Hsa-miR-4520-2-3p: A Potential Modulator of COVID-related ACE2

Molly Ruggles and Nipun Chopra, Ph.D.
Department of Biology, DePauw University, Greencastle, IN

Abstract

Angiotensin-Converting Enzyme (ACE2) is a transmembrane enzyme located in cells in various tissues around the body. Its normal role is the conversion of Angiotensin II to Angiotensin 1-7 leading to vasodilation and a subsequent reduction in blood pressure via the renin-angiotensin-aldosterone system. ACE2 also plays a pivotal role in the infection of COVID-19 as it determines entry of virus into human cells. SARS-CoV-2 uses one of its four structural proteins, the spike (S) glycoprotein, to bind to the ACE2 receptor. This entry into the cell begins the process of infection and spread of the disease; because of the abundance of ACE2 throughout the body, coronaviruses can enter several different organs. This multi-cell-entry via ACE2 is why COVID-19 affects multiple organ systems in the human body. Currently, therapies targeting ACE2 expression are limited. MicroRNA (miRNA)s are short non-coding RNA that downregulate the expression of a variety of proteins, and therefore offer a mechanism by which to inhibit the expression of ACE2. MiRNAs can potentially downregulate ACE2 protein synthesis by binding to the mRNA of ACE2 at a seed sequence. This binding results in mRNA degradation or inhibition of translation. Thus, our goal was to identify miRNA that may potentially target and downregulate ACE2. TargetScan, DIANA-MicroT, and PicTar are three algorithms that predict miRNAs that can bind to the mRNA of ACE2 and downregulate its expression. Together, the prediction tools resulted in 57 shared human miRNAs that target ACE2. We then utilized self-defined parameters to narrow down our list and identified hsa-miR-4520-2-3p as our predicted miRNA to target ACE2. This miRNA will be experimentally verified in the future.

Introduction

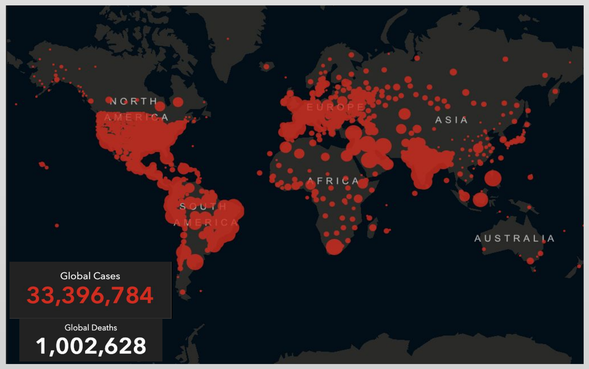

Figure 1. On March 12th, 2020, only three months after the first cases of the coronavirus disease 2019 (COVID19)--caused by SARS-CoV-2--were discovered in Wuhan, China, the disease was declared a pandemic by the WHO. As of September 29th, there have been over 33.3 million confirmed cases of COVID-19 and about 1 million deaths across 188 countries, according to John Hopkins University.

Evidence suggests SARS-CoV-2 originated from bats, and it can be transmitted by human-to-human contact, through aerosols or large respiratory droplets. People being affected by SARS-CoV-2 may be experiencing a range of symptoms including fever, cough, myalgia, shortness of breath, hypoxemia, and acute respiratory distress syndrome. However, people who are positive for COVID-19 may also be asymptomatic, which makes viral spread more effective. Incubation period of SARS-CoV-2 is between 2-14 days.

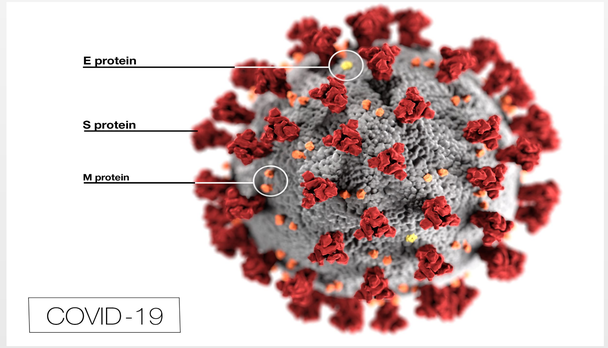

Figure 2. SARS-CoV-2 has a diameter of 150 nanometers and is made up of four structural proteins. One of those four proteins, the spike (S) glycoprotein on SARS-CoV-2 binds to the ACE2 protein. The virus then enters the cell via endocytosis. This image is from the CDC.

