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What is the potential function of microRNAs as biomarkers and therapeutic targets in COVID-19?

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ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of COVID-19, a pandemic associated with substantial morbidity and mortality. Despite of this, no vaccine or approved drug is available to eradicate the virus. In this manuscript, we present an alternative study area that may contribute to development of diagnostic biomarkers and therapeutic targets for COVID-19. We analyzed sixty SARS-CoV-2 genomes to identify regions that could work as virus-encoded miRNA seed sponges and potentially bind to human miRNA seed sites and prevent interaction with their native targets thereby relieving native miRNA suppression. MicroRNAs (miRNAs) are evolutionally conserved single-stranded RNAs that regulate gene expression at the posttranscriptional level by disrupting translation. MiRNAs are key players in variety of biological processes that regulate differentiation, development and activation of immune cells in both innate and adaptive immunity. We find 34 miRNAs for positive-sense viral RNA and 45 miRNAs for negative-sense that can strongly bind to certain key SARS-CoV-2 genes. The disruption and dysfunction of miRNAs may perturb the immune response and stimulate the release of inflammatory cytokines altering the cellular response to viral infection. Previous studies demonstrate that miRNAs have the potential to be used as diagnostic and therapeutic biomarkers. Therefore, its discovery and validation are essential for improving the diagnosis of infection and clinical monitoring in COVID-19.

Dear Editor,

The SARS-CoV-2 outbreak started on December 2019 in China and rapidly spread worldwide. This novel coronavirus can cause severe acute respiratory syndrome and became an international health emergency. Initial outbreaks in China involved 13.8% cases with severe, and 6.1% with critical courses (WHO, 2020). The immune response is vital for the control and resolution of SARS-CoV-02 infections, while it can also lead to cellular damage, associated with an exacerbated immune response. There is a complex network of interaction between the virus and infected host cells, in which microRNAs (miRNAs) have been revealed to play a pivotal role (Girardi et al., 2018; Rupaimoole and Slack, 2017).

MicroRNAs, the smallest endogenous regulatory non-coding RNAs, play a central role in cell differentiation, proliferation and survival by binding to complementary target mRNAs leading to translational inhibition or degradation. miRNAs are the crucial factor in a diverse biological processes such as antiviral defense, oncogenesis and cell development (Bartel, 2004). The miRNA-binding sites within viral genomes are mostly located in the 5'and 3'non-translated regions (NTRs) but have recently been found in the coding regions of viral proteins (Girardi et al., 2018; Trobaugh et al., 2014; Zheng et al., 2013). Various RNA viruses mimic or block the binding between a host miRNA and its target transcript, a phenomenon mediated by the miRNA seed site at the 5′ end of miRNA. We analyzed sixty SARS-CoV-2 genomes to identify regions that could work as virus-encoded miRNA seed sponges and potentially bind to human miRNA seed sites and prevent interaction with their native targets thereby relieving native miRNA suppression.

We searched for miRNAs that shared 100% identity of the 8mer seed region (Ellwanger et al., 2011) with SARS-CoV-02 genome regions, both positive and negative-sense. Currently, the human genome contains 2.654 mature sequences of miRNAs in miRBase database and > 1000 showed 100% of similarity between their seed sequences, important specific gene silencing motifs, and regions from the viral RNA. We refined the search for miRNAs that interact with the SARS-CoV-2 genome with a perfect alignment of 11 nucleotides encompassing the seed region.

Thus, we find 34 miRNAs for positive-sense viral RNA and 45 miRNAs for negative-sense that can strongly bind to certain key SARS-CoV-2 genes. Once a candidate miRNA has been found, it becomes essential to identify its targets to understand the molecular mechanisms underlying the effect on the infection. We therefore conducted a literature review in this study to reveal the potential of these miRNAs in the immunopathogenesis and potential treatment possibilities of SARS-CoV-2 disease. Notably, these miRNAs play an important role in studies with pulmonary and cardiac disorders, including lung cancer, asthma, pneumonia, cardiac fibrosis, among others (Table 1). For example, Bertrams and collaborators analyzed transcriptional networks of peripheral blood mononuclear cells to identify central regulators and potential biomarkers in community-acquired pneumonia (CAP), and acute exacerbations (AE) that are episodes of aggravated chronic obstructive pulmonary disease (COPD) symptoms and often co-occur with respiratory infection. They identified several microRNAs, e.g. miR-545-3p and miR-519c-3p, which separated AECOPD and CAP (Bertrams et al., 2020). Already, Huang and collaborators show that a host miRNA, miR-1290, is induced through the extracellular signal-regulated kinase pathway upon Influenza A (IAV) virus infection and is associated with increased viral titers in human cells and ferret animal models. These findings point to a host species-specific mechanism by which IAV

