Received: 8 June 2021 | Revised: 25 August 2021 | Accepted: 5 September 2021

DOI: 10.1002/acg2.114

ORIGINAL ARTICLE

ADVANCES IN CELL AND GENE THERAPY | WILEY

Identification of homologous human miRNAs as antivirals towards COVID-19 genome

Jitender Singh[1] | Ashvinder Raina[2] | Namrata Sangwan[1] | Arushi Chauhan[1] | Krishan L. Khanduja[1] | Pramod K. Avti[1]

[1]Department of Biophysics, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India

[2]Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India

Correspondence
Pramod K. Avti, MPhil, PhD, MAMS, Associate Professor, Department of Biophysics, Postgraduate Institute of Medical Education and Research (PGIMER), Sector 12, Chandigarh 160012, India.
Email: pramod.avti@gmail.com

Abstract

The COVID-19 fatality rate is ~57% worldwide. The investigation of possible antiviral therapy using host microRNA (miRNA) to inhibit viral replication and transmission is the need of the hour. Computational techniques were used to predict the hairpin precursor miRNA (pre-miRNAs) of COVID-19 genome with high homology towards human (host) miRNA. Top 21 host miRNAs with >80% homology towards 18 viral pre miRNAs were identified. The Gibbs free energy (ΔG) between host miRNAs and viral pre-miRNAs hybridization resulted in the best 5 host miRNAs having the highest base-pair complementarity. miR-4476 had the strongest binding with viral pre-miRNA ($\Delta G = -21.8$ kcal/mol) due to maximum base pairing in the seed sequence. Pre-miR-651 secondary structure was most stable due to the (1) least minimum free energy ($\Delta G = -24.4$ kcal/mol), energy frequency, and noncanonical base pairing and (2) maximum number of stem base pairing and small loop size. Host miRNAs–viral mRNAs interaction can effectively inhibit viral transmission and replication. Furthermore, miRNAs gene network and gene-ontology studies indicate top 5 host miRNAs interaction with host genes involved in transmembrane-receptor signaling, cell migration, RNA splicing, nervous system formation, and tumor necrosis factor-mediated signaling in respiratory diseases. This study identifies host miRNA/virus pre-miRNAs strong interaction, structural stability, and their gene-network analysis provides strong evidence of host miRNAs as antiviral COVID-19 agents.

KEYWORDS
antiviral, COVID-19, Gibbs free energy, hairpin loop, homologous miRNA, hybridization, ontology

1 | INTRODUCTION

The novel CoVs/COVID-19 has been predicted as an epidemic, and the whole world is facing health and economic crises in this pandemic. The newly discovered COVID-19 disease is caused by the transmission of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) from human to human and animal to human. Hence, immediate rescue strategies should include repurposing of drugs for immediate therapeutic remedy. The SARS-CoV-2 is a long single-stranded RNA virus. Many variants of the COVID-19 are being reported over time. Due to the complex nature of COVID-19 genome structure, various components of COVID-19 genome structure (spike protein, main protease, RNA dependent RNA polymerase, nonstructural proteins, membrane proteins, envelop protein) are considered as effective targets for treatment.[1–7] Although several attempts are made to discover the mechanisms that trigger the

Conflict of interest: None declared.

coronavirus, there is still a significant gap in our knowledge of SARS-CoV-2/COVID-19 pathology.[8] One of the recent emerging areas for effective treatment strategies for COVID-19 is the field of microRNAs (miRNAs). miRNAs are genomically encoded small non-coding RNA molecules that are 20–25 nucleotides base pairs in length and regulate the expression of post-transcriptional genes.[9] It is well known that miRNAs are encoded by the intronic region of the DNA from animals, plants, and certain viruses to regulate their various molecular and biological processes.[10,11] Approximately, 30 424 mature miRNAs were found in 206 animals, whereas the human genome encodes approximately 3000 miRNAs.[12] The viral miRNAs not only regulate the host gene expression but also regulate their own gene expression.[13] RNA polymerase-II transcribes miRNA genes and forms primary miRNA in the nucleus. Through enzymatic action of the RNase III ribonuclease disease, primary miRNAs are divided into 70–90 long nucleotide base-pair precursor hairpin called precursor miRNA (pre-miRNA).[13,14] The pre-miRNAs are bound with exportin-5 enzymes and Ran in the nucleus and are exported to the cytoplasm where biogenesis of miRNAs occur.[14] RNase III ribonuclease Dicer further cleaves the pre-miRNAs to the hairpin loop structure known as duplex mature miRNA.[14] The duplex RNA guided strand (active strand) is loaded into an RNA-induced silencing complex (RISC) aimed at degrading or repressing translational activity by messenger RNA.[13] Perfect complementary between the viral mRNA's 3′-untranslated region (UTR) and the mature host miRNA seed sequence (2–7 base pair) is sufficient to lead to cleavage, but imperfect complementary can hinder viral mRNA translation.[13,15] It is known that miRNAs have a potential role in the immunological process and are involved in the regulation of the immune system through the activation of the immune cells.[16] Studies investigated the miRNAs role as an antiviral agent against many diseases, including human immunodeficiency virus-1,[17] herpes simplex virus,[18] dengue,[19] influenza virus,[15] and hepatitis C virus.[20] To prevent and treat the highly pathogenic coronavirus with the help of the antiviral agent is important. As a result, it is highly recommended that new biological strategies for the treatment of viral diseases be created.[21] In the epithelial cells, the expression change of the host miRNAs has a role in the pathogenesis of chronic and severe acute respiratory tract infections.[22]

Our recent studies have shown how the viral RNA stabilizes monomeric and dimeric subunits of the protein complex by suitable interactions that have been mimicked by screening compounds from various drug-approved databases, which show high inhibitory potential against viral mRNA.[1] This study computed any possible human miRNA targets for the SARS-2 (severe acute respiratory syndrome coronavirus-2) genome. It also emphasizes the host miRNAs and COVID-19 viral genome interaction that will help better understand the role of host pathogens and establish new antiviral therapy against COVID-19.

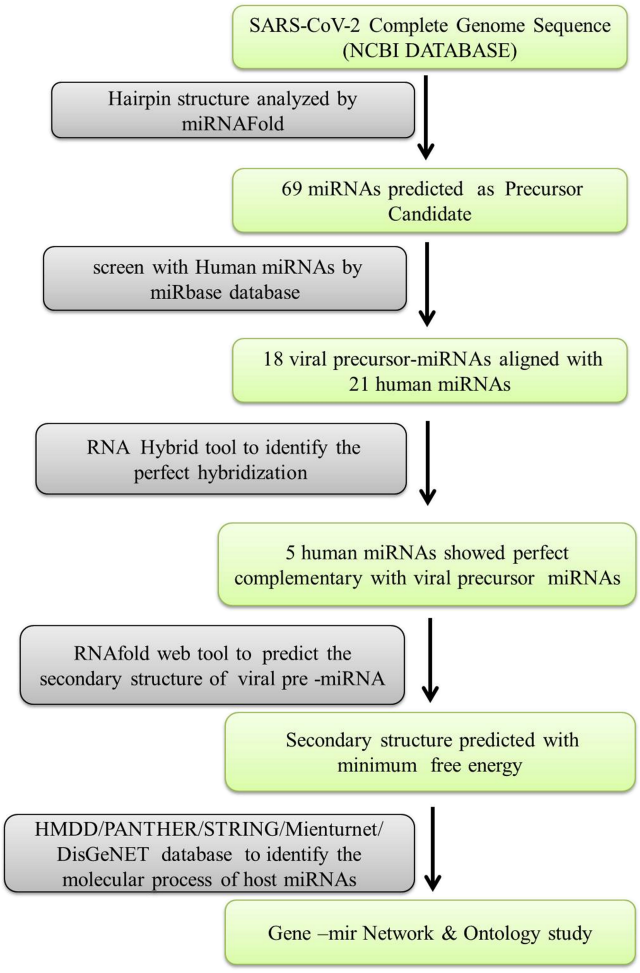

FIGURE 1 Study design of human microRNA (miRNA) prediction on SARS-2 (COVID-19) viral genome

