Spike protein amino acid variants increased miRNA binding and hence reduced virulence of SARS-CoV-2 in Jordan, Middle East

Hazem Haddad (hazem_haddad1981@just.edu.jo)
Princess Haya Biotechnology Centre, Jordan University of Science and Technology, Irbid, Jordan

Ramzi Foudeh
Jordanian Society of Genetic Engineers (JSGE), Amman, Jordan

Walid Al-Zyoud
Department of Biomedical Engineering, School of Applied Medical Sciences, German Jordanian University, Amman, Jordan

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Abstract

Initial epidemiological studies inform the central COVID-19 disease prophet. Some papers have been studying miRNA and viral RNA interaction target predictions over the past few years. In this work, via the mirDB database, we determined the target scores of predicted miRNA to bind with the ss-RNA of SARS-CoV-2 in general and spike gene in specific. Our predicted miRNA targets of the ss-RNA of SARS-CoV-2 might destabilize and hence inhibit the ss-RNA translation of SARS-CoV-2 and prevent viral replication that has been established by more than 80% of asymptomatic infected cases in Jordan due to host miRNA interactions. In respiratory epithelial cells, the high scoring miRNAs prediction covers the RNA from 5' to 3' that explain successful antiviral defenses against ss-RNA of SARS-CoV-2 and lead to new nucleotide deletion mechanisms. The exciting finding here that the substitution 1841A>G at the viral genomic RNA level or the D614G at spike protein level showed a change in the predicted miRNA sequence and an increase in the target score (from 91 to 92) (hsa-miR-4793-5p to hsa-miR-3620-3p).

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused an outbreak in Wuhan city and characterized as a pandemic by the World Health Organization WHO [1]. The first case of SARS-CoV-2 was reported to the Jordanian Ministry of Health on March 2, 2020, for a Jordanian citizen who was in Italy. To the date of this report, there are 522 confirmed cases, 387 recovered and nine deaths of COVID-19 in Jordan, according to the Jordanian Ministry of Health official via its web site launched as a unified source of information about the preventive measurements and symptoms of corona virus-2019 (https://corona.moh.gov.jo/en).

In addition, 18 years ago, severe coronavirus acute breathing syndrome (SARS-CoV) appeared in China. It has spread to over 30 countries, infecting around 8,000 people, killing young people 10%, and aging 50%. No SARS CoV or other up-and-coming CoV vaccines or antivirals such as Middle East respiratory CoV (MERS-CoV) are approved to date [2]. The molecular mechanisms of viral pathogenesis will provide thoughtful help in the search for effective and secure therapeutic strategies against new and well-known human CoVs.

MicroRNAs (miRNAs) are non-coding RNAs that control many function targets within a cell by controlling protein levels through binding to mRNA translation process or mRNA abundance. Many evidence shows that miRNAs contain the RNA virus replication and pathogenesis through direct binding to the RNA virus-mediated and changes in the host transcriptome. Proof of host miRNAs can bind to a wide-ranging of RNA viruses, straight adaptable their pathogenesis through mimics’ cellular miRNAs tolerating direct binding of the miRNA to the viral RNA. Theoretically, the regulation is analogous to that of host miRNAs [3,4].

Many miRNA targeted in Influenza viral RNA segments were linked with the activity of host miRNA-induced antiviral defense. This represents potential treatment with a combination of the five miRNAs through agomir delivery to suppressed viral replication and effectively improved protection against lethal challenge with PR8 in mice [5].

In previous study, severe acute coronavirus syndrome (SARS-CoV) causes human fatal disease and reaction and extensive pulmonary disease. The significance of small non-coding RNAs for SARS-CoV pathologies, sequenced lung RNAs of infected mouse, and three 18 to 22nt small viral RNAs (SvRNAs), were discovered. The three svRNAs originated from the SARS-CoV genomic regions nsp3 (svRNA-nsp3.1 and -nsp3.2), and N (svRNA-N). CoV svRNAs were autonomous, cell type and host species from RNase III; but the extent of viral replication was dependent. In vivo lung pathology and pro-inflammatory cytokine release, antagonim-mediated inhibition of svRNA-N significantly decreased. This indicates that SvRNAs contribute to the pathogenesis of SARS-CoV and demonstrates the potential for antagonists of svRNA-N as antivirals [6].

To understand the early steps of COVID-19 infection, we predicted miRNAs targeting the submitted 29903 bp ss-RNA of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 sequence), the isolate of Wuhan-Hu-1, complete genome. A predicted miRNAs targeting region at 3822 bp ss-RNA of the spike glycoprotein of SARS-CoV-2 was revealed. Also, we predicted miRNAs targeting a variable region of the ss-RNA spike glycoprotein of SARS-CoV-2 sequences from 20 positive nasopharyngeal specimens. These specimens were collected in Jordan and sequenced by Biolab Diagnostic Laboratories (Jordan) & Andersen lab at Scripps Research (USA), who deposited the sequences in GISAID, a maintained global database based in Germany. The perception in this work might help scientists to understand the molecular mechanisms of viral pathogenesis. In addition, it might support the research for effective, safe therapeutic strategies against known human CoVs and new emergent strains with a focus on miRNA-induced antiviral human body defense, which could be a potential treatment development for SARS-CoV-2.

