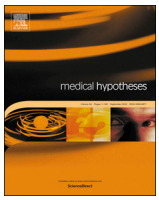
Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Medical Hypotheses 143 (2020) 110203

Contents lists available at ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy



Letter to Editors

A new approach for COVID-19 treatment by micro-RNA

ARTICLE INFO

Keywords:
COVID-19
miRNAs
Virus
Treatment

ABSTRACT

MicroRNAs (miRNAs) naturally occur in plants and all living organisms. They play an important role in gene regulation through binding toa specific region in open reading frames (ORFs) and/or untranslated regions (UTRs) to block the translation processes through either degrading or blocking mRNA resulting in knocking down or suppression of targeted genes. Plants and many organisms protect themselves from viruses through the production of miRNAs, which are complementary to 3UTR of viruses resulting in degrading the viral mRNA or block the translation on ribosomes. As pandemic, COVID-19, and its consequences on the global economy, we hypothesized a new approach for the treatment of COVID-19 paints. This approach includes designing a mix of miRNAs targeting several regions on COVID-19 open reading frame (ORF) and 3 UTR and suitable delivery system targeting respiratory system tissues. These synthesized miRNAs may be delivered to humansinnon-viral delivery systems such as liposomes like exosome (extracellular vesicle), polymer-based carriers, or inorganic nanoparticles, which are considered to be more suitable for human use.

Background

Living organisms are continually facing threats from the environment, including pathogens. They must fight against to survive. Among those, viruses represent which represent a large class of obligatory intracellular parasites. Consequently, viruses and their hosts have occupied in an ever-evolving arms race to be able to maintain their existence [1]. The human genome contains almost 2000 miRNA precursors [2]; each one has the potential to control different targets, including viral transcripts. It has been reported that miRNAs play essential tasks during given virus infection, which can quickly reduce the overwhelming of health care systems during the pandemic.

The fast-spreading of COVID-19 raised a notion that mutations may drive its evolution. Therefore, discovery single nucleotide polymorphisms (SNPs) are considered as a vital step to understanding the viral evolution. The genome of COVID-19 is evoluted from SARS- CoV and MERS CoV6. Comparison of the genome sequences of the COVID-19, SARS-CoV, and MERS-CoV showed that 2019-CoV has better sequence identity with SARS-CoV than the MERS CoV6. Kabir et al. [3] extensively reviewed factors that affect COVID-19 genetic diversity and its evolution. The COVID-19 has a unique amino acid sequence from other coronaviruses entirely in the regions of 1ab polyprotein and surface glycoprotein or S-protein. There are two subunits building S-protein up. One of these subunits binds directly to the host receptor facilitating the virus entry into the host cells. However, the RNA binding domain has a higher homology with SARS-CoV. Although some critical residues for binding to receptors differ, in general, mismatched residues did not change the structural composition. Studies indicate that the human receptor for COVID-19 could be angiotensin-converting enzyme 2 (ACE2). Other coronaviruses, including SARS-CoV, enter human cells through ACE2 [4].

The coronavirus disease 2019 (COVID-19) virus is spreading rapidly worldwide, and the global interested scientists are endeavoring to discover drugs for its efficacious treatment. Chloroquine phosphate, an old drug for the treatment of malaria, is shown to have apparent efficacy and acceptable safety against COVID-19 associated pneumonia in multicenter clinical trials conducted in China [5]. Excessive inflammation, oxidation, and an exaggerated immune response very likely contribute to COVID-19 pathology. This leads to a cytokine storm and subsequent progression to acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) and often death. Melatonin, a well-known anti-inflammatory, and anti-oxidative molecule are protective against ALI/ARDS caused by viral and other pathogens [6]. Most recently, Jackson et al. [7] reported the response of healthy adults human, 18 to 55 years of age, to an mRNA vaccine against SARS-CoV-2. The authors in this report concluded that a synthesized mRNA of COVID 19 vaccine-induced anti–SARS-CoV-2 immune responses in all participants, and no trial-limiting safety concerns were identified. These findings strongly support the idea of targeting the viral mRNA using miRNAs in the human body since large mRNA molecules (about 1273 bp) were delivered in living human cells and induces their biological functions.

