020) that the coronavirus already detected 1200 various types of viruses among 160 are coronavirus. Some of these variants and the genotypic variations in the spike proteins are mentioned in Table 1. (See Figs. 1 and 2.) (See Flowchart 1.)

The evidence regarding transformation the occurrence of recombination events among SARS-CoVs exists in the neighbouring bat population. Such phenomena may be responsible for the series of recombination within the S gene and around ORF8 that led to the origin of the direct progenitor. Moreover, virus spill over occurred from bats to civets and later from people residing near the location or due to indulgence in the wildlife trade of infected animals [4]. The virus interacts with humans due to its survival, development, and distribution: the second stage, viral exposure, route of entry, and dose of virus. The last stage is influenced by genetic factors, the physiological and immunological status of the human host. These last two factors determine the possibility and severity of infection [12]. SARS is an airborne virus, transmitted via as cold and flu do. The virus spreads by an infected person coughing or sneezing, leaving small droplets in the air or stool. So, the person who inhales such droplets or touches the infected surfaces may also get infected [106,107]. Reports state that the receptor-binding domain (RBD) of virus spikes binds to the ACE2 receptor of the potential host cell in the case of human-to-human transmission [16,17]. The most exciting feature is that SARS-CoV-2 and SARS-CoV spikes share RBD sequence similarity, strongly suggesting their standard entry route into the host cells via the ACE2 receptor [17].

3. Role of miRNA in the extracellular signals and transducing mechanism

In general, snRNAs are potential modulators for post-transcriptional modification of mRNA genes. Especially, miRNAs are chiefly intricate transduction mechanisms by transferring the antisense intron binding agents for regulating RNA splicing. Many matrices conditional developments in cellular metabolic processes such as stem cell properties, cell proliferation, differentiation, migration, and apoptosis are regulated by a unique miRNA-dependent modulation of the extracellular signals and cellular receptor [18]. Importantly, miRNAs are prearranged into chromosomes, and their genes emphasize epigenetic regulation by their gene expression and subsequently target gene expressions, E.g. While up-regulating miR-663 to target down-regulation of large matrix protoglycan perlecan (HSPG2). In evidence to this, miRNAs suppress gene expression and are functionally repressive. However, the effects of signaling outputs are sternly reliant on the topology of the pathway [19].

Extracellular vesicles (EVs) are significant resources for intercellular communication, and it helps maintain tissue homeostasis and transfer secreted molecules into the cells [20]. We were representing the primary mechanism of exosomal signaling in figure - 1. Exosomes are considered the best EVs engendered by the endosomes to create multivesicular bodies (MVBs) and help release extracellular matrices through plasma membranes [21]. In addition, EVs are recognized as apoptotic bodies during cellular apoptosis [22] and are also used to reflect EVs’ specific functions of EVs [23]. The exosomes are nanovesicles characterized by novel bio reserves of intercellular signaling; perhaps they tend to translocate cellular messages. According to Naseri et al. 2018 [24], mesenchymal stem cells (MSC) are consistent producers of exosomes [32]. It is also known as appropriate nano-vectors for carrying siRNAs and utilizing *In vitro* and in vivo applications.

The basic principles for extracellular vesicle delivery and their
mechanism of action in regulating gene expressions have been discussed in Fig. 2. Ohno and Kuroda, 2016 [25] reported possible techniques for nucleic acid drugs encapsulation into exosomes, and it has two comprehensive methods such as direct RNAs electroporation [26] and encapsulation of RNAs during the formation stage. This hypothesis evidencing the target miRNA delivery through extracellular vesicles, specially exosome-mediated miRNA, could be used for therapeutic applications.
Flowchart 1. Exosomal mediated miRNA synthesis and target delivery protocol.

Table 2
Binding efficacy between Exosomal ACE2 and of SARS-CoV-2 spike protein.

