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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 (Bostanciklioglu, 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, anti-miR-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), miTarBase (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, Scicenedirect 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 (FANTOMS 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-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).

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 (Rupainoole 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 ACE2.
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 have significant roles in the development and perform diverse regulatory functions in cells including viral infections. Accordingly, more research is needed to identify eligible miRNA targets for 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 needed to identify eligible miRNA targets for SARS-CoV-2 infection.

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### Table 2

| miRNAs     | Cell ontology                        | p value       | Enriched/Depleted |
|------------|--------------------------------------|---------------|-------------------|
| miR-200c-3p| Endo-epithelial cell                 | 1.248e-22     | Enriched          |
|            | Epithelial cell                      | 3.31e-16      | Enriched          |
|            | Respiratory epithelial cell          | 2.36e-13      | Enriched          |
|            | Leukocyte                            | 8.47e-35      | Depleted          |
|            | Hematopoietic cell                   | 3.99e-28      | Depleted          |
|            | Myeloid leucocyte                    | 1.52e-18      | Depleted          |
| miR-200b-3p| Endo-epithelial cell                 | 2.053e-38     | Enriched          |
|            | Epithelial cell                      | 5.82e-24      | Enriched          |
|            | Respiratory epithelial cell          | 8.94e-14      | Enriched          |
|            | Hematopoietic cell                   | 3.17e-23      | Depleted          |
|            | Leukocyte                            | 4.58e-23      | Depleted          |
| miR-429    | Epithelial cell of vascular tree     | 1.17e-16      | Depleted          |
|            | Endo-epithelial cell                 | 4.033e-23     | Depleted          |
|            | 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 leucocyte                    | 2.38e-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                   | 3.09e-18      | Depleted          |
|            | Myeloid leucocyte                    | 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          |

### Appendix A. Supplementary data

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

### Declaration of Competing Interest

The authors declare that there are no conflicts of interest.
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