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Therapeutic potential of miRNAs targeting SARS-CoV-2 host cell receptor ACE2

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ABSTRACT

In late December 2019, several cases of pneumonia of unknown etiology (COVID-19) were reported in Wuhan, Hubei province, China. Based on clinical findings, blood tests and chest radiographs, this disease was diagnosed as a virus-associated pneumonia. Sequence analysis revealed a novel coronavirus, called SARS-CoV-2 (formerly called 2019-nCoV), as the causative agent of pneumonia of unknown etiology. So far, the SARS-CoV-2 infection continues to spread, and this virus poses a serious public health threat. In this study, it was aimed to reveal potential miRNA targets for the regulation of SARS-CoV-2 host cell receptor ACE2. For the identification of potential miRNA targets for the ACE2 gene, TarBase v.8 (DIANA Tools), TargetScan, miRTarBase and miRDB miRNA-target prediction algorithms were used. FANTOM5 CAGE was used for the cellular ontology analysis. Expression levels of these miRNAs were determined using OncomiR Pan-Cancer miRNome Atlas. The results suggest that members of miR-200 family of miRNAs, especially miR-200c-3p, are strong candidate targets for the regulation of ACE2 in respiratory system cells. Consequently, the present study for the first time emphasizes potential use of miRNA-based therapeutics in the battle against SARS-CoV-2 infection and its deadly disease, COVID-19.

1. Introduction

Since the emergence of the novel coronavirus disease in December 2019 in Wuhan, China (Chen and Wang, 2020), the situation has rapidly evolved to a global health problem. The outbreak was announced as a Public Health Emergency of International Concern (PHEIC) on January 30, 2020 and declared as a pandemic on March 11, 2020 by World Health Organization (WHO) (https://www.who.int/news-room/detail/27-04-2020-who-timeline—covid-19). Corona Virus Disease-19 (COVID-19), the illness caused by the SARS-CoV-2 (formerly called 2019-novel coronavirus (2019-nCoV)) infection, is out of control worldwide (Bostancikliogl u, 2020). Although a significant number of individuals infected with the SARS-CoV-2 remain asymptomatic and unrecognized, infected patients typically develop pneumonia which may lead to severe complications such as acute respiratory distress syndrome and multiple organ failure. So far, significant number of adults and children were affected from this disease and pandemic continues to spread widely and rapidly across the world (Rathore et al., 2020; Sacco et al., 2020). As of October 27, 2020, a total of 43,561,060 confirmed cases of COVID-19 and 1,160,389 deaths have been reported globally (https://coronavirus.jhu.edu/map.html). Elderly people and individuals with co-existing respiratory or cardiovascular manifestations appear to be at higher risk of developing severe complications of COVID-19. At present, only supportive care such as oxygen therapy and treatment with antibiotics, is provided to the patients. Some off-label therapies such as certain licensed anti-viral drugs, anti-inflammatory and anti-parasitic agents, and convalescent plasma, are currently used to manage the disease. However, currently, there is no approved effective medication or vaccine available for the treatment of disease caused by the SARS-CoV-2 infection because of lack of evidence. Due to the lack of specific antiviral treatments and insufficient clinical treatments available, virus-related death rates are increasing worldwide.

Moreover, microRNAs (miRNAs) are small regulatory RNA molecules with an average length of 22 nucleotides and do not code for proteins, instead, code for functional RNA molecules for regulatory purposes and control gene expression at post-transcriptional level (Ambros, 2004; Bartel, 2018). Accumulating evidence suggest that miRNAs play chief roles in the regulating of vital cellular processes such as growth, proliferation, differentiation and apoptosis. Deregulation of microRNA expression has been shown to significantly contribute to the

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development and progression of many diseases, especially cancer, diabetes, immune system diseases and neurodegenerative diseases.