2 | MATERIALS AND METHODS

2.1 | COVID-19 complete genome sequence retrieval

The complete genome sequence (NC 045512.2) of COVID-19 was obtained from the NCBI database to predict the pre-miRNAs sequence. A flowchart diagram of the entire theoretical analysis process is shown in (Figure 1) to classify the possible miRNAs.

2.2 | Hairpin precursor analysis

To predict miRNAs hairpin precursors, the entire viral genome was scanned using a miRNA Fold web tool to find the hairpin—structured miRNA precursors to find the expected results (https://evryrna.ibisc.univ-evry.fr/miRNAFold). miRNA fold is now devoted to discovering miRNA precursors in genomes on a wide scale and enables miRNA hairpin structures to be predicted rapidly and with high sensitivity. The scanned hairpins were visualized in the mRNA fold, whereas candidate miRNA precursor 69 sequences of possible hairpin-like forms were extracted. We filtered outputs using

configuration options to prevent bonafide pre-miRNAs structured hairpins with sliding window size (200), minimum hairpin size (30), the maximal thermodynamic value of hairpins (50), and percentage of verified features (95%).

2.3 | Sequence similarity prediction

For the identification of specific homologs, the sequences of the candidate viral miRNA precursors were compared among all host miRNAs using the miRBase database's SEARCH menu (http://www.mirbase.org/search.shtml).[12] In this phase of the research, we identified 18 sequences called potential miRNA precursors (hairpin-like structure) based on their highest sequence resemblance to human miRNAs.

2.4 | miRNAs hybridization prediction

The RNA hybrid (http://bibiserv2.cebitec.uni-bielefeld.de/rnahybrid/) web server was used to investigate the hybridization between viral (COVID-19) pre-miRNAs and possible human mature miRNAs.[23,24] The RNAhybrid method was used to evaluate the effective hybridization between target host miRNA and viral pre-miRNA. RNAhybrid is a method for determining the minimum free energy (MFE) of long and short RNA hybridization and is commonly used to estimate miRNA targets.

2.5 | Prediction of secondary structure

To predict the top best viral pre-miRNAs secondary structures of COVID-19, the RNAfold online tools (http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi) were used. The RNAfold program predicts secondary structures of single-stranded RNA and DNA sequences.[25] An RNA sequence's MFE is the secondary structure that contains the minimum amount of free energy. A loop-based estimation model and the genetic algorithm method are used to predict such a structure.[26] The secondary structure of RNA can be disassembled into loops (internal and external) and external bases (bulges) in a unique manner.

2.6 | Disease association and network analysis

Human MicroRNA Disease Database (HMDD) (https://www.cuilab.cn/hmdd) and mienturnet (http://userver.bio.uniroma1.it/apps/mienturnet/) were used to analyze the miRNA–disease relationship and identify the network association. These online databases provide and organize the scientific evidence for human miRNA and disease connections and networks. miRNAs can control gene expression at two levels: post-transcriptionally by degrading targeted mRNAs or suppressing protein translation and transcriptionally by disrupting regulator RNAs like lncRNAs. Such analyses provide details about the miR-disease networks and their interacting genes involved in various biological activities. STRING online database was employed to evaluate the primary interactions of the predicted host genes.[27]

2.7 | Study of gene ontology

The PANTHER (Protein Analysis by Ancestral Relationships) classification system database (http://www.pantherdb.org) was used to classify the gene ontology of selected target genes involved in the miR-gene network and also to obtain insight into the product of the target genes molecular structure, biological mechanism, and cellular components. DisGeNET (https://www.disgenet.org/) database was used to figure out the associated genes with human diseases evidence via expert-curated collections, genome-wide interaction analyses, research literature, and animal models are all integrated into DisGeNET.[28] The DisGeNET scoring mechanisms include the use of various sources used (level of curation, organisms) and the data from different types of studies are considered for GDAs. In contrast, the score for VDAs is based on sources and literature analysis, and scores vary from 0 to 1.

3 | RESULTS

3.1 | COVID-19 genome retrieval

The complete genome sequence of COVID-19 was acquired from the NCBI with ID (NC 045512.2). The sequenced COVID-19 genome is made up of a single positive-stranded RNA with a length of 29 811 nucleotides. We compared our genome sequence with other COVID-19 strains through the Clustalw multiple sequence alignment (MSA) program. The MSA results show that the sequence ID NC_045512.2 is highly conserved with different selected sequences (Data S1).

3.2 | Prediction of COVID-19 pre-miRNA

Prediction of COVID-19 viral genome pre-miRNA hairpins with their sequence length and score was obtained using miRNA fold computational web tools. A total of 69 probable viral pre-miRNA hairpins were considered as the potential hairpins with the maximum thermodynamic value of hairpin (50 J/mol) and perfect hairpin precursor length (>70 nt) for further analysis.

3.3 | Identifying human miRNAs from precursors

We employed the miRBase database to find the homologs of human miRNAs from the COVID-19 precursors. Every sequence of the viral pre-miRNA was searched for nucleotide resemblance among human miRNAs by using the human miRNA query from the miRBase database's SEARCH menu (http://www.mirbase.org/search.shtml). The search identified 18 potential viral pre-miRNA sequences of the

TABLE 1 Hairpin sequence alignments of viral precursor miRNAs with individual host miRNAs

Sr. no	Hairpin	Alignment score	Alignment between human microRNAs and SARS-2
1	Precursor −1	83	UserSeq 74 gaggcacgucaacaucuuaaagauggcacu 103 hsa-mir-1204 62 gaggcacguccucaccuccaagauguaacu 33
2	Precursor −2	81	UserSeq 62 cucauggucauguuaugguugagcuggu 89 hsa-mir-1261 21 cuuaugggguauuugugguugaucuguu 48
3	Precursor −3	81	UserSeq 1 ugauggcuacccucuugagugcauuaaagaccuucu 36 hsa-mir-3202-1 30 ugauuggagucauuuucagagcauuaaagcucuucu 65 UserSeq 1 ugauggcuacccucuugagugcauuaaagaccuucu 36 hsa-mir-3202-2 51 ugauuggagucauuuucagagcauuaaagcucuucu 16 UserSeq 27 aagaccuucuagcacgugcugguaaagcuucaug 60 hsa-mir-492 20 aggaccaucgaggaccugcgggacaagauucuug 53
4	Precursor −4	88	UserSeq 40 uguggcacugagaauuugacuaaagaaggugc 71 hsa-mir-891a 56 ugugccacugaguauuuuacugaaucaguggc 25
5	Precursor −5	90	UserSeq 13 gaagguuccgaaggucuuaaugacaaccuucu 44 hsa-mir-4476 32 gaaggcugggagggacuuagggacaggcuuuu 1
6	Precursor −6	83	UserSeq 25 cacuucccacagaaguguuaacagaggaaguug 57 hsa-mir-1296 63 cacuccccacagaaggguuuuccuaaaggagaug 31
7	Precursor −7	89	UserSeq 79 uggcagaugcugucauaaaaacuuugcaa 107 hsa-mir-4705 19 ugguaauugcugugauaacaacucagcaa 47
8	Precursor −8	88	UserSeq 60 uuucuuaaagaaagaugcuccauauauagug 90 hsa-mir-519d 45 ucucuuaaacaaagugccucccuuuagagug 75
9	Precursor −9	85	UserSeq 18 uugacaaucuuaagacacuucuuucuuug 46 hsa-mir-1255a 64 uuuagaaucuaaaaaaucuacuuucuuug 36