Methods

The miRDB is an online http://mirdb.org/custom.html database for the target and working annotations of miRNA. All targets in miRDB were anticipated by MirTarget, a bioinformatics tool that was developed through the study of thousands of miRNA target interactions from high-performance research [7,8]. Using http://biomodel.uah.es/en/lab/cybertory/analysis/trans.htm to convert sequences from miRNA to DNA to demonstrate genome sequence alignment using ChromePros Version 2.1.9. Wuhan-Hu-1, full NC 045512.2 accession genome sequence number and MRNAs targeting area 3822 bp ss-RNA spike SARS-CoV-2 Spike. We predicted miRNA targets 29903 bp ss-RNA (SARS-CoV-2 sequence) isolate Wuhan-Hu-1. In addition, miRNAs are predicted to target region on the SARS-CoV-2 sequence Accession no YP 009724390 ss-RNA spike glycoprotein variability from Jordanian positive nasopharyngeal specimens sequenced by Biolab Diagnostic Laboratories (Jordan) & Andersen lab at Scripps Research (USA) who published sequences retrieved from GISAID (https://corona.moh.gov.jo/en) (https://gisaid.org).

Results And Discussion
We predicted top ten score miRNAs targeting between (98-99) the submitted 29903 bp ss-RNA SARS-CoV-2 genome, accession number NC_045512.2, correspondingly, hsa-miR-4288, hsa-miR-195-5p, hsa-miR-16-5p, hsa-miR-15b-5p, hsa-miR-15a-5p, hsa-miR-6838-5p, hsa-miR-497-5p, hsa-miR-424-5p, hsa-miR-3133, hsa-miR-21-3p Table 1. These miRNAs are presented in miRDB, along with associated function annotations. As a recent update, miRDB displays expressions of hundreds of cell lines, and the user may limit their search for the cell line of interest miRNA targets. miRDB offers an integrative analysis of the target prediction and gene ontological data found in Tables 2,3,4,5,6,7,8,9,10 and 11, to promote the prediction of miRNA functions. In addition, we predicted only three miRNAs (hsa-miR-510-3p, hsa-miR-624-5p, hsa-miR-497-5p) with targeting scores of (92, 90 and 84) respectively for the submitted 3822 bp of the ss-RNA spike glycoprotein of SARS-CoV-2 sequence of Wuhan-Hu-1 (from the complete genome NCBI reference sequence: NC_045512.2 Region: 21563-25384) as shown in Table 13. These miRNAs, as well as associated functional annotations, presented in miRDB presents the expression profiles of hundreds of cell lines. To facilitate the prediction of miRNA functions, miRDB offers for integrative analysis of target prediction and Gene Ontology data found in Tables 14 and 15.

Moreover, in our study here we predicted the score of miRNAs targeting regions on many ss-RNA spike glycoprotein of SARS-CoV-2 sequence from Jordanian samples with amino acid substitutions (NCBI Reference Sequence: NC_045512.2 region: 21563-25384 and Nomenclature sequence, Amino Acid Variant (SAV) and annotation used the accession number YP_009724390.1).

The original sequence of the del 432TTA, and the del Y144 have the same miRNA with a target score of 91. The original 1841A target miRNA score (91, 64 and 56) for (hsa-miR-4793-5p, hsa-miR-143-5p and hsa-miR-3133) respectively. The interesting finding here that the 1841A>G, and D614G showed a change in the predicted miRNA and an increase in the target score (from 91 to 92) (hsa-miR-4793-5p to hsa-miR-3620-3p). However, the original and 3415G>T D1139Y showed the same sequence of the miRNA (hsa-miR-548g-3p) and an increase in the target score of 80 to 81. The last substitution of 3499 G>A G1167S showed the same the miRNA sequence of (hsa-miR-155-5p) and a decrease in the target score from 73 to 72. One of the record genomic changes observed in the severe acute respiratory syndrome coronavirus (SARS-CoV-1) isolated from humans after transmission human to human was the acquisition of a specific 29-nucleotide deletion occurred in open reading frame 8 (ORF8). Three top target score of miRNAs prediction (hsa-miR-497-5p, hsa-miR-195-5p and hsa-miR-21-3p) showed on Table 12, 16 and 24 have an expression in the respiratory epithelial cells and effective antiviral defenses against the ss-RNA of SARS-CoV-2 and lead to new mechanisms interaction binding miRNA and SARS-CoV-2 of nucleotide deletion [5-6]& [9].