Due to the wide range of biological functions of miRNAs in various types of disease, manipulation of microRNA regulation, either by synthetic miRNA mimics or inhibitors, pose a strong therapeutic approach for the management of human diseases. Several miRNA-based targeted therapeutics have been introduced into clinical trials. For instance; clinical studies of a mimic of miR-34 (MRX34) for the treatment of HCC, NSCLC and pancreatic cancer were initiated. More importantly, antimiR-122 (miravirsen) has reached phase II trials for the treatment of Hepatitis C infections. Given that miravirsen is a promising miRNA-based therapeutic against hepatitis C virus (HCV) infection, identifying the biology and relevance of the miRNA candidates targeting SARS-CoV-2 host cell receptor ACE2 is of great interest to develop efficient anti-viral agents for the eradication of ongoing SARS-CoV-2 pandemic. Accordingly, in the present study, it was aimed to reveal potential miRNA targets for the regulation of SARS-CoV-2 host cell receptor ACE2.

2. Materials and methods

2.1. Identification of miRNAs targeting ACE2 using target prediction algorithms

TarBase v.8 (DIANA Tools)(Karagkouni et al., 2018), TargetScan (Agarwal et al., 2015), miRTarBase(Chou et al., 2018) and miRDB(Chen and Wang, 2020) miRNA-target prediction algorithms were used to predict potential miRNAs targeting ACE2 (Angiotensin I converting enzyme 2) gene. In screening of miRNAs targeting ACE2 gene, either gene symbol “ACE2”, Ensembl ID or 3’UTR sequence were used.

2.2. Determination of validated miRNA targets of ACE2

For the determination of validated miRNA targets of ACE2, a literature search with the keywords “ACE2”, “miRNA”, “microRNA”, “Angiotensin-converting enzyme 2” in PubMed, Google Scholar, Sciencedirect platforms were performed. Publications until 20 May 2020 were evaluated and miRNAs involved in the direct regulation of ACE2 were listed (Table 1).

2.3. Data acquisition and analysis

Expression of miRNAs, either experimentally validated or predicted to target ACE2, were analyzed using Functional Annotation of The Mammalian Genome Cap Analysis of Gene Expression (FANTOM5 CAGE) public database(Consortium, 2014; Lizio et al., 2019). Cellular ontology findings of miRNAs and lung fibroblast, respiratory epithelial cell were obtained and bronchial epithelial cell data were extracted. These miRNAs were then scanned in the OncomiR (http://www.oncomir.org/) database. Expression levels of these miRNAs in lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) tissues were analyzed in the OncomiR Pan-Cancer miRNome Atlas (Wong et al., 2018). The expression level of 4 of the 5 miRNAs associated with ACE2 were available.

3. Results

To identify, miRNAs targeting host cell receptor ACE2, miRNA target prediction algorithms were used and several miRNAs targeting ACE2 receptor were identified. miRNAs have previously shown to be involved in the regulation of ACE2 receptor, but not predicted to target ACE2 in these databases, were also identified from previous experimental evidences. A total of 24 miRNAs (miR-1208, miR-4318, miR-4314, miR-302c, miR-329, miR-501, miR-655, miR-584, miR-574, miR-383, miR-125b, miR-1915, miR-513b, miR-1303, miR-760, miR-3934, miR-136, miR-218, miR-1251, miR-500a, miR-500b, miR-200a, miR-200b, and miR-200c). miR-200b and miR-200c were found to be highly conserved (Fig. 1A). Binding positions of miR-200c-3p and miR-200b-3p with ACE2 was also presented using the TargetScan algorithm (Fig. 1B). Furthermore, cellular ontologies of these miRNAs were also evaluated. miR-200a-3p, miR-200b-3p, miR-200c-3p, miR-210-3p and miR-429 were found to be highly enriched in respiratory epithelial cells (Supp. Fig. 1). In addition, cellular ontologies of these miRNAs in the other body cells were also presented in Table 2. The expression levels of these miRNAs using OncomiR algorithm in Lung adenocarcinoma (LUAD) and Lung squamous cell carcinoma (LUSC) samples were also analyzed (Supp. Fig. 2). Also, using target correlation interface, miRNAs potentially target ACE2 were determined. It was found that miR-200c-3p is the only target miRNA that regulate ACE2 in cancer cells. Consistently, a significant negative correlation was identified between ACE2 and miR-200c-3p (Correlation coefficient: −0.2910, $p = 7.86e\text{-}26$) in all types of cancer. Also, correlation false discovery rate (FDR) was highly low in all types of cancers (correlation FDR = 1.15e-24).

