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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 transuding 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 performing 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].

Table 1
List of SARS-CoV-2 variants concern.

S. No	Spike Protein Substitutions	First Detected
1.	A67V, 69del, 70del, 144del, E484K, D614G, Q677H, F888L	United Kingdom/Nigeria – December 2020
2.	(L5F*), T95I, D253G, (S477N*), (E484K*), D614G, (A701V*)	United States (New York) – November 2020
3.	D80G, 144del, F157S, L452R, D614G, (T791I*), (T859N*), D950H	United States (New York) – October 2020
4.	L452R, E484Q, D614G	India – February 2021
5.	(T95I), G142D, E154K, L452R, E484Q, D614G, P681R, Q1071H	India – December 2020
6.	T19R, (G142D), 156del, 157del, R158G, L452R, T478K, D614G, P681R, D950N	India – December 2020
7.	T19R, G142D, L452R, E484Q, D614G, P681R, D950N	India – October 2020
8.	E484K, (F565L*), D614G, V1176F	Brazil – April 2020
9.	D80A, D215G, 241del, 242del, 243del, K417N, E484K, N501Y, D614G, A701V	South Africa
10.	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I	Japan Brazil

(*) = detected in some sequences but not all (Source: SARS-CoV-2 Variant Classifications and Definitions, Centre for Disease Control and Prevention (CDC) May 17, 2021)

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 proteoglycan 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 snRNAs and utilizing *Invitro* and in vivo applications.

The basic principles for extracellular vesicle delivery and their

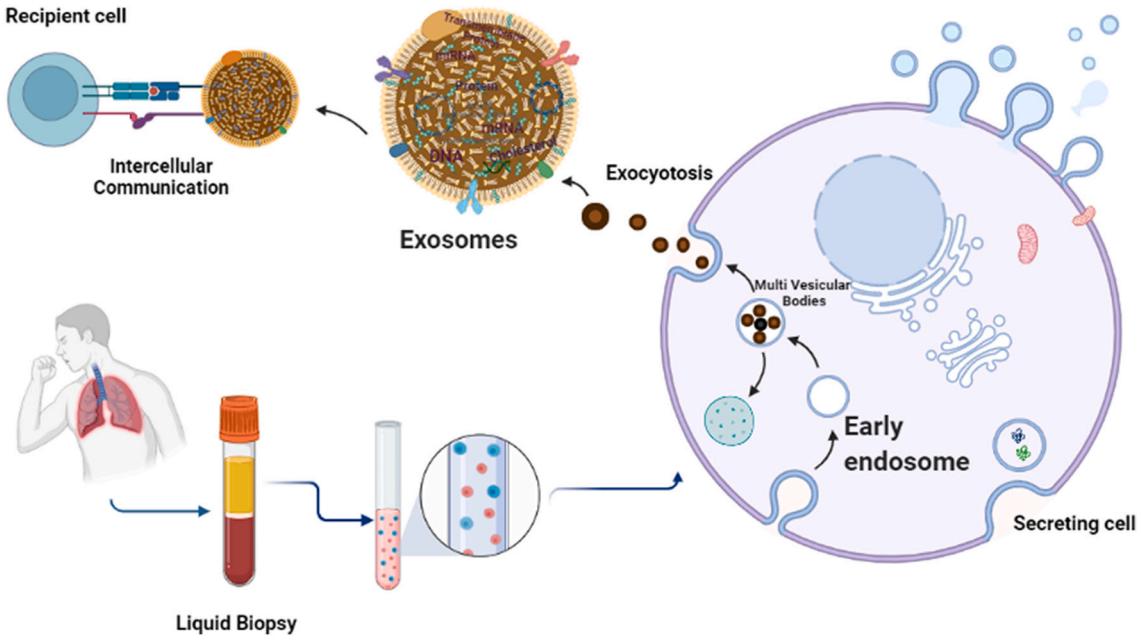


Fig. 1. Schematic representation of the origin, a pathway of exosome secretion, and internalization of exosomes.