| Protein name | HADDOCK values | The binding affinity (ΔG) | Hydrogen bond interaction | Salt bridge interaction |
|--------------|----------------|---------------------------|---------------------------|------------------------|
|              | HaddockScore   | Buried Surface Area       |                           |                        |
| SARS-CoV with HSP 70 | -114.6 +/- 14.5 | 2293.7 +/- 144.7 | -12.3 | ASN318 | LYS112 | 3 | ARG 342 | GLU132 |
|               |                |                          |                           | ARG321 | LYS108 | GLY134 |
|               |                |                          |                           | ARG342 | GLU132 | GLU132 |
|               |                |                          |                           | LYS344 | THR125 | LYS129 |
|               |                |                          |                           | LYS344 | GLU129 | GLU129 |
|               |                |                          |                           | SER346 | ARG 49 | SER235 |
|               |                |                          |                           | ARG347 | ARG 49 | ASN343 |
|               |                |                          |                           | ASN347 | ARG 49 | ASN343 |
|               |                |                          |                           | TYR383 | GLU132 | GLU132 |
|               |                |                          |                           | ARG453 | LEU135 | GLU132 |
|               |                |                          |                           | ARG453 | GLU132 | GLU132 |
|               |                |                          |                           | GLU502 | LYS128 | GLU502 |
|               |                |                          |                           | GLN546 | LYS129 | LYS129 |
|               |                |                          |                           | THR567 | TYR115 | GLY567 |
| 2ack crystal structure with HSP 70 | -104.8 +/- 22.3 | 2321.3 +/- 91.5 | -11.9 | SER325 ASN343 | LYS3 | 10 | ARG 345 | LYS100 |
|               |                |                          |                           | ASN343 THR345 | LYS100 | GLU 117 ASP97 |
|               |                |                          |                           | SER366 LEU441 | LYS128 | GLU132 |
|               |                |                          |                           | LYS529 SER530 | ASN168 LYS77 | ASN141 |
|               |                |                          |                           | SER530 ASN532 |  | THR140 |
|               |                |                          |                           | THR567 | TYR115 | LYS3 |
4. Source exosomes and selective protein binding efficacy in COVID19 treatment

Exosomes have proven to be a drug delivery system and an agent for intercellular communication [27]. Based on the pathway of biogenesis and several physicochemical characteristics, several parallels can be drawn between exosomes and viruses [28]. Based on exosome size and morphology, including spike, it is pretty like that of SARS-CoV2. The correlations between SARS Cov-2 and exosomes concerning spike protein and size are described theoretically in Fig. 3, and the 3-dimensional model is depicted in Fig. 4 and an in-silica analysis in Fig. 4 a. The binding efficacy of the exosome to the spike protein is explained in Table 2. This significantly increases the probability of miRNA transfer between the virus and the vesicles and is the suitable EVS [29]. Estimating the number of exosomes in a given source is necessary to find the ideal sources. This can be done through analysis by nanoparticles that report the size and an approximate number of exosomes [30,31]. This estimation makes it easier to tell which cells to isolate exosomes. As mentioned before, mesenchymal stem cells are an excellent source of exosomes responsible for differentiation. The most common source of MSC is from bone marrow, while it can also be isolated from adipose tissue and umbilical cord jelly [33]. The selective advantage of using stem cells is that they are undifferentiated. This means that the exosomes contained are devoid of any cargo and can be utilized to transport miRNA [34]. Ideally, if the process of loading miRNA into the exosome is perfected, all miRNA can be transferred to the affected cell, halting the translation. Mesenchymal stem cells and their exosomes can be used to initiate the immune system making it advantageous to deal with an infection. Studies have already been studies using these exosomes to treat severe cases of covid-19, which have anti-inflammatory properties, suppress the cytokine storm and induction of ARDS and multiple organ failure. They have also been used to treat several influenza-like viruses, including H5N1. While these are rather different from SARS-COV2, the basic mechanism of action for both the viruses is the same. While the signal transduction pathways are different, miRNA involvement due to the initial protein synthesis can be inhibited in both cases. Convalescent plasma, already known for treating SARS-CoV2 infection [107] can undoubtedly be used to isolate exosomes, which likely play a significant role in the prognosis of the disease. (See Fig. 4.)

Meanwhile, blood plasma contains several cells that can be used to isolate exosomes. In order to target a virus, it is essential to look at sources from the immune system as these are capable of producing antibodies and contain exosomes, making them a candidate for isolation of exosomes such that transformation of the virus can be halted. An essential source of exosomes is immune-derived cells, including T cells, B cells, dendritic cells, macrophages, etc. [34]. T cells have the general purpose of maintaining cellular and humoral immunity, and their exosomes likely have similar properties. In general, T cells have been used to suppress infection by HIV viruses and hence are a useful source of exosomes for the study. There are also several other cells from which exosomes can be isolated. The most common source for isolating exosomes along with stem cells is from dendritic cells. Immature dendritic cells can produce a limited number of exosomes [35], but have been studied in detail. Additionally, B cells have been revealed to be a source

Fig. 3. Exosomal ACE2 for the neutralization of the SARS-CoV-2 spike protein.