Table 1
Experimentally validated miRNA targets of ACE2.

miRNAs	Disease/tissue /cell line	Reference
miR-200c-3p	Acute respiratory distress syndrome	Liu et al. (2017)
Let-7b	Hypoxic pulmonary hypertension	Zhang et al. (2019)
miR-18a	Hypoxia/Reoxygenation-induced injury	Zhang et al. (2018)
miR-30e	Doxorubicin-induced heart failure	Lai et al. (2017)
miRNAs-17, -574-5p, and -214, miR-223 and miR-98	SARS infection in bronchoalveolar stem cells	Mallick et al. (2009)
miR-1246	Acute lung injury	Fang et al. (2017)
miR-181a, miR-378, miR-483	Trophoblast proliferation	Arthurs et al. (2019)
miR-421	Chronic kidney disease	Trojanowicz et al. (2019)
miR-29	Hypertrophic cardiomyopathy	Liu et al. (2019)
miR-200c-3p	Acute respiratory distress syndrome rat models	Li et al. (2018)
miR-21	Lung fibroblasts	Sun et al. (2017)
miR-421	Primary cultures of cardiac fibroblasts	Lambert et al. (2014)
miR-125b	Renal tubular epithelial cells	Huang et al. (2016)
miR-4262	Acute lung injury	Bao et al. (2015)
miR-429, miR-27a	Antenatal maternal hypoxia in mice	Goyal et al. (2015)
miR-143	Aerobic exercise training	Fernandes et al. (2011)

4. Discussion

One of the first antisense oligonucleotide to enter clinical trials was the locked nucleic acid (LNA-)–modified oligonucleotide complementary to 5′ end of miR-122 (miravirsen) for the treatment of HCV. Recently, a phase IIa clinical trial was initiated for the examination of long-term efficacy and safety of miravirsen in patients with chronic HCV genotype 1 infection (Rupaimoole and Slack, 2017). Unlike widely known functions of miRNAs in gene silencing, miR-122 advances the HCV replication thorough binding 5′ non-coding regions of the HCV RNA genome (Jopling et al., 2005). Binding of the miR-122 to 5′ non-coding regions of the HCV viral genome increases its stability in which viral RNA genome acts as a miRNA sponge for miR-122, leading to a reduction in miR-122 abundance at the site of infection and deregulation of liver homeostasis. Studies showed binding of miR-122 serves as a 5′ cap and protect viral RNA genome against Xrn1 exoribonuclease -mediated cleavage (Thibault et al., 2015).

In the light of the miR-122 story in HCV replication, we would also benefit miRNA therapeutics targeting SARS-CoV-2 host cell receptor

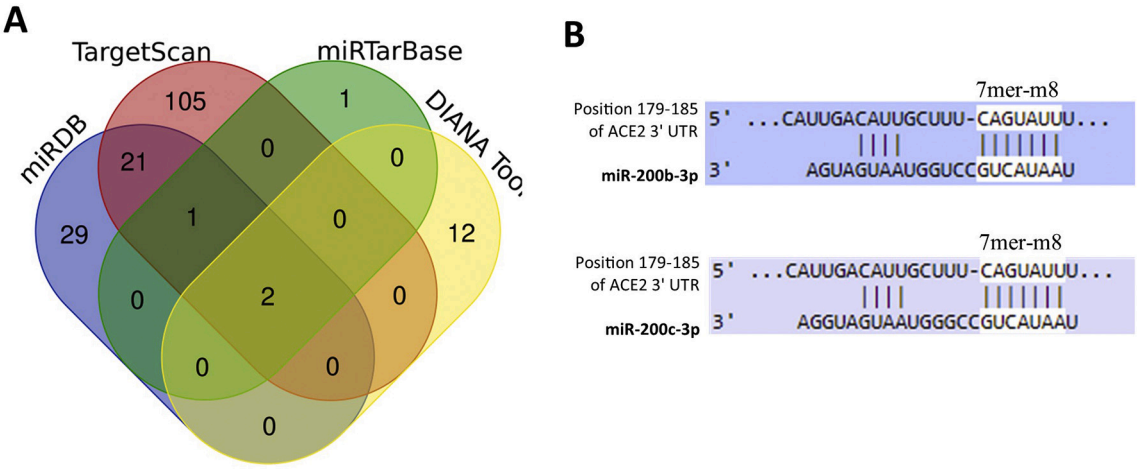

Fig. 1. Prediction of miRNAs targeting ACE2 using TarBase v.8, TargetScan, miRTarBase and miRDB. A. Venn diagram of miRNAs targeting ACE2 B. Complementary binding positions of highly conserved miR-200b-3p and miR-200c-3p with ACE2.