Conclusion

Over the past few years, some articles studied the target prediction of miRNA and viral RNA interaction. In our predictions, more than 80% asymptomatic of the disease raised due to the host miRNA interactions, which have identified the target where the genome replication of the ss-RNA of SARS-CoV-2 has changed to inhibit the translation of the ss-RNA and hence preventing viral replication and stabilization by subsequent generations. The top target score of miRNAs prediction cover from 5' to 3' in the respiratory epithelial cells that might be the reason for an effective antiviral defense against the ss-RNA of SARS-CoV-2 and lead to new mechanisms of nucleotide deletion in the coding region of a protein. The miRNAs found in all tissues have target gene functions, which lead to an identification of novel cellular pathways to block RNA viral replication or even host cell-specific targeting the regulation for the ss-RNA of SARS-CoV-2.

Tables

Table 1: Predicted miRNAs targeting the submitted 29903 bp ss-RNA SARS-CoV-2 genome, accession number NC_045512.2

| Target Rank | Target Score | miRNA Name     |
|-------------|--------------|----------------|
| 1           | 99           | hsa-miR-4288   |
| 2           | 99           | hsa-miR-195-5p |
| 3           | 99           | hsa-miR-16-5p  |
| 4           | 99           | hsa-miR-15b-5p |
| 5           | 99           | hsa-miR-15a-5p |
| 6           | 99           | hsa-miR-6838-5p|
| 7           | 98           | hsa-miR-497-5p |
| 8           | 98           | hsa-miR-424-5p |
| 9           | 98           | hsa-miR-3133   |
| 10          | 98           | hsa-miR-21-3p  |

Table 2: Predicted targets function for hsa-miR-4288 on 29903 bp ss-RNA SARS-CoV-2 genome
### Table 3: Predicted targets function for hsa-miR-195-5p on 29903 bp ss-RNA SARS-COV-2 genome

| Target Rank | Target Score | miRNA Name | Gene Symbol | Gene Description |
|-------------|--------------|------------|-------------|------------------|
| 1           | 100          | hsa-miR-195-5p | PAPPA       | pappalysin 1     |
| 2           | 100          | hsa-miR-195-5p | FASN        | fatty acid synthase |
| 3           | 100          | hsa-miR-195-5p | UNC80       | unc-80 homolog, NALCN channel complex subunit |
| 4           | 100          | hsa-miR-195-5p | FGF2        | fibroblast growth factor 2 |
| 5           | 100          | hsa-miR-195-5p | TNRC6B      | trinucleotide repeat containing 6B |
| 6           | 100          | hsa-miR-195-5p | PTPN4       | protein tyrosine phosphatase, non-receptor type 4 |
| 7           | 100          | hsa-miR-195-5p | PHF19       | PHD finger protein 19 |
| 8           | 100          | hsa-miR-195-5p | DESI1       | desumoylating isopeptidase 1 |
| 9           | 99           | hsa-miR-195-5p | UBE2Q1      | ubiquitin conjugating enzyme E2 Q1 |
| 10          | 99           | hsa-miR-195-5p | LSM11       | LSM11, U7 small nuclear RNA associated |

### Table 4: Predicted targets function for hsa-miR-16-5p on 29903 bp ss-RNA SARS-COV-2 genome

| Target Rank | Target Score | miRNA Name | Gene Symbol | Gene Description |
|-------------|--------------|------------|-------------|------------------|
| 1           | 100          | hsa-miR-16-5p | PAPPA       | pappalysin 1     |
| 2           | 100          | hsa-miR-16-5p | FASN        | fatty acid synthase |
| 3           | 100          | hsa-miR-16-5p | UNC80       | unc-80 homolog, NALCN channel complex subunit |
| 4           | 100          | hsa-miR-16-5p | FGF2        | fibroblast growth factor 2 |
| 5           | 100          | hsa-miR-16-5p | TNRC6B      | trinucleotide repeat containing 6B |
| 6           | 100          | hsa-miR-16-5p | PTPN4       | protein tyrosine phosphatase, non-receptor type 4 |
| 7           | 100          | hsa-miR-16-5p | PHF19       | PHD finger protein 19 |
| 8           | 100          | hsa-miR-16-5p | DESI1       | desumoylating isopeptidase 1 |
| 9           | 99           | hsa-miR-16-5p | UBE2Q1      | ubiquitin conjugating enzyme E2 Q1 |
| 10          | 99           | hsa-miR-16-5p | LSM11       | LSM11, U7 small nuclear RNA associated |