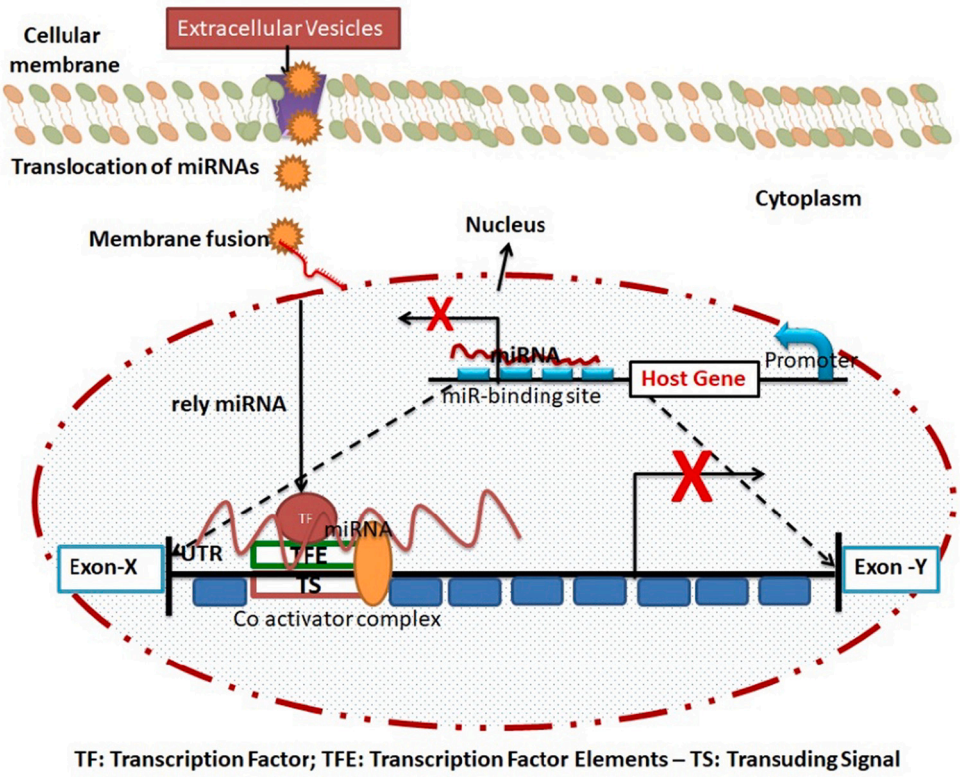
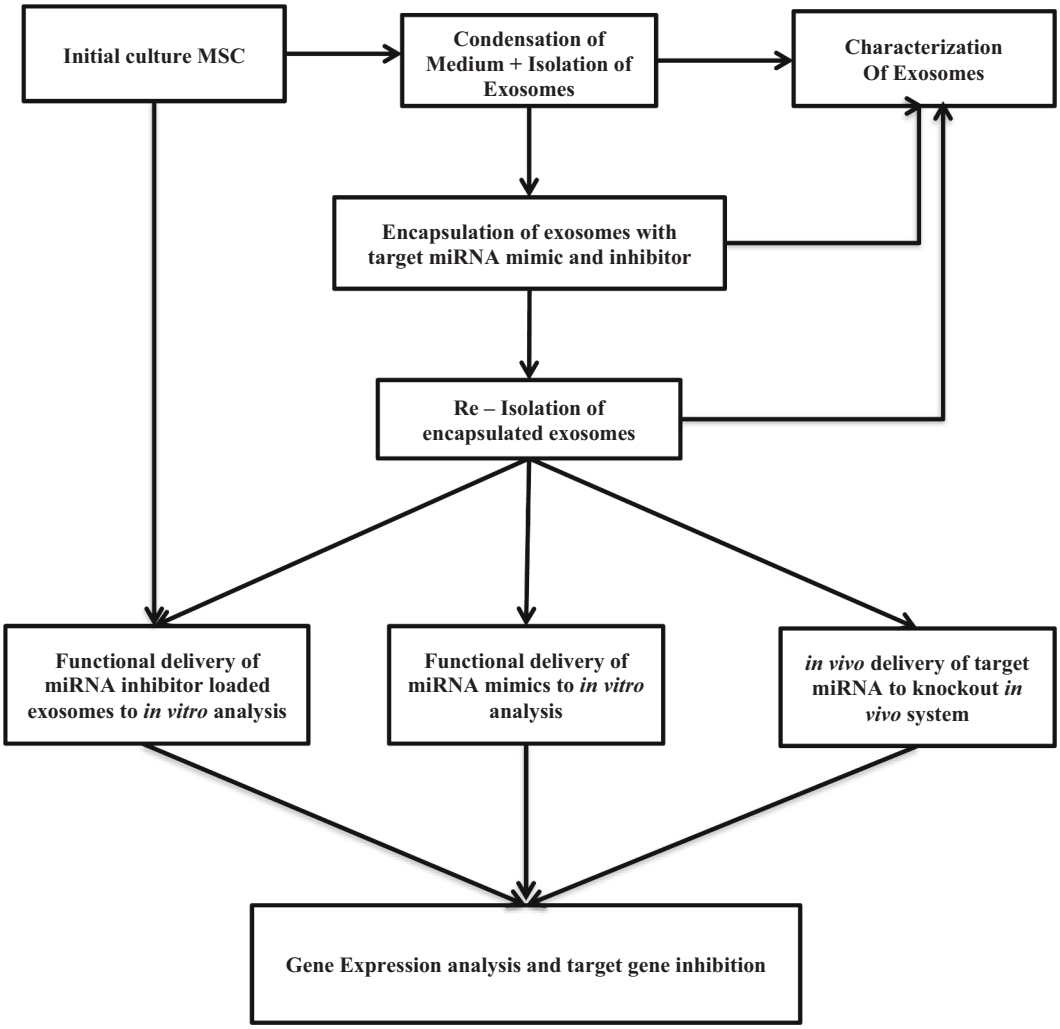