TABLE 1 (Continued)

Sr. no	Hairpin	Alignment score	Alignment between human microRNAs and SARS-2
10	Precursor −10	86	UserSeq 18 cacacgcaaguuguggacaugucaaugacauau 50 hsa-mir-708 69 cacagucuaguuguguucaugugcaagucauuu 37
11	Precursor −11	89	UserSeq 32 cuuucuuaugaacaauuuaagaaagguguucagaua 67 hsa-mir-3167 31 cuuuuuucugaaauucuucaggaaggauuucagaaa 66
12	Precursor −12	88	UserSeq 44 uuauggaccuuuuguugacaggcaaacagcacaa 77 hsa-mir-548d-1 66 uuuuugccauuacuuuuacaggcaaaaaccacaa 33
13	Precursor −13	86	UserSeq 78 guacgcugcuguuauaaaauggagacagguggu 109 hsa-mir-101-2 48 guacaguacugugauaacugaagaaugguggu 79
14	Precursor −14	83	UserSeq 38 auccaaauccuaaaggauuuugugacuuaaaaagguaa 74 hsa-mir-651 43 auaaaaaugcaaaaggaaaguguauccuaaaaggcaa 79
15	Precursor −15	86	UserSeq 5 gguauguggaaagguuauggcuguaguugugaucaacuccgcgaac 50 hsa-mir-548ap 12 gguuggugcaaaaguaauugcggucuuugucauuaaaaccaauaac 57
16	Precursor −16	85	UserSeq 26 uucugcacuguuagcggguacaaucacuucugguu 60 hsa-mir-510 70 uacuccacucuuagagguuucaaucacaccuaauu 36 UserSeq 52 cuucugguuggaccuuuggugcaggugcugcauua 86 hsa-mir-1282 5 cuucucguuugccuuuuucugcuucugcugcauga 39
17	Precursor −17	85	UserSeq 38 aacaacacaguuuaugauccuuugcaaccug 68 hsa-mir-2053 11 aaauacagauuuaauuaacauuugcaaccug 41
18	Precursor −18	88	UserSeq 33 agaaacagcaaacugugacucuucuuccugcugc 66 hsa-mir-3916 42 agaaccagccauuucuuccucuucuuccuucuuc 9

Note: There are 18 precursors hairpin aligned with 21 human microRNAs showing highest alignment score.

COVID-19 virus that showed >80% homology with 21 human miRNAs (Table 1). As primary target miRNAs, human miRNAs with a minimum 30-bp sequence similarity to candidate pre-miRNA were chosen.

3.4 | Hybridization prediction

After distinguishing the human miRNAs from the chosen precursor sequences, hybridization between 18 viral pre-miRNAs and 21 human miRNAs was performed. Result of the hybridization process showed that five human miRNAs that show high sequence complementarity with the shortlisted 3′ region of pre-miRNAs of the COVID-19 genome sequence (Figure 2). The least pairing energy or MFE indicates the stability of strong hybridization process. The MFE of the seed region for hsa-miR-4476 ($\Delta G = -21.8$ kcal/mol), hsa-miR-548d-1 ($\Delta G = -18.5$ kcal/mol), hsa-miR-3202-1 ($\Delta G = -18.1$ kcal/mol), hsa-miR-1296 ($\Delta G = -16.8$ kcal/mol), and hsa-miR-651 ($\Delta G = -14.9$ kcal/mol) showed MFE values (Figure 3).

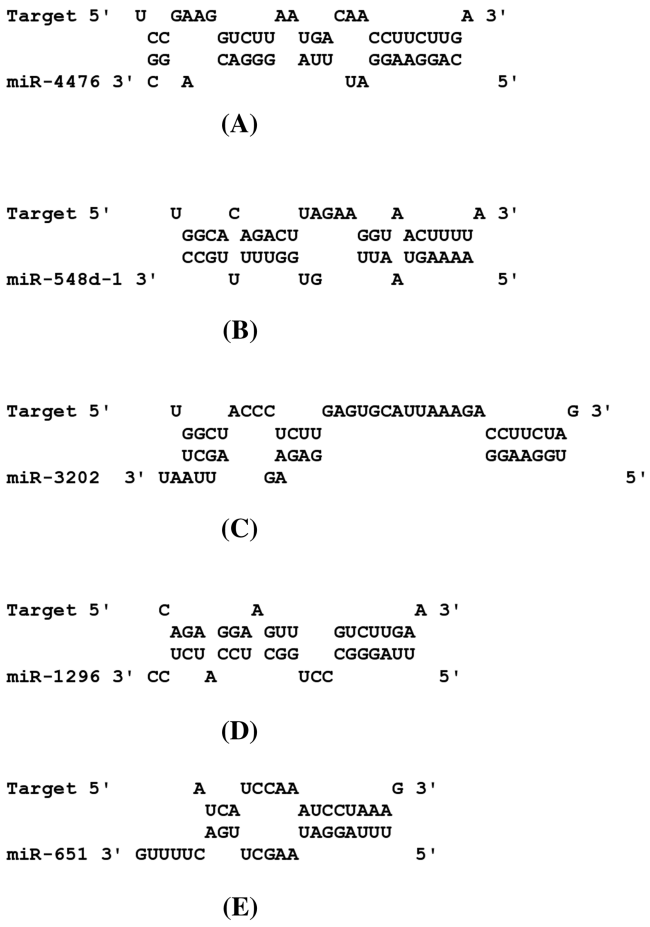

FIGURE 2 Hybridization between host microRNAs (miRNAs) and viral precursor miRNAs (pre-miRNAs) using RNA hybrid program. (A) miR-4476, (B) miR-548-1d, (C) miR-3202, (D) miR-1296, and (E) miR-651