### Table 5: Predicted targets function for hsa-miR-15b-5p on 29903 bp ss-RNA SARS-COV-2 genome

| Target Rank | Target Score | miRNA Name | Gene Symbol | Gene Description |
|-------------|--------------|------------|-------------|------------------|
| 1           | 100          | hsa-miR-15b-5p | PAPPA       | pappalysin 1     |
| 2           | 100          | hsa-miR-15b-5p | FASN        | fatty acid synthase |
| 3           | 100          | hsa-miR-15b-5p | UNC80       | unc-80 homolog, NALCN channel complex subunit |
| 4           | 100          | hsa-miR-15b-5p | FGF2        | fibroblast growth factor 2 |
| 5           | 100          | hsa-miR-15b-5p | TNRC6B      | trinucleotide repeat containing 6B |
| 6           | 100          | hsa-miR-15b-5p | PTPN4       | protein tyrosine phosphatase, non-receptor type 4 |
| 7           | 100          | hsa-miR-15b-5p | PHF19       | PHD finger protein 19 |
| 8           | 100          | hsa-miR-15b-5p | DESI1       | desumoylating isopeptidase 1 |
| 9           | 99           | hsa-miR-15b-5p | UBE2Q1      | ubiquitin conjugating enzyme E2 Q1 |
| 10          | 99           | hsa-miR-15b-5p | LSM11       | LSM11, U7 small nuclear RNA associated |
| Target Rank | Target Score | miRNA Name | Gene Symbol | Gene Description                          |
|-------------|--------------|------------|-------------|------------------------------------------|
| 1           | 100          | hsa-miR-15b-5p | PAPPA       | pappalysin 1                             |
| 2           | 100          | hsa-miR-15b-5p | FASN        | fatty acid synthase                      |
| 3           | 100          | hsa-miR-15b-5p | UNC80       | unc-80 homolog, NALCN channel complex subunit |
| 4           | 100          | hsa-miR-15b-5p | FG2         | fibroblast growth factor 2               |
| 5           | 100          | hsa-miR-15b-5p | TNRC8B      | trimethylguanosine repeat containing 6B   |
| 6           | 100          | hsa-miR-15b-5p | PTPN4       | protein tyrosine phosphatase, non-receptor type 4 |
| 7           | 100          | hsa-miR-15b-5p | PHF19       | PHD finger protein 19                    |
| 8           | 100          | hsa-miR-15b-5p | DESI1       | desumoylating isopeptidase 1             |
| 9           | 99           | hsa-miR-15b-5p | UBE2Q1      | ubiquitin-conjugating enzyme E2 Q1       |
| 10          | 99           | hsa-miR-15b-5p | LSM11       | LSM11, U7 small nuclear RNA associated    |

Table 6: Predicted targets function for hsa-miR-15a-5p on 29903 bp ss-RNA SARS-COV-2 genome

| Target Rank | Target Score | miRNA Name | Gene Symbol | Gene Description                          |
|-------------|--------------|------------|-------------|------------------------------------------|
| 1           | 100          | hsa-miR-15a-5p | PAPPA       | pappalysin 1                             |
| 2           | 100          | hsa-miR-15a-5p | FASN        | fatty acid synthase                      |
| 3           | 100          | hsa-miR-15a-5p | UNC80       | unc-80 homolog, NALCN channel complex subunit |
| 4           | 100          | hsa-miR-15a-5p | FG2         | fibroblast growth factor 2               |
| 5           | 100          | hsa-miR-15a-5p | TNRC8B      | trimethylguanosine repeat containing 6B   |
| 6           | 100          | hsa-miR-15a-5p | PTPN4       | protein tyrosine phosphatase, non-receptor type 4 |
| 7           | 100          | hsa-miR-15a-5p | PHF19       | PHD finger protein 19                    |
| 8           | 100          | hsa-miR-15a-5p | DESI1       | desumoylating isopeptidase 1             |
| 9           | 99           | hsa-miR-15a-5p | UBE2Q1      | ubiquitin-conjugating enzyme E2 Q1       |
| 10          | 99           | hsa-miR-15a-5p | LSM11       | LSM11, U7 small nuclear RNA associated    |

Table 7: Predicted targets function for hsa-miR-6838-5p on 29903 bp ss-RNA SARS-COV-2 genome

| Target Rank | Target Score | miRNA Name | Gene Symbol | Gene Description                          |
|-------------|--------------|------------|-------------|------------------------------------------|
| 1           | 100          | hsa-miR-6838-5p | PAPPA       | pappalysin 1                             |
| 2           | 100          | hsa-miR-6838-5p | FASN        | fatty acid synthase                      |
| 3           | 100          | hsa-miR-6838-5p | UNC80       | unc-80 homolog, NALCN channel complex subunit |
| 4           | 100          | hsa-miR-6838-5p | FG2         | fibroblast growth factor 2               |
| 5           | 100          | hsa-miR-6838-5p | TNRC8B      | trimethylguanosine repeat containing 6B   |
| 6           | 100          | hsa-miR-6838-5p | PTPN4       | protein tyrosine phosphatase, non-receptor type 4 |
| 7           | 100          | hsa-miR-6838-5p | PHF19       | PHD finger protein 19                    |
| 8           | 99           | hsa-miR-6838-5p | UBE2Q1      | ubiquitin-conjugating enzyme E2 Q1       |
| 9           | 99           | hsa-miR-6838-5p | LSM11       | LSM11, U7 small nuclear RNA associated    |
| 10          | 99           | hsa-miR-6838-5p | ANKUB1      | ankyrin repeat and ubiquitin domain containing 1 |