Fig. 2. Mechanism of action behind miRNAs to target genes responsible for signal transducing events. The extracellular vesicles deliver the miRNAs into the cells and then membrane fusion to translocate miRNAs to inhibit the targeted genes. Transcription factor and target Transcription binding site (Elements) to transducing signals to up or down-regulation of miRNAs and based on the expressions, the targeted gene can be either up or down their expression.

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 amiRNA 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		No	SARS CoV spike protein	HSP 70 Protein	No	SARS CoV spike protein	HSP 70 Protein
SARS-CoV with HSP 70	−114.6 +/− 14.5	2293.7 +/− 144.7	−12.3	15	ASN318 ASN321 ARG342 ARG342 LYS344 LYS344 SER346 ASN347 ASN347 TYR383 ARG453 ARG453 GLU502 GLN546 THR567	LYS112 LYS108 GLU132 GLU132 THR125 GLU129 ARG 49 ARG 49 ARG 49 GLU132 LEU135 GLU132 LYS128 LYS159 TYR115	3	ARG 342 LYS 344 GLU 502	GLU132 GLU129 LYS128
2ack crystal structure with HSP 70	−104.8 +/− 22.3	2321.3 +/− 91.5	−11.9	10	SER325 ASN343 ASN343 THR345 SER366 LEU441 LYS529 SER530 SER530 ASN532	LYS3 LYS100 GLU 117 ASP97 ASN168 LYS77 ASN141 THR140 THR140 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. 4a. 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.)