Fig. 4. SARS-CoV Spike protein With HSP 70 protein. Ribbon diagram of the SARS-CoV S1 (blue)/HSP 70 (pink) complex model. The SARS CoV Spike protein (GenBank: QHD43416.1) and the native crystal structure of Heat shock protein (PDB code: 1s3x) were downloaded from the protein data bank (PDB). This model was generated by the fully automatic HADDOCK protein-protein docking server and manually selected based on structural biology knowledge.
for exosomes in vivo while also contributing to the immune response [36]. Immune cell exosomes can be extracted from body fluids such as blood and lymphatic.

Of all the different sources of exosomes discussed above, based on the data presented, the ideal sources for exosomes for this study are MSC. The exosomes have the specific advantage of not carrying any cargo, making the loading of miRNA much easier. These exosomes can transport various materials, being responsible for the regulation of differentiation. Using them is advantageous, given that they contain many exosomes. They have been used for decades to treat diseases like liver fibrosis and chronic kidney disease and have several applications in immunotherapy in oncology. Therefore, exosomes isolated from MSC would be advantageous in this study. Exosomes are extracellular vesicles surrounded by a lipid bilayer, formed as the final product during endocytic internalization of the plasma membrane. An initial endosome is created first, which develops into either late endosomes or multivesicular bodies over time (MVBs). The MVB membranes fold to form intraluminal exosomes. Exosomal cargo depends on the cell type, including membrane-specific domains, mRNA, miRNA, proteins, and DNA.

Finally, MVBs merge with the plasma membrane, causing exosomes to be released [37,38]. Isolating the extracellular vesicle (EVs) straight from the body produces significantly greater yields than EVs obtained from cell culture. Furthermore, getting EVs directly from the body avoids the need for exogenous cell growth mediums, such as a foetal bovine serum, known to reduce the exosomal quantity. As seen by the widespread practice of transfusion medicine, the therapeutic use of EVs directly from the human body is not confined to autologous applications. All cells in the body release significant amounts of EVs (up to 1010/mL) into plasma. Despite this, adverse responses to blood and plasma transfusions are uncommon. There are several ways for isolating exosomes from plasma, serum, and culture media, such as Ultracentrifugation is a traditional technique that uses high centrifugal force to analyse the characteristics of microscopic biological particles [39]. Ultracentrifugation is based on high centrifugal forces (100,000 g) that allow the separation of various components according to size. Centrifugation is a technique for the sedimentation of particulate particles in solution, such as vesicles, and is regarded as the best approach for isolating exosomes [40]. Precipitation-based EV isolation uses several commercial EV isolation kits, including the ExoQuick and Invitrogen Total Exosome Isolation Reagent, miCURYTM Exosome Isolation Kit, which are based on membrane particle precipitation [41]. In general, the polymeric precipitation approach has been used to estimate the amount of RNA and protein. The isolation of exosomes by immunological separation Technique is an alternative approach for isolating exosomes based on multiple proteomic investigations of the molecular makeup of exosomes. Exosomal component investigations have revealed the presence of several proteins on the exosomal membrane [42]. Due to the immune-affinity interactions between proteins and antibodies, which should be specific for a specific marker present on the exosomal membrane [43]. Rapid isolation with a novel synthetic peptide is another novel approach that has been also revealed that can aggregate exosomes from the media in less than 30 min. Under stress situations, such as cancer, infection, cardiovascular, neurological, and metabolic diseases in human or animal body fluids, the peptide Vn 96 can identify EVs produced from cells due to their high Hsps [44]. Reangiotensin convertingsearchers recently discovered circulating exosomes expressing the SARS-CoV-2 viral entry receptor angiotensin-converting enzyme 2 (ACE2) in plasma from healthy donors and COVID-19 patients [45].

5. Predicted miRNAs in SARS CoV-2 and its significant pathways

CoV-2 is single-stranded RNA and 30 kb lengths, made up of genetic materials consisting of large RNA viruses [46]. However, small miRNAs have suppressed gene expression through transcription [47]; miRNAs suppress RNA transcripts by guiding miRISC, the so-called miRNA response element (MRE). It induces RNA degradation or translation [48,49]. The miRNA between MRE and base sequence of six to eight 5’ end of matured miRNA known to be seed miRNA [50] This miRNA-mRNA is correlated with antiviral mechanism in cells [55]. The predicted miRNA is to be better understood of Cov-2- SARS infection. The severe acute respiratory disease of Cov-2 was first isolated from Wuhan-hu-1. Several studies predicted miRNA targeted regions containing ssRNA bp of spike glycoprotein. Supporting miRNA in the human host cell antiviral defence has the potential targets for the SARS-CoV-2 genome. Our brief review has predicted miRNA with MREs in the SARS-CoV-2 genome. The others reported miRNA [56,57] to create a list of confidence of miRNA predicted to target the SARS-CoV-2 genome. The prediction of miRNAs of antiviral deference mechanism is to understand better scientists developing the target-specific drugs in future research for improved therapeutic points for the well-known human
respiratory COVID-19. Studying known miRNAs and their functions is essential to predict the prospective miRNA targeting viral transduction. This is represented in Table 3. Due to the increase in corona deaths in India and other parts of the world, we conclude that this miRNA development is a better improvement of drug design against SARS-CoV-2 antiviral immune booster in the cell deference mechanism. Finally, in medical, miRNA is considered a therapeutic drug to target SARS-CoV-2 in the pandemic.