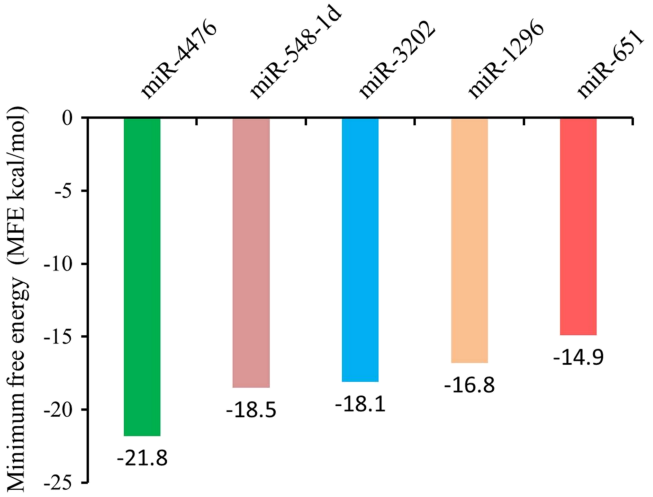

FIGURE 3 Bar graph showing the minimum free energy values of host microRNA (miRNA) target with five viral pre-miRNAs. Hybridization between severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) precursor miRNA (pre-miRNA) with host miRNAs (miR-4476 = −21.8 kcal/mol, miR-548-1d = −18.5 kcal/mol, miR-3202 = −18.1 kcal/mol, miR-1296 = −16.8 kcal/mol, miR-651 = −14.9 kcal/mol)

3.5 | Secondary structure assessment

The secondary structure of viral pre-miRNAs was analyzed using the RNAfold (http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi) web server with the default parameters after the findings established possible top 5 best human miRNAs. The most reliable secondary structure of COVID-19 hairpin sequences was predicted using the RNAfold program. The pre-miRNA precursor on either end is flanked upstream and downstream by ∼200 and ∼100 bp in the series used for prediction analysis. According to the results, the thermodynamic ensemble's pre-miR-651 free energy is $\Delta G = -24.40$ kcal/mol, the MFE structure's frequency is 4.56%, and the ensemble diversity is 12.81. The thermodynamic ensemble free energy of pre-miR-548-1d is −21.82 kcal/mol. The MFE structure's frequency is 10.04%, and the ensemble diversity is 8.16. Pre-miR-3202 has a thermodynamic ensemble free energy of −18.67 kcal/mol, a frequency of 10.80% for the MFE structure, and an ensemble richness of 6.49. Pre-miR-1296 has the thermodynamic ensemble free energy of −16.66 kcal/mol, a frequency of 4.14% for the MFE configuration in the ensemble, and diversity of 17.57. The thermodynamic ensemble free energy of pre-miR-4476 is −16.58 kcal/mol, the frequency of the MFE configuration in the ensemble is 28.04%, and the ensemble diversity is 3.21 (Figures 4–6).

3.6 | Disease association and network analysis

HMDD (https://www.cuilab.cn/hmdd) was used to find out the host miRNA with the disease association, miR network,. This is a

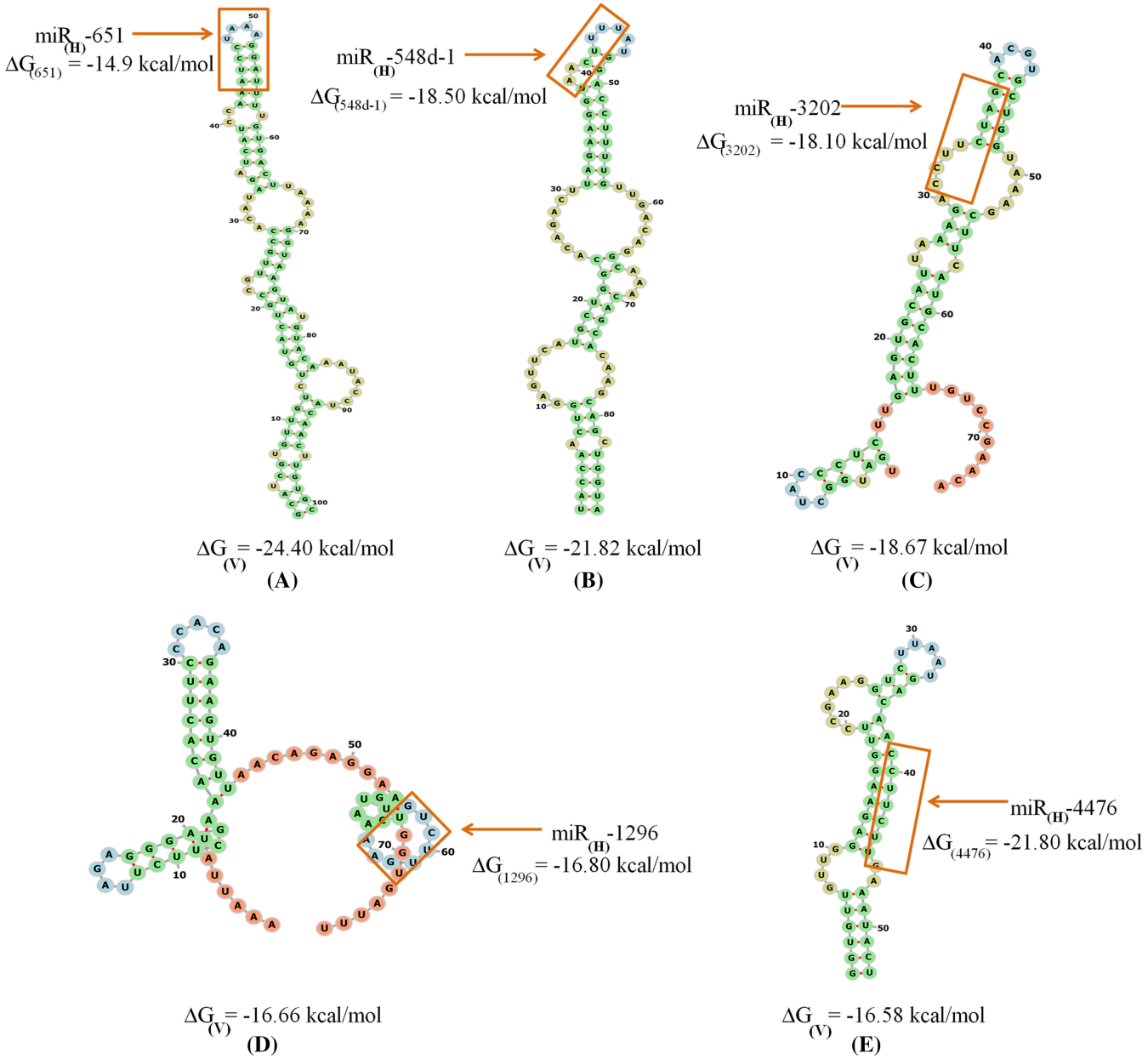

FIGURE 4 Predicated secondary structure of potential hairpins candidate of SARS-2 with RNAfold web program. (H, human; V, viral) only secondary structures were depicted. (A)–(E) represent pre-miR-651, pre-miR-548-1d, pre-miR-3202, pre-miR-1296, pre-miR-4476, and their thermodynamic ensemble's free energy, the ensemble's frequency of the minimum free energy (MFE) structure, and ensemble diversity

repository for experimentally validated proof of human miRNA and disease interactions. We found the top 5 human miRNAs that are involved in multiple diseases (Table 2). The predicted miR-gene network showed a strong association between specific genes (SDC4, SMU1, NAV2, and SPATA2). It is depicted to play a role in cell migration, RNA splicing, nervous system development, tumor necrosis factor-mediated signaling pathway, and molecular adaptor activity (Figure 7). We further performed STRING database search to identify the physical interaction among the four genes identified from HMDD analysis. Results showed that there were many other essential genes having interactions with these selected four genes involved in the various biological process like positive regulation of extracellular exosome assembly, exosomal secretion, catabolic process, cell migration, angiogenesis, etc., molecular function like, wnt signaling pathway, integrin binding, growth factor receptor binding, heparin-binding, and signaling receptor binding. (Figure S1).