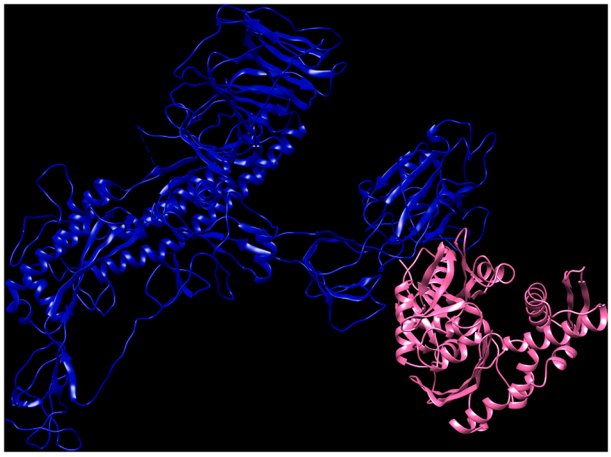


Fig. 4. SARS-CoV Spike protein With HSP 70 protein. Ribbon diagram of the SARS-CoV S1 (blue)/HSP 70 (pink) complex model. The SARS CoV Spike protein (GenBank: QHD43416.1) and the native crystal structure of Heat shock protein (PDB code: 1s3x) were downloaded from the protein data bank (PDB). This model was generated by the fully automatic HADDOCK protein-protein docking server and manually selected based on structural biology knowledge.

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

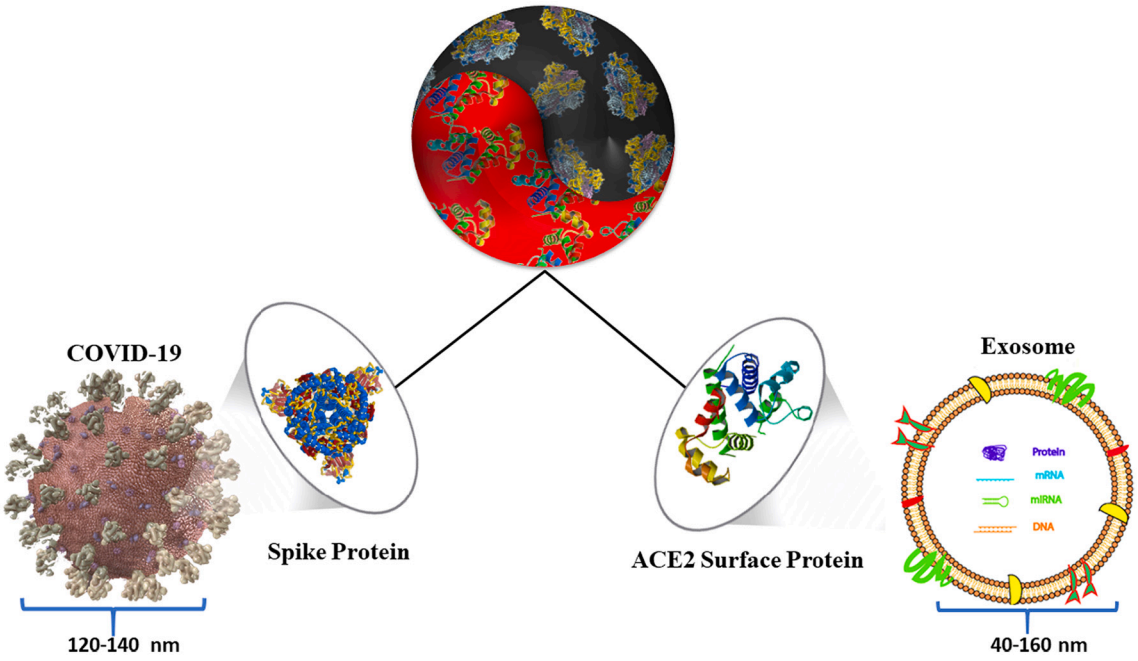


Fig. 3. Exosomal ACE2 for the neutralization of the SARS-CoV-2 spike protein.