MicroRNAs are a class of non-coding RNAs that play important roles in regulating gene expression. These molecules comprise a group of small non-coding RNAs 18 ~ 25 nucleotides (nt) in length that post-transcriptionally regulate gene expression via binding to the 3′-untranslated regions (3′-UTRs) of target gene mRNA [8,9]. The majority of miRNAs are transcribed from DNA sequences into primary miRNAs and processed into precursor miRNAs, and finally mature miRNAs. In most cases, miRNAs interact with the 3′ UTR of target mRNAs to induce mRNA degradation and translational repression resulting in protein biosynthesis inhibition. However, the interaction of miRNAs with other regions, including the 5′ UTR, coding sequence, and gene promoters, have also been reported [10].

The extracellular miRNAs can be delivered to target cells, and they may act as autocrine, paracrine, and/or endocrine regulators to modulate cellular activities [11]. Contrary to cellular RNA species, extracellular miRNAs are highly stable, resisting degradation at room temperature for up to 4 days and in deleterious conditions such as boiling,

https://doi.org/10.1016/j.mehy.2020.110203
Received 18 May 2020; Received in revised form 7 August 2020; Accepted 17 August 2020

multiple freeze-thaw cycles, and high or low pH8 [12,13].

The previous studies indicated that there is a direct binding of miRNA to viral RNA. This binding has negative effects on the virus and consequently preventing its translation on host ribosome's or virus genome degradation [1]. If there is a target site for miRNA on the virus genome, it is likely that this site or its sequences will be removed/changed (introduced -SNPs) very quickly adapted in the virus progeny. For that reason, our hypothesis was developed to stop this natural evolution by targeting many sites on the viral genome simultaneously.

Hypothesis

Recently, a complete genome sequence for a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) strain isolated from an oropharyngeal swab specimen of a Nepalese patient with coronavirus disease 2019 (COVID-19) was published in GenBank (accession number NC_045512), excluding the poly(A) tail of the genome [14]. Taking this advantage, we hypothesized that designing a synthetic miRNA complemented to COVID-19 at 3′ UTR and another essential region, including ORF9, which encodes a nucleocapsid phosphoprotein (N). This protein is a basic RNA-binding protein encoded in the most 3′ protein of the COVID genome, plays both structural and non-structural roles in infection. Most of N complexes with genome RNA to form the viral capsid and interacts with the viral membrane protein during assembly. Therefore, we assume targeting this gene may reduce or stop the process of viral particle assembly. The 3′ UTR region and ORF9 of COVID-19 were identified using the sequence (Acc.#: NC_045512). We hypothesize if we can disrupt the binding between eukaryotic translation initiation factors that bind with viral mRNA by miRNAs from 3′ and 5′ UTR regions could inhibit or suppress the translation process. Designing other miRNAs from ORF could stop the translation or degrading the viral RNA. Therefore, Thei-score designer (www.med.nagoya-u.ac.jp/neurogenetics/i_Score/i_score.html) was used to deduce some candidates of miRNAs complemented of COVID-19. These miRNA was tested by blasting against the human genome to check whether they are complemented to any human genes or not. The ones that give no significant matching with the human genome were selected (examples given in Table 1). We assume these miRNAs (Table 1)could be delivered to the human respiratory system by a suitable delivery system resulting in reduction or inhibition of the virus life cycle.

Testing hypothesis

In order to test whether this hypothesis is positive or negative, these miRNAs will be synthesized using any DNA synthesizer technology with high purity of HPLC coupled with PAGE technologies. We assume to perform an *in vitro* experiment using cell culture techniques. These cells will be infected with COVID-19 thereafter, the miRNA will be delivered by a suitable method (discussed after). Application of quantitative RT-PCR, the infected cells will be isolated and washed several time; the total RNA acid will be isolated and converted to cDNA using suitable means. Comparing the quantitative RT-PCR results with the control treatment (cells without miRNA treatment, and non-infected cells), we can evaluate our hypothesis.