3.7 | Gene-ontology analysis (GO analysis)

The PANTHER (Protein Analysis by Evolutionary Relationship) classification scheme (http://www.pantherdb.org) was to gain insight into the molecular structures, biological mechanisms, and cells of used to

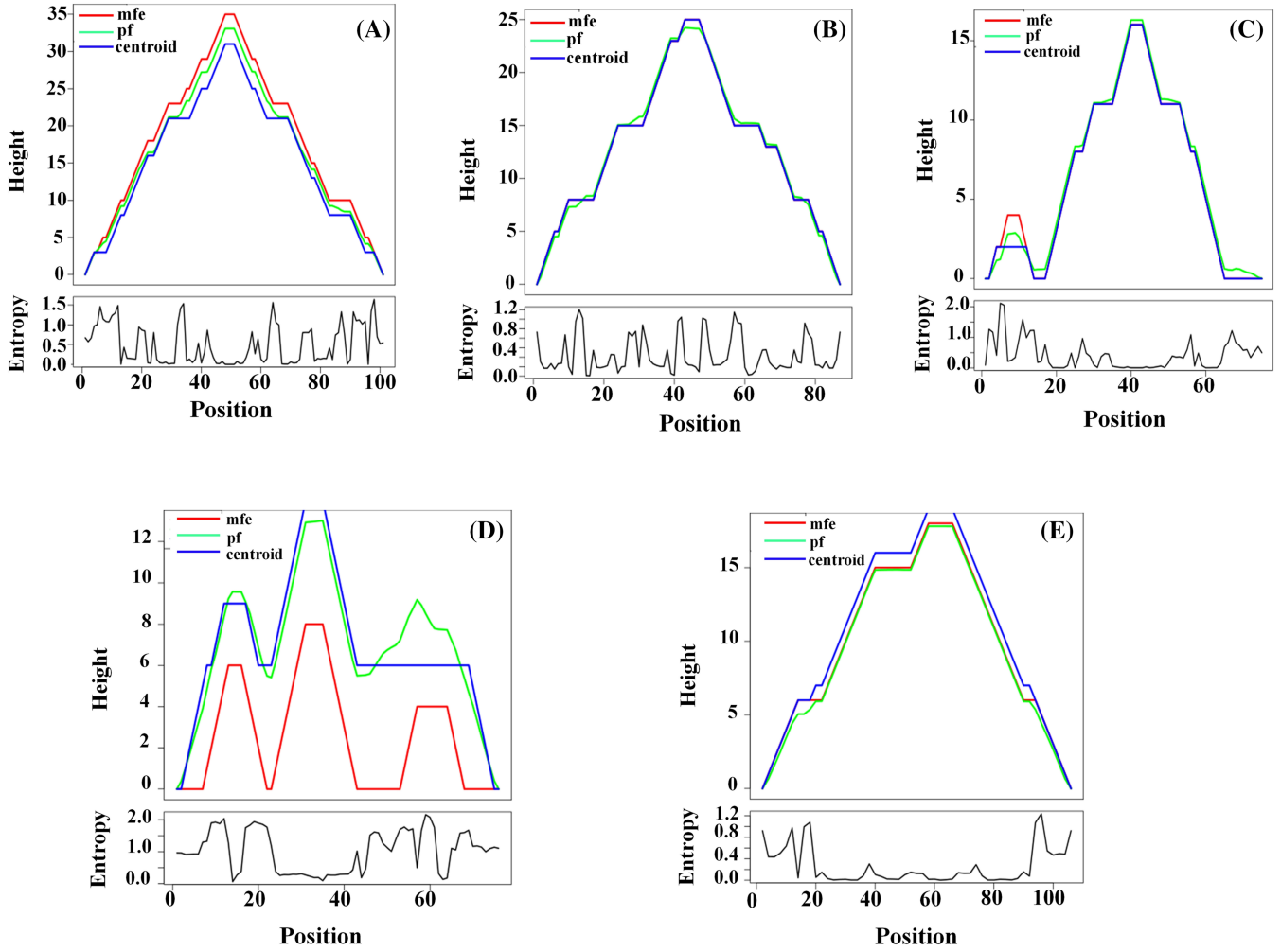

FIGURE 5 Predicted secondary structure mounting plot of the entire selected precursor microRNA (miRNA) hairpin, that is, (A) pre-miR-651, (B) pre-miR-548-1d, (C) pre-miR-3202, (d) pre-miR-1296, and (E) pre-miR-4476. The minimum free energy (MFE), the thermodynamic ensemble of RNA (pf), and the centroid structures were represented by the red, green, and blue lines, respectively

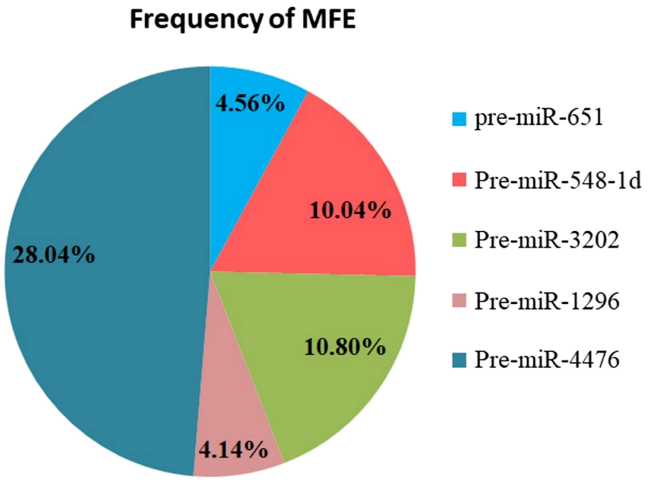

FIGURE 6 Pie chart showing the frequency of minimum free energy (MFE) of five viral pre-miRNAs sequences

conduct gene ontology analysis on the obtained target genes. It was necessary to predict the gene ontology to gain further insight into the related functions of its genes participation in PANTHER classification to learn more about the connection between COVID-19 and the projected human miRNA that all humans share. The cluster of target gene (SDC4, SMU1, NAV2, and SPATA2) products was shown to play key roles in cell migration, RNA splicing, nervous system growth, tumor necrosis factor-mediated signaling cascade, and molecular adaptor behavior in terms of molecular functions (Table 3). The results from the DisGeNET database were filtered out for the respiratory tract disease associated with the identified four host genes. Gene SDC4 is involved in neoplasms of the lungs, respiratory tract diseases, and infections; NAV2 is involved in respiratory tract diseases, immune system diseases; SPATA2 is implicated in nonsmall cell lung carcinoma, primary malignancy of the lungs, and SMU1 is involved in cardiomyopathy, genetic, neonatal cancers and defects, neoplasms, and respiratory system disorders (Table 4).