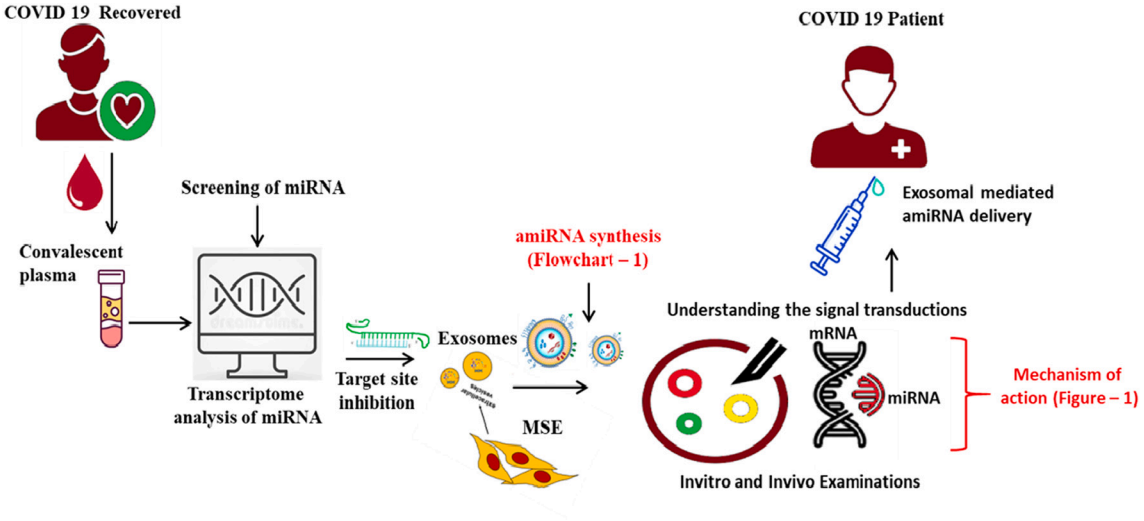


Fig. 5. Mechanism of action to target genes responsible for viral transformation. The process takes place in the internalization of antibody-mediated exosomes into endosomes, then membrane fusion to rely upon genetic materials (snRNA, miRNA, etc.) to inhibit viral transformation. This delivery would provide extracellular signaling through noncoding RNAs and has played an integral role in the mechanism of miRNAs-mediated exosomes; it contains anti-codon information for RNA splicing and/or transcriptional regulator factors.

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 enable 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, miRCURYTM 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 ss-RNA 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