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Table 1
MicroRNAs that plays a significant role in different mechanisms with based on the scientific literature. In silico analyzes were not considered. Many of the microRNAs that exhibited a perfect alignment of 11 nucleotides encompassing the seed region have not yet been found with their expression changed. Therefore, there were no scientific data about them.

microRNA	Function	Model	Type	Year	Reference
miR-18b-5p	hsa-miR-18b-5p of CD8 + T cells exhibited a positive correlation with miR-BHRF1–2-5P and miR-BART2-5P.	Infectious mononucleosis pediatric patients	in vivo	2015	https://doi.org/10.1186/s12985-015-0441-y
miR-34c-3p	Overexpression led to loss of PMP70 and PEX2, proteins important for biogenesis of peroxisomes.	HIV/AIDS patients with HIV-associated neurocognitive disorder (HAND)	in vivo	2017	https://doi.org/10.1371/journal.ppat.1006360
miR-193a-3p	overexpression reduced inflammation and apoptosis in pulmonary injury model	WI-38 cells Normal Human Fetal Lung Fibroblast	in vitro	2019	PMCID: PMC6789249
miR-203a-5p	Was reported as a tumor suppressor and disregulated in many malignancies including nasopharyngeal carcinoma	Nasopharyngeal carcinoma cell lines.	in vitro	2017	https://doi.org/10.1186/s13046-017-0604-3
miR-208b-5p	higher expression in ventricles over atria in cardiac tissue	Human cardiac tissue (autopsy)	in vivo	2016	https://doi.org/10.1016/j.ijcard.2016.02.145
miR-219a-5p	downregulates SOX5 and reduces progression of NSCLC by decreasing cell viability, migration and invasion and increasing apoptosis	Non-small-cell lung cancer (NSCLC) cells	in vitro	2020	https://doi.org/10.1111/1759-7714.13274
miR-367-3p	downregulation leads to increased expression of CD69 and may be associated with cardiac fibrosis	Biopsy of right atrial appendage and fibroblast 3 T3 cells	in vivo/ in vitro	2018	https://doi.org/10.3892/mmr.2018.9234
miR-489-3p	suppresses cell proliferation and promotes apoptosis	A549 cells (NSCLC)	in vitro	2020	https://doi.org/10.1016/j.prp.2020.152823
miR-498-5p	induces NF-κB activation by inhibition of NKRF protein	16HBE cells and 293-T cells	in vitro	2020	https://doi.org/10.1016/j.ecoenv.2020.110455
miR-519c-3p	one of the microRNAs used as biomarkers to differentiate between community-acquired pneumonia (CAP) and chronic obstructive pulmonary disease (COPD)	Blood from CAP and COPD patients	in vivo	2020	https://doi.org/10.1038/s41598-019-57,108-0
miR-548as-3p	miRNAs-mediated novel mechanism for NF-κB signaling in NSCLC metastasis	NSCLC	in vitro	2019	https://doi.org/10.2174/1871520619666190206165215
miR-590-3p	Targets p50 subunit of NF-κB blocking IL-6 and TNF-a expression	Rat model of experimental autoimune myocarditis	in vivo	2015	https://doi.org/10.1159/000433596
miR-668-3p	suppressed mediators of inflammation and oxidative stress and enhanced cell viability through the SDF-1/CXCR4 signaling pathway	Rat H9c2 cardiomyocytes	in vitro	2020	10.12659/MSM.919601
miR-1226-3p	reduced AQP5 expression leading inhibition of breast cancer cell migration	Human breast cancer MDA-MB-231 cells	in vivo	2019	https://doi.org/10.1096/fj.201902434R
miR-1290	reduced expression of vimentin gene. Downregulation of vimentin increased influenza A viral polymerase activity	NSCLC	in vitro	2019	https://doi.org/10.1016/j.omtn.2019.04.028
miR-1304-5p	circ-FOXM1/miR-1304-5p/PPDPF/MACC1 signaling was essential for the development and progression of NSCLC	NSCLC	in vitro	2019	https://doi.org/10.1016/j.bbrc.2019.03.213
miR-3065-3p	may repress MDGA1 and repress cell adhesion, being relevant to airway epithelial homeostasis in asthma	Primary normal human bronchial epithelial cells (NHBE) and asthmatic bronchial epithelial cells (DHBE)	in vitro	2017	10.18632/oncotarget.19752
miR-3613-3p	downregulate CMPK1 and in turn reduces IFN-α and IFN-β	Samples from chronic hepatitis B patients and Hepatoma cancer cell lines	in vivo/ in vitro	2019	https://doi.org/10.1016/j.meegid.2019.103919
miR-4718	downregulation induced cell proliferation and prevented apoptosis in vitro	Hepatoma cancer cell lines (Huh7 and HepG2)	in vivo/ in vitro	2019	https://doi.org/10.3390/biomedicines7040087
miR-5582-5p	overexpression inhibited the proliferation and migration, meanwhile promoted apoptosis in NSCLC cells	NSCLC	in vitro	2019	PMCID: PMC6945143
miR-5692a	was overexpressed in hepatocellular carcinoma(HCC) and associated with regulation of MMP9, promoting increased proliferation and invasion while decreasing apoptosis	HCC patients and cell lines	in vivo/ in vitro	2018	10.26355/eurrev_201808_15623.