5.1. Pathways

The study of molecular changes of SARS-CoV-2 is to help the virulence and virus replication [51]. The mutational change in the nucleotide and amino sequence measures the molecular diversity pathways. The evolutionary theory of crucial viral proteins such as the S- and N-protein, several NSP, and accessory proteins is associated with mutational sequence changes. Virulence influences the mutational changes, mainly the S-protein nucleotide changes in N-protein. In methylation pathways, gene expression cannot alter the nucleotide sequence. DNA methylation involves mostly CpG islands, which are part of the promoter gene sequences [58], and the methylation pattern of CpG islands regulates the level of gene transcription [59]. For years, it has been known that viral infections use epigenetic mechanisms in general and especially CpG methylation to find ways to induce endocytosis and syncytium development.

The strategy the virus needs to develop is first to fuse itself within the host’s cell membrane and induce host cell-cell fusion. This process is called endocytosis invasion of neighbour cell membrane so-called syncytium [60]. Syncytium formation leading to the creation of giant multinucleated cells in the placenta makes this tissue impermeable and generates mother-child immune tolerance [61]. Syncytin genes are hypomethylated and therefore functionally active in the mammalian placenta, whereas they are hypermethylated and thus silenced in other tissues, where syncytium formation may cause various diseases, i.e., schizophrenia, multiple sclerosis, and diabetes type 1 [62]. CpG methylation of syncytin genes in non-placental tissues is obligatory for preventing the expression of syncytium-forming proteins [63]. Several viruses use the human syncytin genes to fuse themselves with the host’s cell membrane and/or induce cell-cell fusion in the infiltrated tissues [64].

How a virus can use epigenetic mechanisms to fuse itself with host cells is given by how the Epstein-Barr virus and cytomegalovirus can affect human health [107]. Both viruses can demethylate the host syncytin 1 and 2 genes, increasing gene transcription and causing the formation of syncytium in tissues where those genes usually are hypermethylated and silenced [65]. This process can cause multiple sclerosis and even amyotrophic lateral sclerosis [66]. Syncytium formation by SARS-CoV-2 is many times faster than in the 2002 SARS-CoV, and syncytium formation is highly responsible for the virulence factor and induction of a cytokine storm of any virus in general and SARS-CoV-2 especially [67–69].

6. A prospective link between signaling stimulation and amiRNA-mediated exosomes delivery for targeting mRNA splicing

After the virus enters the body through droplet inhalation, they attach to specific host cells through adhesion to ACE-2, beginning the infection cycle as was discussed above [86]. The primary process which we want to target is that of virus transformation. The SARS-CoV-2 transformation in the human model has been studied in vivo conditions and can certainly be used to develop therapeutic solutions to the infection [87]. Although the strains rapidly mutated through different factors that cause spike protein changes and nucleocapsid, the infection pattern remains the same [88]. Hence, our treatment strategy is to target the host factors that contribute to the transmission of the virus, mainly
Table 3

Predicted sites of binding of the miRNA antiviral defence mechanism of SARS-CoV-2 genome.