Table 8: Predicted targets function for hsa-miR-497-5p on 29903 bp ss-RNA SARS-COV-2 genome.
| Target Rank | Target Score | miRNA Name  | Gene Symbol | Gene Description                           |
|-------------|--------------|-------------|-------------|--------------------------------------------|
| 1           | 100          | hsa-miR-497-5p | PAPPA       | pappalysin 1                              |
| 2           | 100          | hsa-miR-497-5p | FASN        | fatty acid synthase                        |
| 3           | 100          | hsa-miR-497-5p | UNC80       | unc-80 homolog, NALCN channel complex subunit |
| 4           | 100          | hsa-miR-497-5p | FGFR2       | fibroblast growth factor 2                |
| 5           | 100          | hsa-miR-497-5p | TNRC6B      | tri nucleotide repeat containing 6B        |
| 6           | 100          | hsa-miR-497-5p | PTPN4       | protein tyrosine phosphatase, non-receptor type 4 |
| 7           | 100          | hsa-miR-497-5p | PHF19       | PHD finger protein 19                      |
| 8           | 99           | hsa-miR-497-5p |UBE2Q1       | ubiquitin-conjugating enzyme E2 Q1         |
| 9           | 99           | hsa-miR-497-5p |LSM11        | LSM11, U7 small nuclear RNA associated     |
| 10          | 99           | hsa-miR-497-5p |ANKUB1       | ankyrin repeat and ubiquitin domain containing 1 |

Table 9: Predicted targets function for hsa-miR-424-5p on 29903 bp ss-RNA SARS-COV-2 genome.

| Target Rank | Target Score | miRNA Name  | Gene Symbol | Gene Description                           |
|-------------|--------------|-------------|-------------|--------------------------------------------|
| 1           | 100          | hsa-miR-424-5p | PAPPA       | pappalysin 1                              |
| 2           | 100          | hsa-miR-424-5p | FASN        | fatty acid synthase                        |
| 3           | 100          | hsa-miR-424-5p | UNC80       | unc-80 homolog, NALCN channel complex subunit |
| 4           | 100          | hsa-miR-424-5p | FGFR2       | fibroblast growth factor 2                |
| 5           | 100          | hsa-miR-424-5p | TNRC6B      | tri nucleotide repeat containing 6B        |
| 6           | 100          | hsa-miR-424-5p | PTPN4       | protein tyrosine phosphatase, non-receptor type 4 |
| 7           | 100          | hsa-miR-424-5p | PHF19       | PHD finger protein 19                      |
| 8           | 99           | hsa-miR-424-5p |UBE2Q1       | ubiquitin-conjugating enzyme E2 Q1         |
| 9           | 99           | hsa-miR-424-5p |LSM11        | LSM11, U7 small nuclear RNA associated     |
| 10          | 99           | hsa-miR-424-5p |ANKUB1       | ankyrin repeat and ubiquitin domain containing 1 |

Table 10: Predicted targets function for hsa-miR-3133 on 29903 bp ss-RNA SARS-COV-2 genome.

| Target Rank | Target Score | miRNA Name  | Gene Symbol | Gene Description                           |
|-------------|--------------|-------------|-------------|--------------------------------------------|
| 1           | 100          | hsa-miR-3133 | HOOK3       | hook microtubule tethering protein 3       |
| 2           | 100          | hsa-miR-3133 | RIMS2       | regulating synaptic membrane exocytosis 2  |
| 3           | 100          | hsa-miR-3133 | TGFBRAP1    | transforming growth factor-beta receptor-associated protein 1 |
| 4           | 99           | hsa-miR-3133 | RPRD1A      | regulation of nuclear pre-mRNA domain-containing 1A |
| 5           | 99           | hsa-miR-3133 | TFAP2B      | transcription factor AP-2 beta              |
| 6           | 99           | hsa-miR-3133 | PTTPK       | protein tyrosine phosphatase, receptor type K |
| 7           | 99           | hsa-miR-3133 | MAP2        | microtubule-associated protein 2           |
| 8           | 99           | hsa-miR-3133 | NRF1        | nuclear respiratory factor 1              |
| 9           | 99           | hsa-miR-3133 | KAT6A       | lysine acetyltransferase 6A                |
| 10          | 99           | hsa-miR-3133 | URI1        | URI1, prefoldin-like chaperone             |