upregulates miR-1290 to disrupt vimentin expression and retain virus ribonucleoprotein in the nucleus, thereby enhancing viral polymerase activity and viral replication (Huang et al., 2019).

The differentially expressed (DE) miRNAs exhibit promising potential for COVID-19 screening. MiRNAs levels can be monitored at different stages during the progression of the infection on severely ill patients. DE miRNA between early stages of infection and late stages could potentially be used to aid anticipate prognosis. Thus, these small RNA molecules may function as favorable clinical biomarkers for distinguishing the different clinical progressions of COVID-19, treatment strategy selection, and outcomes. For example, SARS-CoV-2 infection leads to fast activation of innate immune cells and change in the levels of many pro-inflammatory effector cytokines, such as TNF, IL-1β, IL-6, IL-8, G-CSF and GM-CSF, as well as chemokines, such as MCP1, IP10 and MIP1α, that are elevated in patients with COVID-19 (Huang et al., 2020). The deregulated expression of some miRNAs can modulate translation of transcripts occasioning in a decrease in the levels of immunomodulating factors that can inhibit or originate the inflammatory response, thus acting as molecular brake to regulate inflammation (Boldin et al., 2011). The expression levels of these miRNAs may offer promising diagnostic value and severity prediction of inflammatory response for COVID-19.

The role of cellular miRNAs is crucial when they are proviral, or when a longer, persistent infection is established. A fantastic example of use microRNAs is a significant reduction in virus titres (> 300-fold) without no sign of a rebound in viral titres, even after discontinuation of treatments in the Hepatitis C virus infection (Rupaimoole and Slack, 2017). The miRNA-based therapeutics for Hepatitis C virus infection is performed using a antimiR (AntimiR-122), which had designed to bind directly to the mature strand of the targeted miRNA-122 and thus to induce a functional blockade. The miR-122 upregulates the replication of the Hepatitis C virus RNA genome, having a key role in promoting viral RNA stability (Jopling, 2005). miRNAs can be used as an entry gate into regulatory networks that could be explored to find new unconventional therapeutic targets. Moreover, the use of antisense oligonucleotides to block viral genome, can be the most straightforward and easy to implement way toward new drugs therapy. For example, in one study researchers developed four artificial microRNAs (amiRNAs) that were designed to target different regions of Chikungunya virus (CHIKV) genome. These amiRNAs significantly inhibited CHIKV replication significantly (up to 99.8%) (Saha et al., 2016). In medicine, miRNAs have been revealed as novel, highly promising biomarkers and as attractive tools and targets for novel therapeutic approaches. As candidate miRNAs begin to proceed toward clinical trials in virology field, the landscape of both diagnostic and interventional medicine will arguably continue to evolve.

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.

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