TABLE 2 Hybridization between host microRNAs and viral miRNA (SARS-2) using RNA hybrid program

miRNA name	Evidence code	Disease name	PMID
hsa-mir-4476	circulation_biomarker_diagnosis	Carcinoma, Biliary Tract	25706130
hsa-mir-1296	circulation_biomarker_prognosis	Carcinoma, Colon	27485175
hsa-mir-1296	other	Prostate Neoplasms	20332239
hsa-mir-1296	target gene	Carcinoma, Gastric	28099468
hsa-mir-1296	target gene	Colorectal Carcinoma	30026827
hsa-mir-1296	Tissue expression	Carcinoma, Hepatocellular	30064138
hsa-mir-548d-1	genetics knock down promote	Medulloblastoma	21793975
hsa-mir-548d-1	other	Esophageal Neoplasms	21743970
hsa-mir-548d-1	other	Pancreatic Neoplasms	21743970
hsa-mir-651	circulation_biomarker_prognosis	Carcinoma, Breast	27959953
hsa-mir-651	tissue_expression	Carcinoma, Hepatocellular	26229398

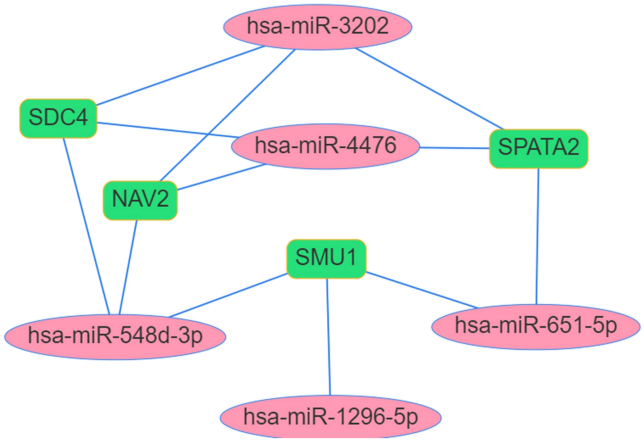

FIGURE 7 Network diagram showing the interaction between host microRNAs (miRNAs) and genes using mienturnet database

4 | DISCUSSION

The infection of a virus is one of the most serious global challenges to human health. Humans are infected with the current pandemic coronavirus (COVID-19), which has resulted in severe respiratory infections,[29,30] with symptoms such as diarrhea, cough, and breathing difficulties.[31] However, a lack of thorough understanding of the fundamental molecular pathways limits developing the novel therapeutics. MiRNAs are genomically encoded, small non-coding RNA, which are approximately ~22 nucleotides long that normally target the mRNAs and regulate their expression at the post-transcriptional level. Targeting the viral mRNA by the host miRNA is an essential mechanism for regulating further the viral expression in the host. Hence, recognizing target viral mRNA with high complementation with the host miRNA seed sequence is an essential mechanism for inhibiting the viral progression.[32] miRNA is an important type of regulatory RNA, which mainly inhibits gene expression at the post-transcriptional level.[33] According to our study, the COVID-19 genome sequence with NCBI ID: NC_045512.2 was 100% conserved compared with other variants (MT435086, MT339041, MT066156, and MT507794.1).[34]

Several computational methods were used to predict the top 5 human miRNAs: miR-4476 (−21.8 kcal/mol), miR-548-1d (−18.5 kcal/mol), miR-3202 (−18.1 kcal/mol), miR-1296 (−16.8 kcal/mol), and miR-651 (−14.9 kcal/mol), which get strongly hybridized with the viral pre-miRNA with stable MFE. The miR-4476 strongly binds with the target viral pre-miRNA pre-miR-4476 sequence at the 3′ end region with the least free energy (ΔG) of −21.8 kcal/mol. The hybridized structure showed high complementarity with the seed sequence of host miRNA having eight base pair long region. Due to this strong complementary region, the host miRNA-4476 can be considered as strong anti-viral agent among the top 5 identified host mi-RNA and can strongly inhibit the target sequence (Figure 2A). In comparison with other host miRNAs, the miR-548-1 having target with pre-miR-548-1d, which has the binding minimum energy of −18.5 kcal/mol (Figure 2B), can also be considered a good target. The stability of miR-548-1 is slightly lower than the miRNA-4475 due to the shorter seed sequence length of only six base pair long as compared with miR-4476 having eight base pairs. Similar results were observed for the other targets but with less stable free energies and less seed sequence base pairing, complementary base pairing, and supplementary base-pairing resulting in the overall lower stability of the other structures. Therefore, it could be assumed that the length of seed sequence, complementarity, and supplementary base pairing is essential and critical for stabilizing the hybridized viral pre-miRNA structure (Figure 3). However, a recent study by Sharma et al. (2020) predicted 22 potential miRNA from 5 genomes of SARS-CoV-2 linked with 12 host miRNAs.[34] It is known that having G-U pairs in the seed sequence at viral 3′ pre-miRNAs decreases the stability of the target structure.[35] Our hybridization results showed a minimum number of G-U pairs in the seed sequences of 3′ of all five viral pre-miRNAs that strongly contribute to the top 5 host miRNAs binding with viral pre-miRNAs with overall superior stabilities. Usually, the secondary structure of most miRNA's plays key transcriptional roles in modulating the miRNA-mRNA associations that translates to the various miRNAs having different degrees of gene susceptibility. RNA's secondary structure guides mRNA folding, structural stability protection, and RNA-binding proteins recognition sites and functions as a substrate for enzymatically active

TABLE 3 Genes having ontology (PANTHER family, PANTHER protein class) functions in various molecular and biological processes

Sr. no	Gene symbol	Gene name	Panther family	Panther protein class	Biological –process
1	SDC4	SYNDECAN-4	Nuclear Transcription Factor Y Subunit Alpha (Pthr12632:Sf6)	transmembrane signal receptor	cell migration
2	SMU1	WD40 repeat-containing protein SMU1 SMU1 ortholog	Wd40 Repeat-Containing Protein Smu1 (Pthr22848:Sf2)	—	RNA splicing
3	NAV2	Neuron navigator 2 NAV2 ortholog	Neuron Navigator 2 (Pthr12784:Sf6)	—	nervous system development
4	SPATA2	Spermatogenesis-associated protein 2	Spermatogenesis-Associated Protein 2 (Pthr15326:Sf8)	—	tumor necrosis factor-mediated signaling pathway,regulation of programmed cell deathregulation of signal transduction,protein K63-linked deubiquitinatio

TABLE 4 Shortlisted four host genes showing association with respiratory tract disease, results obtained from DisGeNET database