Table 11: Predicted targets function for hsa-miR-21-3p on 29903 bp ss-RNA SARS-COV-2 genome.
| Target Rank | Target Score | miRNA Name | Gene Symbol   | Gene Description                        |
|-------------|--------------|------------|---------------|-----------------------------------------|
| 1           | 99           | hsa-miR-21-3p | STK38L       | serine/threonine kinase 38 like         |
| 2           | 98           | hsa-miR-21-3p | PCDH19       | protocadherin 19                        |
| 3           | 96           | hsa-miR-21-3p | LAMP1        | lysosomal associated membrane protein 1 |
| 4           | 96           | hsa-miR-21-3p | GRIA2        | glutamate ionotropic receptor AMPA type subunit 2 |
| 5           | 96           | hsa-miR-21-3p | TOGARAM1     | TOG array regulator of axonemal microtubules 1 |
| 6           | 96           | hsa-miR-21-3p | ATP1B1       | ATPase Na+/K+ transporting subunit beta 1 |
| 7           | 96           | hsa-miR-21-3p | TSC22D2      | TSC22 domain family member 2            |
| 8           | 96           | hsa-miR-21-3p | NAP1L5       | nucleosome assembly protein 1 like 5    |
| 9           | 95           | hsa-miR-21-3p | UBE4B        | ubiquitination factor E4B               |
| 10          | 95           | hsa-miR-21-3p | ZNF326       | zinc finger protein 326                 |

Table 12: Predicted miRNAs targeting region on 29903 nt SARS-COV-2 complete genome
| miRNA Name | Previous Name | miRNA Sequence 5' - 3' | (length) |
|------------|---------------|------------------------|----------|
| hsa-miR-15b-5p | hsa-miR-15b | uagcagcacaucaugguuuaca | 22 |
| hsa-miR-16-5p | hsa-miR-16 | uagcagcacuuaauauuggcg | 22 |
| hsa-miR-497-5p | hsa-miR-497 | cagcagcacacugugguuugu | 21 |
| hsa-miR-3133 | | uaaagaacucuuaaaacccaau | 22 |
| hsa-miR-424-5p | hsa-miR-424 | cagcagcaauucauguuuugaa | 22 |
| hsa-miR-497-5p | hsa-miR-497 | cagcagcacacugugguuugu | 21 |
| hsa-miR-21-3p | hsa-miR-21 | caacaccagucgaugggcugu | 21 |

Table 13: Predicted miRNAs targeting the submitted 29903 nt SARS-COV-2 complete genome, accession number NC_045512.2
| Target Rank | Target Score | miRNA Name | Gene Symbol | Gene Description |
|-------------|--------------|------------|-------------|------------------|
| 1           | 92           | hsa-miR-510-3p | CNOT6    | CCR4-NOT transcription complex subunit 6 |
| 2           | 90           | hsa-miR-624-5p | NEXMIF   | neurite extension and migration factor |
| 14          | 84           | hsa-miR-497-5p |           |                   |

Table 14: Predicted targets function for hsa-miR-510-3p on 3822 bp ss-RNA spike glycoprotein of SARS-COV-2 sequence, accession number NC_045512.2 REGION: 21563-25384.

| Target Rank | Target Score | miRNA Name | Gene Symbol | Gene Description |
|-------------|--------------|------------|-------------|------------------|
| 1           | 100          | hsa-miR-510-3p | CNOT6    | CCR4-NOT transcription complex subunit 6 |
| 2           | 99           | hsa-miR-510-3p | NEXMIF   | neurite extension and migration factor |
| 3           | 99           | hsa-miR-510-3p | RBMS3    | RNA binding motif single stranded interacting protein 3 |
| 4           | 99           | hsa-miR-510-3p | DENND6A  | DENN domain containing 6A |
| 5           | 99           | hsa-miR-510-3p | SNAP91   | synaptosome associated protein 91 |
| 6           | 99           | hsa-miR-510-3p | BCLAF1   | BCL2 associated transcription factor 1 |
| 7           | 99           | hsa-miR-510-3p | LATS2    | large tumor suppressor kinase 2 |
| 8           | 99           | hsa-miR-510-3p | ELOVL7   | ELOVL fatty acid elongase 7 |
| 9           | 98           | hsa-miR-510-3p | ZFHX3    | zinc finger homeobox 3 |
| 10          | 98           | hsa-miR-510-3p | FMR1     | fragile X mental retardation 1 |