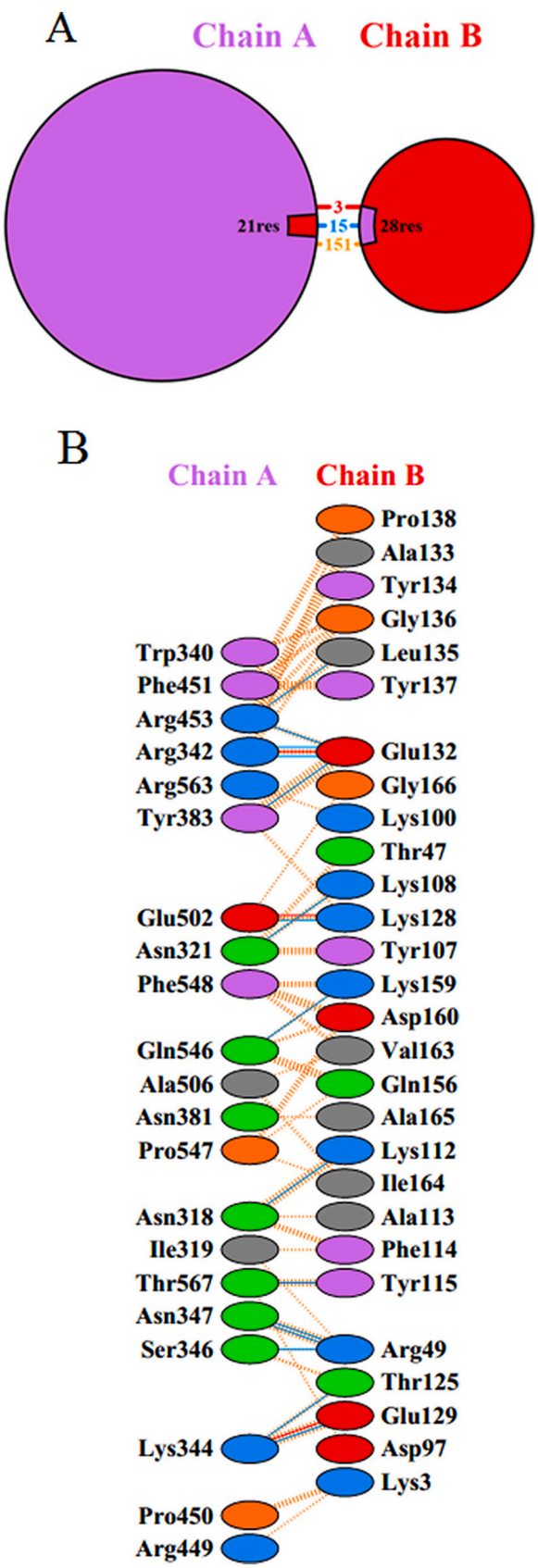


Fig. 4. (A). In silico interaction analysis of the SARS CoV spike protein/ HSP 70 protein. (A) The interaction was between the SARS CoV spike protein, and the HSP 70 protein was made through salt bridges, hydrogen bonds and non-bonding contacts, (3 B) amino acid interaction and its respective bonds were noticed between two proteins

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 deference mechanism of SARS-CoV-2 genome.

S. No.	Predicted miRNA	Process of human gene	Disease involved	References
1	hsa-miR-6891-5p	Cellular process, biological regulation, response to stimulus, signaling, metabolic process	Reproduction, immune system	[69]
2	hsa-miR-05220	Different KEGG pathways, potential target sites on SARS-CoV-2	Chronic myeloid leukemia, cancer	[70]
3	hsa-miR-05205	Proteoglycans	cancer	[70]
4	has-miR-05218	different KEGG pathways	Melanoma,	[70]
5	**miR-8066** and **miR-5197-3p**	Related to cytokine-cytokine receptor interaction; related to morphine addiction and metabolism of xenobiotics by cytochrome P450 mechanisms.	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	hsa-miR-195-5p	fatty acid synthase	obesity, metabolic syndrome, inflammation, cardiovascular disease, and cancer	[72]
8	hsa-miR-195-5p	fibroblast growth factor 2	human breast cancer	[72]
9	hsa-miR-424-5p	protein tyrosine phosphatase, non-receptor type 4	Developmental defects, neoplastic disorders, and immunodeficiency.	[72]
10	hsa-miR-3133	regulating synaptic membrane exocytosis 2	Cone-Rod Synaptic Disorder, Congenital Nonprogressive and Scoliosis	[72]
11	hsa-miR-3133	transcription factor AP-2 beta	Char syndrome and Patent Ductus Arteriosus	[72]
12	hsa-miR-3133	protein tyrosine phosphatase, receptor type K	Extragonadal Germ Cell Cancer and Eye Lymphoma	[72]
13	hsa-miR-3133	nuclear respiratory factor 1	autism spectrum disorders	[72]
14	miR-199a	regulate TMPRSS2 expression in the liver, stomach, and uterine corpus, since this protease is critical for the entry of SARS-CoV-2, SARS-CoV, and MERS-CoV entry into cells	lung adenocarcinoma (LUAD), endometrial uterine corpus endometrial	[73]
15	miR-16, miR-29, and miR-30	lung epithelial A549 cells	pulmonary disease, lung cancer	[74]
16	hsa-miR-145	Up-modulated by Vitamin D	immune/ inflammation	[11]
17	hsa-miR-222	X-linked miRNA involved in a negative feedback loop with ERα. Downmodulated by androgen.	immunity and cancer	[76]
18	hsa-let-7a-g/i	Up-modulated by Estrogen/ERα	autoimmune diseases, insomnia, allergic skin rashes,	[76]
19	MiR-208	Necessary for cardiomyocyte hypertrophy	Heart diseases	[78]
20	miR-8066	Bind and activate NfκB-mediated TLR-8 expression and induce cytokine synthesis	chronic inflammatory diseases	[74]
21	miR-5197-3p	therapeutic potential, since they bind with high-affinity to SARS-CoV2 guide RNA	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	hsa-miR-589-3p	Involved in a mitochondrial organization and can target cFOS gene	Glioblastoma cell migration	[81]
24	hsa-miR-4282	Participated in epigenetic control through chromatin remodeling	Involved in proliferation, invasion, and metastasis of breast cancer through Myc.	[81]
25	hsa-miR-5193	Involved in interferon gamma signaling and CDK mediated phosphorylation and removal of cdc6	HBV related hepatocellular carcinoma	[82]
26	hsa-miR-5011-5p	Linked to the occurrence of glioblastoma	Cancer	[83]
27	hsa-miR-6835-3p	Plays a role in cell growth and proliferation through the ornithine decarboxylase pathway	Ovarian cancer	[84]
28	hsa-miR-190a-3	Plays a role in the regulation of several cellular processes through regulation of production of nfk-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 [09]. 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

immune-derived exosomes or those isolated from convalescent plasma would not be ideal for transferring miRNA. The proposed protocol for exosomal mediated artificial miRNA delivery is exhibited in Flow chart 1 and Fig. 5.