Gene name	Disease	Disease class	Score	PMID
SDC4	Non-Small Cell Lung Carcinoma	Neoplasms; respiratory tract diseases	0.31	28971587
	Pneumonia	Infections; respiratory tract diseases	0.2	31600983
	Community acquired pneumonia	Infections; Respiratory Tract Diseases	0.1	29368632
NAV2	Childhood asthma	Respiratory tract diseases; immune system diseases	0.1	23829686
SMU1	Congenital chromosomal disease	Congenital, hereditary, and neonatal diseases and abnormalities	0.1	31385554
	Influenza	Infections; respiratory tract diseases	0.1	31076555
SPATA2	Non-Small Cell Lung Carcinoma	Neoplasms; respiratory tract diseases	0.06	30249170
	Primary malignant neoplasm of lung	Neoplasms; respiratory tract diseases	0.02	30173128
	Carcinoma of lung	Neoplasms; respiratory tract diseases	0.02	28446615
	Malignant neoplasm of lung	Neoplasms; respiratory tract diseases	0.02	28446615

reactions. From the results of the secondary structure of viral pre-miRNA sequence, we predict that the pre-miR-651 has the highly stable structure due to the MFE ($\Delta G = -24.40$ kcal/mol), three internal loops, one hairpin loop, two bulges, and a minimal number of non-canonical base pairs with maximum base-pairing stems in comparison to other secondary structures (Figure 4A). For miRNA at equilibrium, the structure having the least free energy is the most stable. Similarly, the pre-miR-548-1d has two internal loops, one hairpin loop, three bulges, and a minimum number of non-canonical base-pairing and least number of stem base paring so, the pre-miR-548-1d is less stable than pre-miR-651 (Figure 4B). A similar trend is observed for the other pre-miRNAs and accordingly is arranged in the decreasing order of stability (Figure 4). The viral pre-miRNA sequence's centroid structure is the secondary structure having the shortest base-pair distance. Our results suggest that pre-miR-651 (Figure 5A) has the best centroid structure because this pre-miRNA structure showed minimum base-pair distance and has MFE and minimum thermodynamic ensemble (pf), which leads to higher structural stability as compared with other precursor secondary structures (Figure 5A).[36] The MFE structure's frequency in the ensemble of secondary structures, as well as the ensemble's variety, indicates whether the bases are fundamentally unpaired, weakly paired, or firmly paired. Our results show that the frequency of pre-miR-651 is 4.56%, the value of MFE is −24.40 kcal/mol with maximum number of strong bases pairing, minimum number of non-canonical paired bases and has longer length of pre-miRNA sequence as compared with other frequencies of the predicted secondary structures, which results in having a very high stable structure (Figure 6).

The interaction analysis from the host miRNAs-viral miRNA might help identify the key regions of the formed duplex secondary structure between the miRNA and mRNA. This secondary structure with their base-pair interactions will help understand the type of drug candidates for developing either the new chemical entities or screening the approved drugs as repurposing druggable candidates.

As we observed a strong interaction of the viral pre-miRNAs with the top 5 host miRNAs, we further tried to keep their role in other gene regulations. The network analysis showed that all the selected top 5 host miRNAs are involved in various pathological diseases also show strong interaction with 4 other genes (SDC4, NAV2, SMU1, and SPATA2) (Table 2 and Figure 7).[37–41] Cell migration, RNA splicing,

nervous system growth, tumor necrosis factor-mediated signaling cascade, modulation of programmed cell death, signal transduction regulation, and protein K63-linked deubiquitination are all regulated by these genes (Table 3). Further, STRING online database search of these four genes (SDC4, NAV2, SMU1, and SPATA2) suggested their roles in having primary interactions (Figure S1) with various biological processes and also has the disease association link including the respiratory tract disease (Table 4). Recent research has discovered that host miRNA plays a role in a variety of regulatory pathways, including growth, virus protection, hematopoiesis, cell proliferation, and apoptosis, as well as ACE2 expression during COVID-19 infection. Viral miRNAs have been shown to cause mRNA degradation in host cells, regulating various molecular pathways.[42,43] As a result, it is crucial to annotate the novel coronavirus's miRNA in order to achieve a deeper understanding of the virus and create new anti-miR/antiviral therapy tools. Based on our theoretical findings, we believe that the host miRNA-4476 plays a significant role in the host-pathogen interaction and that recognizing this relationship would aid in the development of future antiviral therapeutics.

5 | CONCLUSION

We predicted 21 possible candidate human miRNAs using computational approaches and a collection of bioinformatics techniques that demonstrated >80% homology with 18 viral pre-miRNA sequences. These 21 miRNAs were further utilized to hybridize with the viral pre-miRNAs and predict the best probable antiviral agents based on their structural binding energies. A total of five human miRNAs showed perfect complementarity with the 3′ region of the precursor sequence of COVID-19. The human miR-4476 had strongest interaction due to the long seed sequence size and maximum complementarity with viral-pre-miR showing a stable structure with the MFE of about −21.80 kcal/mol. These hybridization results suggest that the miRNAs from viral precursor's sequences would be the best target candidates by the human miRNAs. Thus, the five host miRNAs identified in this study may be exploited for use as antiviral agents. Similarly, secondary structure prediction results suggest that pre-miR-651 is a highly stable structure ($\Delta G = -24.40$ kcal/mol MFE), the minimum frequency of ensemble, due to the maximum number of stem base pairing, which helps stabilize the structure.

The interaction analysis from the host miRNAs-viral mRNA might help in identifying the critical regions of the formed duplex secondary structure between the miRNA and mRNA. This type of duplex secondary structure with its high base-pair complementarity interactions will help understand the type of drug candidates needed to develop the new chemical entities or screening the approved drugs for repurposing as druggable candidates. Therefore, these findings not only help obtain insight into the structure–activity relationship of the viral pre-miRNAs and the host miRNAs to develop as antiviral agents but also provide information about the strong physical interactions among them that can inhibit the viral replication and transmission efficiently.

CLINICAL IMPLICATIONS

The clinical implications for the present treatment strategies for SARS-CoV-2 are in progress globally with the use of mRNA or viral vector based vaccines. However, the ability of SARS-CoV-2 variant generation is one of the major issue facing the clinical implications with the above vaccines. Therefore, the present study focus could provide effective solution due to the expression of host miRNAs efficiently targeting the pre-miRNAs of SARS-CoV-2 genome. This targeting approach of specific host miRNA expression could effectively consider any variant of pre-miRNAs of SARS-CoV-2 expressed in the host.

FUNDING INFORMATION

None.

AUTHOR CONTRIBUTION

JS was responsible for the execution, analysis, and writing of the manuscript; AR was responsible for the manuscript writting, NS was responsible for the manuscript writing, result analysis, and discussion; AC was responsible for the manuscript editing and result analysis; KLK was responsible for the study design; PKA conceived the study, design, analysis, writing and revising the manuscript.

ETHICS STATEMENT

Not required.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated during the current study

ORCID

Pramod K. Avti https://orcid.org/0000-0001-5603-4523

REFERENCES

1. Chauhan A, Avti P, Shekhar N, et al. Structural and conformational analysis of SARS-CoV-2 N-CTD revealing monomeric and dimeric active sites during the RNA binding and stabilization: insights towards potential inhibitors for N- CTD. *Comp. Boil. Med*. 2021;134:104495.
2. Avti P, Chauhan A, Shekhar N, et al. Computational basis of SARS-CoV-2 main protease inhibition: an insight from molecular dynamic simulation based findings. *J Bio Mol Stru Dyn*. 2021;13:1-11.
3. Prajapat M, Sarma P, Shekhar N, et al. Drug targets for corona virus: A systematic review. *Indian J Pharmacol*. 2020;52(1):56-65.
4. Sarma P, Shekhar N, Prajapat M, Avti P, et al. In-silico homology assisted identification of inhibitor of RNA binding against 2019-nCoV N-protein (N terminal domain). *J Biomol Struct Dyn*. 2020;18:1-9.
5. Singh J, Malik D, Rania A. Computational investigation for identification of potential phytochemicals and antiviral drugs as potential inhibitors for RNA-dependent RNA polymerase of COVID-19. *J Biomol Struct Dyn*. 2020;17:1-16.
6. Singh J, Malik D, Raina A. Immuno-informatics approach for B-cell and T-cell epitope based peptide vaccine design against novel COVID-19 virus. *Vaccine*. 2021;39(7):1087-1095.
7. Singh J, Malik D, Raina A. Molecular docking analysis of azithromycin and hydroxychloroquine with spike surface glycoprotein of SARS-CoV-2. *Bioinformation*. 2021;17(1):11-22.