Table 15: Predicted targets function for hsa-miR-624-5p on 3822 bp ss-RNA spike glycoprotein of SARS-COV-2 sequence, accession number NC_045512.2 REGION: 21563-25384.
Table 16: Predicted miRNAs targeting region on 3822 bp ss-RNA spike glycoprotein of SARS-COV-2 sequence

| Target Rank | Target Score | miRNA Name | Previous Name | miRNA Sequence     | miRNA Sequence 5' | Gene Symbol | Gene Description |
|-------------|--------------|------------|---------------|--------------------|-------------------|-------------|------------------|
| 1           | 96           | hsa-miR-624-5p |               | 5' - uaguaccaguaccuuguguuca - 3' (length = 22) |                   | ARL4A       | ADP ribosylation factor like GTPase 4A |
| 2           | 96           | hsa-miR-624-5p |               | 5' - uaguaccaguaccuuguguuca - 3' (length = 22) |                   | SOWAHC      | sosonowah ankyrin repeat domain family member C |
| 3           | 95           | hsa-miR-624-5p | SYT1          | 5' - zinc finger protein 800 - 3' (length = 22) |                   | STK3B       | serine/threonine kinase 3B |
| 4           | 95           | hsa-miR-624-5p | CLOCK         | 5' - lymbyocyte cytosolic protein 1 - 3' (length = 22) |                   | SYT1        | synaptotagmin 1 |
| 5           | 95           | hsa-miR-624-5p |               | 5' - zinc finger protein 800 - 3' (length = 22) |                   | STK3B       | serine/threonine kinase 3B |
| 6           | 95           | hsa-miR-624-5p |               | 5' - lymbyocyte cytosolic protein 1 - 3' (length = 22) |                   | STK3B       | serine/threonine kinase 3B |
| 7           | 95           | hsa-miR-624-5p |               | 5' - zinc finger protein 800 - 3' (length = 22) |                   | STK3B       | serine/threonine kinase 3B |
| 8           | 95           | hsa-miR-624-5p |               | 5' - lymbyocyte cytosolic protein 1 - 3' (length = 22) |                   | STK3B       | serine/threonine kinase 3B |
| 9           | 94           | hsa-miR-624-5p |               | 5' - lymbyocyte cytosolic protein 1 - 3' (length = 22) |                   | STK3B       | serine/threonine kinase 3B |
| 10          | 94           | hsa-miR-624-5p |               | 5' - lymbyocyte cytosolic protein 1 - 3' (length = 22) |                   | STK3B       | serine/threonine kinase 3B |

Table 17: Original and del 432 TTAdel, Y144 have the same miRNA and target score

| Target Rank | Target Score | miRNA Name          |
|-------------|--------------|---------------------|
| 1           | 91           | hsa-miR-106a-1-3p   |

Table 18: Original 1841 A target miRNA score

| Target Rank | Target Score | miRNA Name          |
|-------------|--------------|---------------------|
| 1           | 91           | hsa-miR-4793-5p     |
| 2           | 64           | hsa-miR-143-5p      |
| 3           | 56           | hsa-miR-3133        |

Table 19: 1841A>G D614G showed a change in the sequence of the miRNA and increase in the target score from 90 to 91 on variable ss-RNA spike glycoprotein of SARS-COV-2 sequence from Jordanian samples

| Target Rank | Target Score | miRNA Name          |
|-------------|--------------|---------------------|
| 1           | 92           | hsa-miR-3628-3p     |
| 2           | 55           | hsa-miR-3133        |
| 3           | 54           | hsa-miR-21-3p       |
### Table 20: Original 3415G target miRNA score

| Target Rank | Target Score | miRNA Name       |
|-------------|--------------|------------------|
| 1           | 80           | hsa-miR-548g-3p  |
| 2           | 72           | hsa-miR-627-5p   |
| 3           | 61           | hsa-miR-506-5p   |

**Table 21**: 3415G>T D1139Y show same the miRNA and increase the target score 80 to 81 on variation ss-RNA spike glycoprotein of SARS-COV-2 sequence from Jordanian samples

| Target Rank | Target Score | miRNA Name       |
|-------------|--------------|------------------|
| 1           | 81           | hsa-miR-548g-3p  |
| 2           | 72           | hsa-miR-627-5p   |
| 3           | 61           | hsa-miR-506-5p   |

### Table 22: Original 3499 G target miRNA score.

| Target Rank | Target Score | miRNA Name       |
|-------------|--------------|------------------|
| 1           | 73           | hsa-miR-155-5p   |
| 2           | 62           | hsa-miR-6765-5p  |
| 3           | 53           | hsa-miR-551b-5p  |

**Table 23**: 3499 G>A G1167S show same the miRNA and decrease the target score 73 to 72 on variation ss-RNA spike glycoprotein of SARS-COV-2 sequence from Jordanian samples

| Target Rank | Target Score | miRNA Name       |
|-------------|--------------|------------------|
| 1           | 72           | hsa-miR-155-5p   |
| 2           | 62           | hsa-miR-6765-5p  |
| 3           | 51           | hsa-miR-668-3p   |