To determine the specific miRNA which can be used to arrest the viral infection, a detailed study of the process and the host genes involved is essential. The one pathway in which transcription factors were up-regulated in response to nucleocapsid entry was that of the AP1 transcription factor complex. The pathway is involved in several processes, including induction of apoptosis, cytokine production, and bacterial and viral infections [92]. The complex includes c-Fos, ATF2, CREB-1 and Fos B. A variety of micro-RNAs can target these transcription factors and inhibit their transcription. According to "Zhang," the gene C-fos can be targeted by hsa-miR-589-3p [93]. Similarly, hsa-miR-4282 (Griffiths-Jones et al., 2006) and hsa-miR-548e-5p can be used to target the ATF2 gene. Lastly, CREB1 can be transcriptionally inhibited by various miRNA with great efficiency including hsa-miR-3682-5p, hsa-miR-6835-3p [94], hsa-miR −5011-5p, hsa-miR-190a-3p, hsa-miR-5004-5p [95] and hsa-miR-12,136 [96]. The gene is important for several phosphorylation pathways and regulation of the cAMP pathway.

There are several possible pathways through which SARS-CoV2 can initiate viral transduction. The target gene was selected based on the criteria that it should regulate the proposed viral transduction pathway. The target gene, CREB1, is involved in the AP-1 pathway, initially associated with the coronavirus transduction by Runtao He et al. [97] in 2003. AP-1 is a pathway that is a transcription pathway that responds to several viral infections, including influenza [52] [98] and HTLV-1 [99]. The pathway can induce proliferation and differentiation or apoptosis, the former being beneficial for the virus [108]. Within the miRNAs that could adhere to the target gene with 100% efficacy, intraselection was also required. The miRNA was selected on the criteria that it should inhibit the target gene and several other proposed mechanisms that can connect to it. The selected miR190a-3p can interfere in several known metabolic pathways [100]. The first transduction pathway is the clathrin-mediated endocytosis which involves the AP1 pathway, involving the target gene in the nucleus. The initial cytoplasmic pathway followed through the viral transduction pathway is through cAMP followed pKA followed the initiation of AP-1 pathway. Based on the receptor being ACE-2, another possible pathway can be Ras, or in this case RAPH-1 along with MAPK3which is inhibited by the miRNA [101]. The pathways may have to be changed based in the viral transduction pathways based on the ethnicity of the individual. However, the CREB-1 pathway is common among all individuals and ethnicity, and hence there is a higher probability of the virus infecting through the same in all individuals.As the pathway chosen is with respect to the viral transduction pathway and not the host response, it will likely be common among all individuals.

Meanwhile, it also targets PTEN and AKT-3, which can be used to inhibit the PI3K pathway. Similarly, the SMAD4 gene could be inhibited to stop the TGF- beta signaling pathway [102]. The Wnt pathway can also be targeted through Wnt-3 is another proliferative pathway [103]. These are the cytoplasmic pathways that could be targeted, the last step being the inhibition of CREB1, preventing the process of viral transduction [104]. Several general pathways can be regulated with the help of miR190a-3p. This is the most important pathway, occurring within the nucleus, the AP-1 pathway. This is the point for transcriptional inhibition, where the viral transduction can be inhibited [105]. Hence the study focuses on transcriptional inhibition of viral transduction through artificial miRNA to target mRNA or gene.

Flowchart 1 shows the main root to isolate exosomes, encapsulating the artificial miRNA and delivery for understanding the mechanism for target inhibition of traducing signal and their event.

7. Conclusions

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 transcription of the target gene mRNA 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.

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