Table 2
Cell ontologies of miRNAs targeting ACE2 in the FANTOM5 CAGE database.

miRNAs	Cell ontology	*p* value	Enriched/Depleted
miR-200c-3p	Endo-epithelial cell	1.248e-22	Enriched
	Epithelial cell	3.316e-16	Enriched
	Respiratory epithelial cell	2.36e-13	Enriched
	Leukocyte	8.47e-35	Depleted
	Hematopoietic cell	3.39e-28	Depleted
	Myeloid leukocyte	1.52e-18	Depleted
miR-200b-3p	Epithelial cell	2.053e-38	Enriched
	Endo-epithelial cell	5.82e-24	Enriched
	Respiratory epithelial cell	8.94e-14	Enriched
	Hematopoietic cell	3.17e-23	Depleted
	Leukocyte	4.58e-23	Depleted
	Epithelial cell of vascular tree	1.17e-16	Depleted
miR-429	Endo-epithelial cell	4.033e-23	Enriched
	Epithelial cell	1.44e-21	Enriched
	Respiratory epithelial cell	6.22e-17	Enriched
	Leukocyte	5.14e-23	Depleted
	Hematopoietic cell	5.70e-24	Depleted
	Myeloid leukocyte	2.36e-12	Depleted
miR-200a-3p	Endo-epithelial cell	5.39e-26	Enriched
	Epithelial cell	1.72e-23	Enriched
	Respiratory epithelial cell	4.40e-15	Enriched
	Leukocyte	1.50e-18	Depleted
	Hematopoietic cell	3.69e-18	Depleted
	Myeloid leukocyte	2.11e-12	Depleted
miR-210-3p	Endo-epithelial cell	1.938e-11	Enriched
	Respiratory epithelial cell	6.207e-10	Enriched
	Endodermal cell	3.425e-8	Enriched
	Neuroectodermal cell	6.858e-8	Depleted
	Leukocyte	2.159e-7	Depleted
	Hematopoietic cell	3.552e-7	Depleted

ACE2. Herein, it was demonstrated that members of miR-200 family of miRNAs, especially miR-200c-3p, are strong candidate targets for the regulation of ACE2 in respiratory system cells. It was also identified that several other miRNA targets for the post-transcriptional regulation of ACE2 in cells. Our results suggest that human encoded miRNAs could be the finest candidates for development of miRNA-based therapeutics in the management of SARS-CoV-2 infection. Studies can also focus on the miRNAs directly targeting genomic RNA of SARS-CoV-2. More recently, it has been suggested that SARS-CoV-2 genome can be targeted by combination of several miRNAs and these miRNAs can be delivered using more suitable patient-friendly, high-efficacy delivery systems such as polymer-based carriers, liposome-like exosomes, or inorganic nanoparticles (El-Nabi et al., 2020). Although it is difficult to make a definitive judgment with the present data, it is evident that findings obtained in the present study will guide future studies in the fight against SARS-CoV-2 infection and enable identification of miRNA targets for antiviral intervention.

5. Conclusions, future perspectives and limitations

As the pandemic continues to spread, identification and developing drug targets with high-efficacy targeting SARS-CoV-2 infection is of great interest. This deadly disease is currently responsible from the death of more than a million people. In the present study, using a bioinformatic approach, several members of the miR-200 family of miRNAs were identified to be good candidates for targeting SARS-CoV-2 host cell receptor ACE2. However, validation of these results by wet-lab approaches is one of the main limitation of the current study. Therefore, future studies should focus on the role of these miRNAs in SARS-CoV-2 infection. miRNAs expressed ubiquitously across species and play significant roles in the development and perform diverse regulatory functions in cells including viral infections. Accordingly, more research is need to identify eligible miRNA targets for SARS-CoV-2 infection. Considering the fact that this virus affects various organ systems from gastrointestinal system to central nervous system, drug targets in development should be more advantageous compared currently available treatment options.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mgene.2020.100831.

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