### Table 24: Predicted miRNAs targeting region on variation ss-RNA spike glycoprotein of SARS-COV-2 sequence from Jordanian samples
| Sample number with accession on GISAID | Sequence variation ss-RNA spike glycoprotein of SARS-CoV-2 sequence | Single Amino Acid Variation | miRNAs targeted by spike glycoprotein of SARS-CoV-2 sequence |
|---------------------------------------|---------------------------------------------------------------|-----------------------------|---------------------------------------------------------------|
| NCBI Reference Sequence: NC_045512.2 | AATAACGCTACTAATGGTTATAAATCTGGTAAATTTGCTAATCCATTATTGCATTGTTTATATTACCAAAAACAACAAAGTTGGATGGAAAGT | original | miRNA Names 196a-3p, 3620-3p, 548g-3p, 155-5p, 4793-5p, 196a-1-3p |
| Sample 23: hCoV-19/Jordan/SR-02/2020 EPI_ISL_430000/2020-03-30 | AATAACGCTACTAATGGTTATAAATCTGGTAAATTTGCTAATCCATTATTGCATTGTTTATATTACCAAAAACAACAAAGTTGGATGGAAAGT | del 432 TTA, del Y144 | miRNA Names 196a-3p, 3620-3p, 548g-3p, 155-5p, 4793-5p, 196a-1-3p, 3620-3p, 548g-3p, 155-5p |
| NCBI Reference Sequence: NC_045512.2 | GGAACAAATAACTTTACCAACCAGTGTCGCTGCTCTTATACGAGGATTTAACTGAGACAGAAGTTCCCTGTTTGCTATTCATGCAGATCAACTTACTCCTACTTGGCGTGT | Original 1841A | miRNA Names 4793-5p, 3620-3p, 548g-3p, 155-5p, 4793-5p, 3620-3p, 548g-3p, 155-5p |
| Sample 3, 5, 7, 8, 9, 10, 11, 12, 13, 15, 18, 20 and 21 | GGAACAAATAACTTTACCAACCAGTGTCGCTGCTCTTATACGAGGATTTAACTGAGACAGAAGTTCCCTGTTTGCTATTCATGCAGATCAACTTACTCCTACTTGGCGTGT | | miRNA Names 3620-3p, 548g-3p, 155-5p, 4793-5p, 3620-3p, 548g-3p, 155-5p, 4793-5p, 3620-3p, 548g-3p, 155-5p |
| NCBI Reference Sequence: NC_045512.2 | ACTGTGATGGTTAATAAGAGCAATTGCAACACCACAGTTTATAGATCTCTCTTGCAACCTGAAATTAGCTTACATCAAGGAGTTAGATAAATATTTTAAGAATCATACA | Original 3415G | miRNA Names 548g-3p, 155-5p, 4793-5p, 3620-3p, 548g-3p, 155-5p, 4793-5p, 3620-3p, 548g-3p, 155-5p |
| Sample 21: hCoV-19/Jordan/SR-03/2020 EPI_ISL_429993/2020-03-16 | ACTGTGATGGTTAATAAGAGCAATTGCAACACCACAGTTTATAGATCTCTCTTGCAACCTGAAATTAGCTTACATCAAGGAGTTAGATAAATATTTTAAGAATCATACA | 3415G>T D1139Y | miRNA Names 548g-3p, 155-5p, 4793-5p, 3620-3p, 548g-3p, 155-5p, 4793-5p, 3620-3p, 548g-3p, 155-5p |
| NCBI Reference Sequence: NC_045512.2 | TCACCAGATGTTAGTTTACCATGAGACATCTCTGGCATTATGCTTAACTCAATTTCCATTCAAAAAAGAATTTGACCGCCTCAATGAGGTGCGCAAGATTTAATG | Original 3499 G | miRNA Names 155-5p, 4793-5p, 3620-3p, 548g-3p, 155-5p, 4793-5p, 3620-3p, 548g-3p, 155-5p, 4793-5p |
| Sample 16: hCoV-19/Jordan/SR-03/2020 EPI_ISL_429998/2020-03-28 | TCACCAGATGTTAGTTTACCATGAGACATCTCTGGCATTATGCTTAACTCAATTTCCATTCAAAAAAGAATTTGACCGCCTCAATGAGGTGCGCAAGATTTAATG | 3499 G>A G1167S | miRNA Names 155-5p, 4793-5p, 3620-3p, 548g-3p, 155-5p, 4793-5p, 3620-3p, 548g-3p, 155-5p, 4793-5p |

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**Declarations**

The authors declare no competing interests.