The main role of ACE2 is the conversion of Angiotensin II to Angiotensin 1-7, which opposes the actions of Angiotensin II. Angiotensin II is a peptide hormone that causes vasoconstriction, increased blood pressure, and sodium retention. Angiotensin 1-7 is a peptide, with one less amino acid than Angiotensin II, that causes vasodilation and decreased blood pressure. Thus, the normal function of ACE2 is important to maintain homeostasis in the human body. ACE2 is prevalent in many tissues throughout the body, including the lungs, esophagus, stratified epithelial cells, parts of the large intestine, and the brain; in fact ACE2 is capable or reducing levels of β-amyloid (Aβ) – a protein involved in the pathogenesis of Alzheimer's disease (AD). The expression of ACE2 throughout the body is what can lead to multi-tissue infection when binding with SARS-CoV-2.

MicroRNAs (miRNA) offer a novel approach for ACE2 inhibition. MiRNAs are ~22 nucleotide, single-stranded, non-coding RNAs that regulate gene expression by binding to the messenger RNA (mRNA) of a protein at the miRNA seed sequence (Agarwal 2015). A seed sequence is a region of 2-8 nucleotides on the miRNA that is complementary to the within the 3' untranslated region (UTR) within the mRNA. After miRNA binds to mRNA, the RNA-induced silencing complex (RISC) interferes with translation of ACE2 mRNA, resulting in either mRNA degradation or inhibition of ACE2 translation. Interference of translation allows miRNAs to downregulate ACE2 protein expression in order to decrease the number of opportunities available for the virus to infect a cell.

Materials & Methods

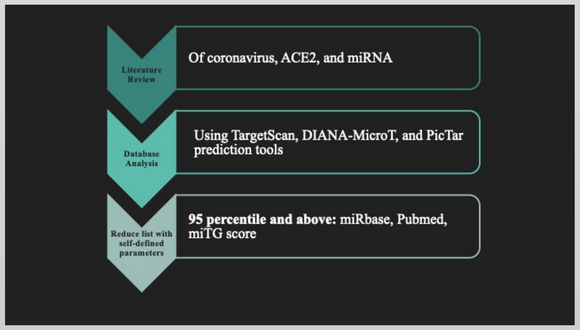

Figure 3. Workflow for research project.

Results

Separately, TargetScan, DIANA-MicroT, and PicTar algorithms resulted in 281, 60, and one miRNA(s) predicted to target the ACE2 protein, respectively. 57 miRNAs were shared across the algorithms as potential targets for ACE2. 56 of the predicted miRNAs were shared among TargetScan and DIANA-MicroT, and one miRNA, miR-200c-3p was shared among all 3 algorithms.

MiRNA Ranked by Context++ Score Percentile

miRNA	context++ score	context++ score percentile	Pct	miTG score	PicTar score	Probabilities	Free Energies (kcal/mol)
hsa-miR-362-5p	-0.39	99	N/A	0.9143045131			
hsa-miR-500b-5p	-0.39	99	N/A	0.8737869004			
hsa-miR-1305	-0.23	99	N/A	0.8731140447			
hsa-miR-4520-2-3p	-0.47	98	N/A	0.9298492547			
hsa-miR-6790-5p	-0.34	98	N/A	0.7320984971			
hsa-miR-4658	-0.31	98	N/A	0.708402343			
hsa-miR-3529-3p	-0.28	98	N/A	0.9225692671			
hsa-miR-7850-5p	-0.16	97	N/A	0.9095734643			
hsa-miR-4693-5p	-0.32	97	N/A	0.7645048533			
hsa-miR-4786-5p	-0.31	97	N/A	0.706960058			
hsa-miR-421	-0.29	97	N/A	0.8678968541			
hsa-miR-4270	-0.31	97	N/A	0.9001629924			
hsa-miR-200c-3p	-0.23	96	0.4	0.8111477856	2.32	0.89	-19.9
hsa-miR-200b-3p	-0.23	96	0.4	0.8078639157			
hsa-miR-632	-0.22	96	N/A	0.9236769912			
hsa-miR-3909	-0.38	96	N/A	0.8589104112			
hsa-miR-3684	-0.39	96	N/A	0.706732382			
hsa-miR-4773	-0.2	96	N/A	0.9600978585			
hsa-miR-6852-3p	-0.36	96	N/A	0.8220092235			
hsa-miR-345-3p	-0.18	95	N/A	0.8277265777			

Table 1. Above is a table showing a list of 20 miRNAs (narrowed down from the original 57) that are predicted to target ACE2. The miRNAs are ranked from the highest context++ score percentile to lowest. The table shows the specific scores assigned to these miRNA from TargetScan, DIANA-MicroT, and PicTar, yellow, blue, and orange, respectively.