| S. No. | Predicted miRNA | Process of human gene | Disease involved | References |
|--------|-----------------|-----------------------|-----------------|------------|
| 1      | has-miR-6891-5p | Cellular process, biological regulation | Reproduction, immune system | [69] |
| 2      | has-miR-05220   | Different KEGG pathways, potential target sites on SARS-CoV-2 | Chronic myeloid leukemia, cancer | [70] |
| 3      | has-miR-05205   | Proteoglycans | Cancer | [70] |
| 4      | has-miR-05218   | Different KEGG | Melanoma, | [70] |
| 5      | miR-8066 and miR-5197-3p | Related to cytokine- | TGF-β and mucin-type O-glycan biosynthesis; Vitamin digestion-absorption | [17] |
| 6      | miR-3934-3p     | Glycosaminoglycan biosynthesis | Short long bones, joint dislocations or laxity and scoliosis; skin, congenital heart defects | [71] |
| 7      | has-miR-195-5p  | Fatty acid synthase | Obesity, metabolic syndrome, inflammation, cardiovascular disease, and cancer | [72] |
| 8      | has-miR-195-5p  | Fibroblast growth factor 2 | Human breast cancer | [72] |
| 9      | has-miR-424-5p  | Protein tyrosine phosphatase, receptor type 4 | Developmental defects, neoplastic disorders, and immunodeficiency. | [72] |
| 10     | has-miR-3133    | Regulating synaptic membrane exocytosis | Cne-Rod Synaptic Disorder, Congenital Nonprogressive and Scoliosis | [72] |
| 11     | has-miR-3133    | Transcription factor AP-2 beta | Char syndrome and Patent Ductus Arteriosus | [72] |
| 12     | has-miR-3133    | Protein tyrosine phosphatase, receptor type K | Extragonadal Germ Cell Cancer and Eye Lymphoma | [72] |
| 13     | has-miR-3133    | Nuclear respiratory factor 1 | Autism spectrum disorders | [72] |
| 14     | miR-199a        | Regulate TMEM82 expression in the liver, stomach, and uterine corpus | Lung adenocarcinoma (LUAD), endometrial uterine corpus endometrial | [73] |

Table 3 (continued)

| S. No. | Predicted miRNA | Process of human gene | Disease involved | References |
|--------|-----------------|-----------------------|-----------------|------------|
| 19     | MiR-208         | Necessary for cardiomyocyte hypertrophy | Heart diseases | [78] |
| 20     | miR-8066        | Bind and activate Nrf8-mediated TLR-8 expression and induce cytokine synthesis | Chronic inflammatory diseases | [74] |
| 21     | miR-5197-3p     | Viral COVID 19 | | [79] |
| 22     | miR-29          | Exhibited various binding sites on ORF1ab, nucleocapsid, and spike sequences. Spike region is necessary for viral entry and is a promising target for antiviral therapy | Viral COVID 19 | [80] |
| 23     | has-miR-589-3p  | Involved in a mitochondrial organization and can target FO3 gene | Glioblastoma cell migration | [81] |
| 24     | has-miR-4282    | Participated in epigenetic control through chromatin remodeling | Involved in proliferation, invasion, and metastasis of breast cancer through Myc. HBV related hepatocellular carcinoma | [81] |
| 25     | has-miR-5193    | Involved in interferon gamma signaling and CDK mediated phosphorylation and removal of cdk6 | Cancer | [82] |
| 26     | has-miR-5011-5p | Linked to the occurrence of glioblastoma | | [83] |
| 27     | has-miR-6835-3p | Plays a role in cell growth and proliferation through the ornithine decarboxylase pathway | Ovarian cancer | [84] |
| 28     | has-miR-190a-3  | Plays a role in the regulation of several cellular processes through regulation of production of Pfk-beta | Glioblastoma | [85] |

focusing on viral transformation. The molecule of interest for this study is miRNA, which is responsible for gene silencing at the transcriptional, translation, and epigenetic levels [89]. Our study used to screen the miRNA involved in the viral transduction process from convalescent plasma-derived exosomes instead of those from the immune due to their high potency. This miRNA can be identified through the transcriptome analysis of potential target genes to understand viral transduction mechanisms. This selected miRNA can be synthesized and encapsulated with exosomes derived from mesenchymal stem cells. However, the ideal state of the exosomes will be nonspecific, so that any cargo can be loaded into it [90]. Additionally, a source with abundant exosomes would be required for this process. Exosomes isolated from mesenchymal stem cells would undoubtedly be suitable [90]. As mesenchymal stem cells are crucial for the establishment of most microenvironments, they contain a significant number of empty exosomes to be filled with cargo during differentiation [91]. Although certainly efficient for immunotherapy, any other
The method explored in our study is one of the approaches to stop the viral transduction. The virus is known to spread zoonotically and mutate to form new strains, requiring new protocols for treatment. This property of the virus makes it quite challenging to reduce its spread. Our proposal included applying MSC derived exosome-mediated drug delivery system of miRNA to infected cells. Inhibition of transduction of the target gene through artificially synthesized miRNA will block the AP-1 pathway responsible for spread by blocking the target gene CREB1. The goal is to arrest the process of viral transduction. This approach does not involve fighting against the immune response but combating the spread of the virions to other infected cells. The proposition is not a treatment method but can be an approach to stop the spread of the infection. The principle in this study can be applied to treat any infected cells in any part of the body and for any viral infection. Arresting the transduction process can be a new approach to dealing with viral infections. The use of exosomes to deliver artificial miRNA can be an efficient protocol for controlling the spread of the viral infection through the body, minimizing the damage to the patient’s health.