Table 1
the sequences of deduced antisense miRNA using i-score web tools:

Targeted Area	Sense miRNA (5′–3′)
ORF9	GGAUGAUUUCUCCAAACAA
	GCAUAUUGACGCAUACAAA
	GAAGGCUGAUGAAACUCAA
	CCAUCAAAUUGGAUGACAA
	CAAGGUUUACCCAAUAAUA
3′UTR	CCCUAAUGUGUAAAAUUAA
	CCUAAUGUGUAAAAUUAAU
	GAAGAGCCCUAAUGUGUAA
	UAAUCAGUGUGUAACAUUA
5′UTR	CAGUAUAAUUAAUAACUAA
	GGGUGUGACCGAAAGGUAA
	GAUCUGUUCUCUAAACGAA
	CAGGUAACAAACCAACCAA

Discussion

The previous studies revealed that plant miRNAs might target their genes in the consumers and have been detected not only in blood circulation but also in murine tissue [15]. In mice, it has been clear that plant miRNAs down-regulated at least one endogenous target in the liver, the low-density lipoprotein receptor adapter protein one 1 (LDLRAPI), within hours of dietary intake [16]. Based on these results, the extracellular miRNAs may be used to regulate different molecular pathways controlling different metabolic and developmental processes. Therefore, the synthetic miRNA could be delivered appropriately, sticking out the specific organ in the human body and entered the cells targeting the particular gene. The main obstacle to the use of this approach is how to deliver the synthetic miRNAs to a specific organ.

The delivery of exogenous plant miRNAs to circulation and murine tissue is not clear.

Some studies indicated that exogenous miRNA makes a complex called vesicle-associated-miRNA that may enter the cells by either phagocytosis, endocytosis, or direct fusion with the plasma membranes. On the other hand, vesicle-free miRNA may be delivered by specific receptors on the cell surface [17]. Some studies indicated that miRNAs enter the cells by endocytosis and micropinocytosis [18]. This process depends on clathrin, but not on caveolae or lipid rafts in PC12 cells [18]. However, A549-P studies showed that endocytosis of exogenous miRNAs is mediated by caveolae- and lipid raft-dependent pathways [19].

Furthermore, vesicle-free miRNAs that are linked with HDL are delivered by HDL and BI (SR-BI) receptors, located in the plasma membrane of the target cells [20,21]. miRNAs show an ability to transfer between co-cultured cells mediated direct cell-cell contact and gap junctions [22]. Although these studies suggest that extracellular miRNAs can interact with recipient cells via multiple mechanisms, the factors that determine the specificity of such interactions are not clear.

Lipid-based nanocarriers are widely used for delivering nucleic acids *in vivo* because their ability to be modified chemically to conjugate with moieties and fluorescent probes. Cationic lipids may be selected from commercially available products, such as Lipofectamine®, due to their hydrophilic head and a hydrophobic tail [23]. Many studies revealed that the validation of the use of cationic liposomes as carriers for transporting miRNA *in vivo*. Currently, large numbers of cationic lipids are available for nucleic acid drug delivery. However, the main disadvantage of using Cationic lipids is low delivery efficiency. To overcome this obstacle, most recently novel lipids have been synthesized, and new tools for constructing lipid nanocomplexes have been established. Subsequently, polyethylene glycol (PEG), a frequently used functional group, was conjugated to cationic lipids to escape phagocytosis of the RES when administered systemically [24].

Studies at Tokyo University confirmed that miRNA-126-loaded by modified PEG liposomes binds with entrapping ultrasound, promoting angiogenesis, and improving blood flow in an experimental hindlimb ischemia model [25]. A novel system contains miRNA-10b antagomirs and a pH-responsive liposome modified with the antimicrobial peptide [D]-H_6L_9 (D-Lip). It has been reported that paclitaxel could delay 4 T1 tumor growths and reduce lung metastases in a murine breast cancer model [26]. Exploring miRNA profile in cells infected with HCV indicated that the top hits miR-25, miR-130a/b, and let-7a were down-regulated by the virus both in cultured cells and liver tissues of infected patients. These results suggest that HCV counteracts their proven antiviral capacity by reducing their levels [27]. In another study, miR-

485-5p was one of the most upregulated ones not only upon Newcastle Virus (NDV) infection but also in cells infected with Influenza A virus (IAV) H5N1 or transfected with a synthetic dsRNA, polyI:C [28].

Conflict of interests

The authors declare that there is no conflict of interests

Funding

There was no funding for this work.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2020.110203.

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