8. Mirzaei R, Mahdavi F, Badrzadeh F, et al. The emerging role of microRNAs in the severe acute respiratory syndrome coronavirus2 (COVID-19) infection. *Int Immunopharmacol*. 2020;90:107204.
9. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 2004;2(2):281-297.
10. Ambros V. microRNAs: tiny regulators with great potential. *Cell*. 2001;7(7):823-826.
11. Carrington JC, Ambros V. Role of microRNAs in plant and animal development. *Science*. 2003;5631:336-338.
12. Kozomara A, Griffiths-Jones S. miRBase: annotating high confidence microRNAs using deep sequencing data. *Nucleic Acids Res*. 2014;42(D1):68-73.
13. Sullivan CS, Ganem D. MicroRNAs and viral infection. *Mol Cell*. 2005;1:3-7.
14. Du T, Zamore PD. microPrimer: the biogenesis and function of microRNA. *Development*. 2005;21(21):4645-4652.
15. Zhang H, Li Z, Li Y, et al. A computational method for predicting regulation of human microRNAs on the influenza virus genome. *BMC Syst Biol*. 2013;7:1-14.
16. Chandan K, Gupta M, Sarwat M. Role of host and pathogen-derived microRNAs in immune regulation during infectious and inflammatory diseases. *Front Immunol*. 2020;10:1-14.
17. Sanghvi VR, Steel LF. RNA silencing as a cellular defense against HIV-1 infection: progress and issues. *FASEB j*. 2012;10:3937-3945.
18. Wu Z, Zhu Y, Bisaro DM, Parris DS. Herpes simplex virus type 1 suppresses RNA-induced gene silencing in mammalian cells. *J Virol*. 2009;813(13):6652-6663.
19. Hussain M, Asgari S. MicroRNA-like viral small RNA from dengue virus 2 autoregulates its replication in mosquito cells. *Proc. Natl. Acad. Sci*. 2014;7:2746-2751.
20. Pedersen IM, Cheng G, Wieland S, et al. Interferon modulation of cellular microRNAs as an antiviral mechanism. *Nature*. 2007;449(7164):919-922.
21. Mirzaei R, Mahdavi F, Badrzadeh F, et al. The emerging role of microRNAs in the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. *Int Immunopharmacol*. 2021;90:107204.
22. Dang T, Tyagi S, D'Cunha G, et al. Computational prediction of microRNAs in marine bacteria of the genus Thalassospira. *PloS One*. 2019;14(3):e0212996. https://doi.org/10.1371/journal.pone.0212996
23. Krüger J, Rehmsmeier M. RNAhybrid: microRNA target prediction easy, fast and flexible. *Nucleic Acids Res*. 2006;34:34451-34454.
24. Rehmsmeier M, Steffen P, Hochsmann M, Giegerich R. Fast and effective prediction of microRNA/target duplexes. *RNA*. 2004;10(10):1507-1517.
25. Gruber AR, Lorenz R, Bernhart SH, Neubock R, Hofacker IL. The Vienna RNA websuite. *Nucleic Acids Res*. 2008;36(Web Server):70-74.
26. Zuker M, Stiegler P. Optimal computer folding of large RNA sequences using thermodynamics and auxiliary information. *Nucleic Acids Res*. 1981;1(1):133-148.
27. Szklarczyk D, Morris JH, Cook H, et al. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. *Nucleic Acids Res*. 2017;45(D1):362-368.
28. Piñero J, Ramírez-Anguita JM, Saüch-Pitarch J, et al. The DisGeNET knowledge platform for disease genomics: 2019 update. *Nucleic Acids Res*. 2020;48:845-855.
29. Guo YR, Cao QD, Hong ZS, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak—an update on the status. *Mil Med Res*. 2020;7(1):1-10.
30. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor Recognition by the Novel Coronavirus from Wuhan: an Analysis Based on Decade-Long Structural Studies of SARS Coronavirus. *J Virol*. 2020;94(7):1-9.
31. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med*. 2020;8(8):727-733.
32. Damas ND, Fossat N, Scheel TKH. Functional Interplay between RNA Viruses and Non-Coding RNA in Mammals. *Noncoding RNA*. 2019;5(1):1-25.
33. Harwig A, Das AT, Berkhout B. Retroviral microRNAs. *Curr Opin Virol*. 2014;7:47-54.
34. Sarma A, Phukan H, Halder N, Madanan MG. An in-silico approach to study the possible interactions of miRNA between human and SARS-CoV2. *Comput Biol Chem*. 2020;88:107352.
35. Afonso-Grunz F, Müller S. Principles of miRNA-mRNA interactions: beyond sequence complementarity. *Cell Mol Life Sci*. 2015;16(16):3127-3141.
36. Ding Y, Chan CY, Lawrence CE. RNA secondary structure prediction by centroids in a Boltzmann weighted ensemble. *RNA*. 2005;8(8):1157-1166.
37. Tao Y, Ma C, Fan Q, Wang Y, Han T, Sun C. MicroRNA-1296 facilitates proliferation, migration and invasion of colorectal cancer cells by targeting SFPQ. *J Cancer*. 2018;13(13):2317-2326.
38. Majid S, Dar AA, Saini S, et al. Regulation of minichromosome maintenance gene family by microRNA-1296 and genistein in prostate cancer. *Cancer Res*. 2010;7(7):2809-2818.
39. Kojima M, Sudo H, Kawauchi J, et al. MicroRNA markers for the diagnosis of pancreatic and biliary-tract cancers. *PLoSOne*. 2015;10(2):e0118220.
40. Heyn H, Schreek S, Buurman R, Focken T, Schlegelberger B, Beger C. MicroRNA miR-548d is a superior regulator in pancreatic cancer. *Pancreas*. 2012;2(2):218-221.
41. Chang JT, Wang F, Chapin W, et al. Identification of microRNAs as breast cancer prognosis markers through the Cancer Genome Atlas. *PLoS One*. 2016;11(12):e0168284.
42. Khokhar A, Noorali S, Sheraz M, et al. Computational analysis to predict functional role of hsa-miR-3065-3p as an antiviral therapeutic agent for treatment of triple infections: HCV, HIV-1, and HBV. *Libyan J Med*. 2012;7(1):19774.
43. Widiasta A, Sribudiani Y, Nugrahapraja H, Hilmanto D, Sekarwana N, Rachmadi D. Potential role of ACE2-related microRNAs in COVID-19-associated nephropathy. *Noncoding RNA Res*. 2020;4(4):153-166.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Singh J, Raina A, Sangwan N, Chauhan A, Khanduja KL, Avti PK. Identification of homologous human miRNAs as antivirals towards COVID-19 genome. *Adv Cell Gene Ther*. 2021;4(4):e114. doi: 10.1002/acg2.114