Declaration of Competing Interest

Authors declare no conflict of interest regarding any financial and personal relationships with other people or organizations that could inappropriately influence (bias) this work.

References

[1] Kenneth Lundstrom, Debamalya Barh, Bruce D. Uhal, Kazuo Takayama, Alaa A. Aljabali, Tarek M. Abd, Amos Lal El-Azziz, El rashidy M. Redwann, Parisie Adadi, Gaurav Chauhan, Samendra P. Shechen, Gajendra K. Azad, Nima Rezaei, Angé Solano-Aroca, Nicolao G. Bazan, Sk S. Hasan, Pritam K. Panda, Pabitra Pal Choudhury, Damiano Pizzolo, Ramesh Kandimalla, Wagner Baetas-da-Cruz, Yogendra K. Mishra, Giorgio Palu, Murtaza M. Tambuwala, Vladimir N. Ovseevsky, COVID-19 vaccines and thrombosis—roadblock or dead-end street? Biomolecules 11 (7) (2021) 1020, https://doi.org/10.3390/biom11071020.

[2] R. Procipio Pinheiro, M.A. Gaubeur, A.M. Irezoriote, S.O. Saleh, F. Hojaji, M. Andrade, A.L. Jacomo, F.E. Akamatsu, Anatomical study of the innervation of the masseter muscle and its correlation with myofascial trigger points, J. Pain Res. 2 (13) (2020) 3217–3226, https://doi.org/10.2147/JPRA.S265717.

[3] E.J. Fay, R.A. Langlois, MicroRNA-attenuated virus vaccines, Non-coding RNA 4 (4) (2018) 25, https://doi.org/10.1089/ncrn.2018.0025.

[4] G. Hu, K.M. Drescher, X.M. Chen, Exosomal miRNAs: biological properties and therapeutic potential, Front. Genet. 20 (2012), https://doi.org/10.3389/fgene.2012.00056, 3,56.

[5] M. Fani, M. Zandi, S. Ebrahimii, S. Solezani, S. Abbasii, The role of miRNAs in COVID-19 disease, Furor. Virul. (2021), https://doi.org/10.2127/vft.2020-0389, 2217/vft-2020-0389.

[6] T. Abu-Ismied, N. Alfajiri, A. Mohammed Ibrahim, M. Nooshad Javed, K. Mustafa Salem, F. Hyder Pothiti, Kamal M. Amjad, Micro-RNAs in the regulation of immune response against SARS COV-2 and other viral infections, J. Adv. Res. (2020), https://doi.org/10.1016/j.jare.2020.11.013.

[7] C. Hum, J. Loiselie, N. Ahmed, et al., MicroRNA mimics or inhibitors as antiviral therapeutic approaches against COVID-19, Drugs 81 (2021) 517–533, https://doi.org/10.1007/s40265-021-01474-5.

[8] E.J. Bungulawa, W. Wang, T. Yin, et al., Recent advancements in the use of exosomes as drug delivery systems, J. Nanobiotechnol. 16 (2018) 81, https://doi.org/10.1186/s12951-018-0403-9.

[9] A. Carbonelli, P. Lisan, J.A. Daros, Multi-targeting of viral RNAs with synthetic trans-acting small interfering RNAs enhances plant antiviral resistance, Plant J. 100 (4) (2019) 729–737.

[10] R. Chen, Q. Liu, D. Ge, Emerging coronaviruses: genome structure, replication, and pathogenesis, J. Med. Virol. 2 (2020) 418–423, https://doi.org/10.1002/jmv.25681.

[11] H. Chen, J. Guo, C. Wang, F. Luo, X. Yu, W. Zhang, J. Li, D. Zhao, D. Xu, Q. Gong, J. Liao, H. Yang, W. Hou, Y. Zhang, Clinical characteristics and intratissue vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records, Lancet. 395 (2020) 809–815, https://doi.org/10.1016/S0140-6736(20)30630-3.

[12] R.K. Ploegwright, C.R. Parrish, H. McCullum, et al., Pathways to zoonotic spillover, Nat. Rev. Microbiol. 15 (8) (2017) 502–510, https://doi.org/10.1038/nrmicro.2017.45.

[13] Y. Cai, J. Zhang, T. Xiao, H. Peng, S.M. Sterling, R.M. Walsh Jr., S. Rawson, S. Ritts-Volloch, B. Chen, Distinct conformational states of SARS-CoV-2 spike protein, Science. 21 4251 (2020), https://doi.org/10.1126/science.abd4251.