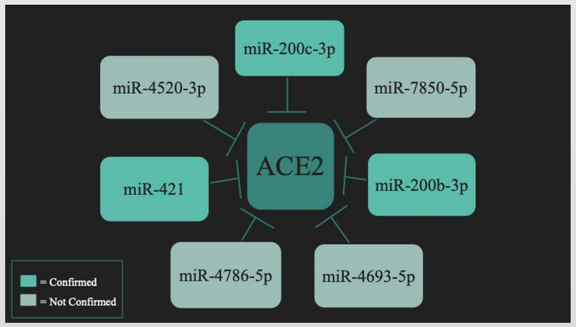

Figure 4. Filtered list of miRNA that potentially target ACE2. If there was experimental evidence on PubMed relating that a miRNA downregulated ACE2, then that miRNA was confirmed. If there were articles about ACE2 linked to the miRNA but no direct relationship, then that miRNA was unconfirmed.

MiRNA Finalized by Parameters and Ranked by miTG Score

miRNA	context++ score	context++ score percentile	Pct	miTG score	PicTar score	Probabilities	Free Energies (kcal/mol)
hsa-miR-4520-2-3p	-0.47	98	N/A	0.9298492547			
hsa-miR-7850-5p	-0.16	97	N/A	0.9095734643			
hsa-miR-4693-5p	-0.32	97	N/A	0.7645048533			
hsa-miR-4786-5p	-0.31	97	N/A	0.706960058			
hsa-miR-421	-0.29	97	N/A	0.8678968541			
hsa-miR-200c-3p	-0.23	96	0.4	0.8111477856	2.32	0.89	-19.9
hsa-miR-200b-3p	-0.23	96	0.4	0.8078639157			
hsa-miR-632	-0.22	96	N/A	0.9236769912			
hsa-miR-362-5p	-0.39	99	N/A	0.9143045131			
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hsa-miR-3529-3p	-0.28	98	N/A	0.9225692671			
hsa-miR-6852-3p	-0.36	96	N/A	0.8220092235			

Table 2. List of 20 miRNAs (narrowed down from the original 57) that are predicted to target ACE2. The varying shades of red indicate when a group of miRNAs are eliminated from being the sole miRNA (determined by the self-defined parameters) to target ACE2. The darkest red indicates the first level of elimination, while the lightest red indicates the last level of elimination. The miRNAs are ranked from the highest miTG score to the lowest across each group. The miRNA highlighted in green is the determined miRNA that targets ACE2.

Conclusion

Based on our *in silico* methodology, hsa-miR-4520-2-3p was narrowed as a potential miRNA targeting ACE2. This finding was based on a complete literature review, production of a list of miRNAs that target ACE2 by the three algorithms TargetScan, DIANA-MicroT, and PicTar, and self-defined parameters that further narrowed down the list of miRNAs predicted to target ACE2.

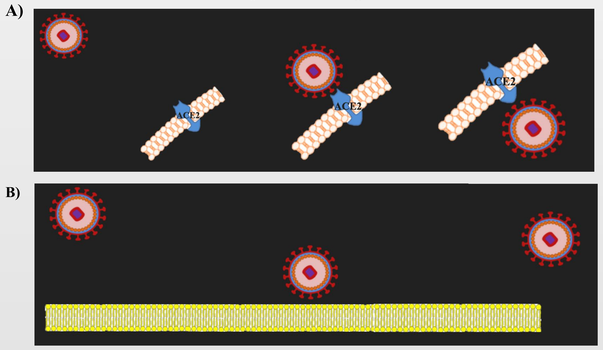

Figure 5. A) SARS-CoV-2 utilizes S-protein binding to ACE2 protein to facilitate entry into the cell in absence of miRNA. B) Presence of miRNA eliminates ACE2, preventing SARS-CoV-2 from gaining entry into the cell.

However, finding a miRNA that can suppress ACE2 is quite difficult. This is due to the reason that each miRNA is not exclusively bound to a specific mRNA. Thus, while the results were consistent with each other and allowed for the finding of one miRNA that could potentially target ACE2 in an experimental setting, hsa-miR-4520-2-3p could be a false positive when experimentally tested.

Other limitations within this research were:

- The algorithms used limited results of the study, resulting in the possibility of both Type I and Type II errors.
- The parameters defined by the researchers within this experiment were limiting
- The composition of miRNA has a short target sequence, hindering easy transfection of miRNA into cells for treatment of SARS-CoV-2

Future Research

Experimental research with the determined hsa-miR-4520-2-3p in order to determine if the miRNA does indeed target ACE2. Results of this future experiment could support or eliminate this miRNA as a potential regulator of ACE2.

References

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