[14] X. Fan, D. Cao, L. Kong, X. Zhang, Cryo-EM analysis of the post-fusion structure of the SARS-CoV spike glycoprotein, Nat Commun. 17 11 (1) (2020) 3618, https://doi.org/10.1038/s41467-020-17371-6.
[73] Hazem Haddad, Walid Al-Zyoud, miRNA target prediction might explain the reduced transmission of SARS-CoV-2 in Jordan, Middle East, Noncoding RNA Res. (2020) 123-143, https://doi.org/10.1007/s13239-020-00092-6.

[74] S. Nersisyan, M. Shkurnikov, A. Turchinovich, E. Knyazev, A. Tonevitsky, Integrative analysis of miRNA and mRNA sequencing data reveals potential regulatory mechanisms of ACE2 and TMPRSS2, PloS One 15 (7) (2020), e0235867, https://doi.org/10.1371/journal.pone.0235867.

[75] S. Chang, L. Gao, Y. Yang, et al., miR-145 mediates the proapoptotic and gene regulatory effects of vitamin D3 by directly targeting EF2 in gastric cancer cells, Oncotarget. 6 (10) (2015) 7675–7685, https://doi.org/10.18632/oncotarget.6304.

[76] Ajay Francis Christopher, Raman Preet Kaur, Gurneepreet Kaur, Amandeep Kaur, Vikas Gupta, Parveen Banal, MicroRNA therapeutic discovery of novel targets and developing specific therapy, Prospect. Clin. Res. 7 (2) (2016) 68–74, https://doi.org/10.1016/j.prct.2016.06.001.

[77] Juan Bautista De Sanctis, Alexis Garcia, Jenny Garmendia, Dolores Moreno, Marial Jordi, Danuta Radzioch, Importance of miRNA in SARS-CoV-2 infection, Gac. Méd. Caracas 128 (Suppl 1) (2020), https://doi.org/10.47307/GMC-2020.128.s1.S.517-522.

[78] Saeideh Farajinejad Farsangi, Maryam Moazam Jazi, Farzameh Rostamzadeh, Mortaza Hadizadeh, High affinity of host human microRNAs to SARS-CoV-2 genome: An in-silico analysis, Noncoding RNA Res. 5 (4) (2020) 222–231, https://doi.org/10.1007/s12864-020-007318-9.

[79] V. Cesarini, D.A. Silvestris, V. Tassinari, S. Tomaselli, S. Alon, E. Eisenberg, F. Locatelli, A. Gallo, ADAR2/miR-589-3p axis controls glioblastoma cell migration/invasion, Nucleic Acids Res. 46 (4) (2018) 2045–2059, https://doi.org/10.1093/nar/gkx1257.

[80] Z. Song, Q. Guo, H. Wang, L. Gao, S. Wang, D. Liu, B. Lin, miR-5193, regulated by FUT1, suppresses proliferation and migration of ovarian cancer cells by targeting RPS6KB1, Pathology-Res. Practice 216 (11) (2020) 1531-46, https://doi.org/10.1016/j.prp.2020.153146.

[81] W.Y. Wang, W.C. Lu, Reduced expression of hsa-miR-338-3p contributes to the development of glioma cells by targeting mitochondrial 3-oxoacyl-ACP synthase molecular sponge of miR-190a-3p, Aging 11 (5) (2019) 1456, https://doi.org/10.14336/OT2.S26873.

[82] Z. Yiming, Y. Hang, S. Bing, X. Hua, H. Bo, L. Honggui, L. Shu, Antagonistic effect of miR-190a-3p on the VDR/CREB1 pathway on cadmium-induced apoptosis in porcine spleen, Preliminary report, Ann. N. Y. Acad. Sci. 1108 (2007) 567–568, https://doi.org/10.1126/2007.1108.567.

[83] H. Zhang, M.R. Rostami, P.L. Leopold, J.G. Mezey, S.L. Onganer, The prediction of miRNAs in SARS-CoV-2 genomes: hsa-miR databases identify 7 key miRs linked to host responses and virus pathogenicity-related KEGG pathways significant for comorbidities, Viruses 12 (6) (2020) 614, https://doi.org/10.3389/fmicb.2017.02686.

[84] S. Ludwig, C. Ehrhardt, E.R. Neumeier, M. Kracht, U.R. Rapp, S. Pleschka, Influenza virus-induced AP-1-dependent gene expression requires activation of the JNK signal transduction pathway, Biol. Chem. 276 (14) (2001) 10990–10998, https://doi.org/10.1074/jbc.M009922200.

[85] T.B. Hansen, J. Kjems, J.B. Bramsen, Enhancing miRNA annotation confidence in miRBase by continuous cross dataset analysis, RNA Biol. 8 (3) (2011) 378–383, https://doi.org/10.4161/rna.8.3.14323.

[86] M.D. Sacar Demirci, A. Adan, Computational analysis of microRNA-mediated interactions in SARS-CoV-2 infection, PeerJ 8 (2020), e9369, https://doi.org/10.7717/peerj.9369.

[87] M. Parera, B. Clotet, M.A. Martinez, Genetic screen for monitoring severe acute respiratory syndrome coronavirus 3C-like protease, J. Virol. 78 (24) (2004) 14057–14061, https://doi.org/10.1128/JVI.78.24.14057-14061.2004.

[88] M. El Gazzar, C.E. McCall, MicroRNAs distinguish translational from gene regulatory effects of vitamin D3 by directly targeting EF2 in gastric cancer cells, Oncotarget. 3048, https://doi.org/10.3389/fmicb.2017.02686.

[89] S. Nersisyan, M. Shkurnikov, A. Turchinovich, E. Knyazev, A. Tonevitsky, Integrative analysis of miRNA and mRNA sequencing data reveals potential regulatory mechanisms of ACE2 and TMPRSS2, PloS One 15 (7) (2020), e0235867, https://doi.org/10.1371/journal.pone.0235867.

[90] S. Ludwig, C. Ehrhardt, E.R. Neumeier, M. Kracht, U.R. Rapp, S. Pleschka, Influenza virus-induced AP-1-dependent gene expression requires activation of the JNK signal transduction pathway, Biol. Chem. 276 (14) (2001) 10990–10998, https://doi.org/10.1074/jbc.M009922200.

[91] T.B. Hansen, J. Kjems, J.B. Bramsen, Enhancing miRNA annotation confidence in miRBase by continuous cross dataset analysis, RNA Biol. 8 (3) (2011) 378–383, https://doi.org/10.4161/rna.8.3.14323.

[92] S. Gheisgheis-Jones, R.J. Grocock, S. van Dongen, A. Bateman, A.J. Enright, miRbase: microRNA sequences, targets and gene nomenclature, Nucleic Acids Res. 34 (Database issue) (2006) D140–D144, https://doi.org/10.1093/nar/gkj112.

[93] E. Ladwig, K. Okamura, A.S. Flynn, J.O. Westholm, E.C. Lai, Discovery of hundreds of mirtrons in mouse and human small RNA data, Genome Res. 22 (9) (2012) 1634–1645, https://doi.org/10.1101/gr.135533.111.

[94] T.B. Hansen, J. Kjems, J.B. Bramsen, Enhancing miRNA annotation confidence in miRBase by continuous cross dataset analysis, RNA Biol. 8 (3) (2011) 378–383, https://doi.org/10.4161/rna.8.3.14323.

[95] M.D. Sacar Demirci, A. Adan, Computational analysis of microRNA-mediated interactions in SARS-CoV-2 infection, PeerJ 8 (2020), e9369, https://doi.org/10.7717/peerj.9369.

[96] S. Ludwig, C. Ehrhardt, E.R. Neumeier, M. Kracht, U.R. Rapp, S. Pleschka, Influenza virus-induced AP-1-dependent gene expression requires activation of the JNK signal transduction pathway, Biol. Chem. 276 (14) (2001) 10990–10998, https://doi.org/10.1074/jbc.M009922200.

[97] T.B. Hansen, J. Kjems, J.B. Bramsen, Enhancing miRNA annotation confidence in miRBase by continuous cross dataset analysis, RNA Biol. 8 (3) (2011) 378–383, https://doi.org/10.4161/rna.8.3.14323.

[98] M.D. Sacar Demirci, A. Adan, Computational analysis of microRNA-mediated interactions in SARS-CoV-2 infection, PeerJ 8 (2020), e9369, https://doi.org/10.7717/peerj.9369.

[99] S. Ludwig, C. Ehrhardt, E.R. Neumeier, M. Kracht, U.R. Rapp, S. Pleschka, Influenza virus-induced AP-1-dependent gene expression requires activation of the JNK signal transduction pathway, Biol. Chem. 276 (14) (2001) 10990–10998, https://doi.org/10.1074/jbc.M009922200.

[100] T.B. Hansen, J. Kjems, J.B. Bramsen, Enhancing miRNA annotation confidence in miRBase by continuous cross dataset analysis, RNA Biol. 8 (3) (2011) 378–383, https://doi.org/10.4161/rna.8.3.14323.