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Computationally predicted SARS-COV-2 encoded microRNAs target NFKB, JAK/STAT and TGFB signaling pathways

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ABSTRACT

Recently an outbreak that emerged in Wuhan, China in December 2019, spread to the whole world in a short time and killed >1,410,000 people. It was determined that a new type of beta coronavirus called severe acute respiratory disease coronavirus type 2 (SARS-CoV-2) was causative agent of this outbreak and the disease caused by the virus was named as coronavirus disease 19 (COVID19). Despite the information obtained from the viral genome structure, many aspects of the virus-host interactions during infection is still unknown. In this study we aimed to identify SARS-CoV-2 encoded microRNAs and their cellular targets. We applied a computational method to predict miRNAs encoded by SARS-CoV-2 along with their putative targets in humans. Targets of predicted miRNAs were clustered into groups based on their biological processes, molecular function, and cellular compartments using GO and PANTHER. By using KEGG pathway enrichment analysis top pathways were identified. Finally, we have constructed an integrative pathway network analysis with target genes. We identified 40 SARS-CoV-2 miRNAs and their regulated targets. Our analysis showed that targeted genes including *NFKB1, NFKBIE, JAK1–2, STAT3–4, STAT5B, STAT6, SOCS1–6, IL2, IL8, IL10, IL17, TGFBR1–2, SMAD2–4, HDAC1–6* and *JARID1A-C, JARID2* play important roles in NFKB, JAK/STAT and TGFB signaling pathways as well as cells' epigenetic regulation pathways. Our results may help to understand virus-host interaction and the role of viral

Abbreviations: ACE-2, angiotensin-converting enzyme 2; AKT1, AKT serine/threonine kinase 1; BCL2, BCL2 apoptosis regulator; CDKL2, cyclin dependent kinase like 2; CDK1, cyclin dependent kinase 1; CTNNB1, catenin beta 1; COVID19, new type corona virus disease; CXCL9, C-X-C motif chemokine ligand 9; CXCL1, C-X-C motif chemokine ligand 1; CXCL10, C-X-C motif chemokine ligand 10; CXCL11, C-X-C motif chemokine ligand 11; CXCL16, C-X-C motif chemokine ligand 16; E2F1, E2F transcription factor 1; EIF4A1, eukaryotic translation initiation factor 4A1; GRB2, growth factor receptor bound protein 2; HDAC1, histone deacetylase 1; HDAC2, histone deacetylase 2; HDAC3, histone deacetylase 3; HIF1A, hypoxia inducible factor 1 subunit alpha; ICTV, International Committee on Taxonomy of Viruses; IL2, interleukin 2; IL5, interleukin 5; IL7, interleukin 7; IL8, interleukin 8; IL10, interleukin 10; IL13, interleukin 13; IL15, interleukin 15; IL16, interleukin 16; IL17A, interleukin 17 A; IL21, interleukin 21; IL22, interleukin 22; IL24, interleukin 24; IL25, interleukin 25; IL33, interleukin 33; IKBKE, inhibitor of nuclear factor kappa B kinase subunit epsilon; IFNGR2, interferon gamma receptor 2; JAK1, Janus kinase 1; JAK2, Janus kinase 2; JARID1A, lysine demethylase 5A; JARID1C, lysine demethylase 5C; JARID2, Jumonji and AT-rich interaction domain containing 2; JARID1B, lysine demethylase 5B; SARS-CoV-2, severe acute respiratory disease coronavirus type 2; KEGG, Kyoto Encyclopedia of Genes and Genomes; MAPK1, mitogen-activated protein kinase 1; MAPK3, mitogen-activated protein kinase 3; MAPK4, mitogen-activated protein kinase 4; MAPK6, mitogen-activated protein kinase 6; MAPK7, mitogen-activated protein kinase 7; NFKB1, nuclear factor kappa B subunit 1; NFKBIE, NFKB inhibitor epsilon; NOS3, nitric oxide synthase 3; PANTHER, protein analysis through evolutionary relationships; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PTEN, phosphatase and tensin homolog; RHOA, ras homolog family member A; RB1, RB transcriptional corepressor 1; SOCS1, suppressor of cytokine signaling 1; SOCS3, suppressor of cytokine signaling 3; SOCS4, suppressor of cytokine signaling 4; SOCS5, suppressor of cytokine signaling 5; SOCS6, suppressor of cytokine signaling 6; SP1, Sp1 transcription factor; STAT6, signal transducer and activator of transcription 6; STAT4, signal transducer and activator of transcription 4; STAT3, signal transducer and activator of transcription 3; STAT5B, signal transducer and activator of transcription 5B; SMAD2, SMAD family member 2; SMAD3, SMAD family member 3; SMAD4, SMAD family member 4; SUMO1, small ubiquitin like modifier 1; SUMO2, small ubiquitin like modifier 2; SOS1, SOS Ras/Rac guanine nucleotide exchange factor 1; TGFBR1, transforming growth factor beta receptor 1; TGFBR2, transforming growth factor beta receptor 2; TBP, TATA-box binding protein; TMPRSS11A, transmembrane serine protease 11A; TMPRSS4, transmembrane serine protease 4; TNFRSF21, TNF receptor superfamily member 21; WHO, World Health Organization.

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miRNAs during SARS-CoV-2 infection. As there is no current drug and effective treatment available for COVID19, it may also help to develop new treatment strategies.

1. Introduction

The outbreak that emerged in Wuhan, China in December 2019, spread to the whole world in a short time. It was determined that a new type of beta coronavirus called severe acute respiratory disease coronavirus type 2 (SARS-CoV-2) was causative agent of this outbreak (Wang et al., 2020). The disease caused by the virus was named coronavirus disease 19 (COVID-19) by International Committee on Taxonomy of Viruses (ICTV) as its similarity to the one that caused the SARS outbreak (SARS-CoVs). Later, it was declared as a pandemic in March 2020 by World Health Organization (WHO). COVID-19 spreads all over the world except Antarctica in four months and transmitted to >60 million people and killed over 1,410,000 people. COVID-19 is mainly transmitted between people through "respiratory droplets" when symptomatic people sneeze or cough (Huang et al., 2020; Li et al., 2020). The disease starts as an acute pneumonia in most of the patients with clinical symptoms including fever, cough, myalgia and fatigue. In severe forms of the disease most patients develop and die. The cytokine storm was reported to be associated with disease severity (Guo et al., 2020; Huang et al., 2020).

SARS-CoV-2 is a positive-stranded RNA virus belongs to Coronaviridae family. Its single-stranded RNA genome contains 29,891 nucleotides with a 5′-cap structure and 3′-poly-A tail, encoding for 9860 amino acids. Its genome shows 89% nucleotide identity with bat SARS-like-CoVZXC21 and 79.2% with that of human SARS-CoV (Chan et al., 2020; Lu et al., 2020). Sequence analysis of SARS-CoV-2 revealed that ORF1ab located in the first part of the viral genome translates polyproteins pp1a and pp1ab that responsible for encoding 16 nonstructural proteins (NSP). The remaining virus genome encodes several accessory proteins and four major structural proteins the spike (S) protein, nucleocapsid (N) protein, membrane (M) protein, and the envelope (E) protein. The S protein is responsible for facilitating entry of the CoV into the target cells. To infect humans it binds to Angiotensin-Converting Enzyme 2 (ACE-2) and enters cells (P. Zhou et al., 2020).

Despite the information obtained from the viral genome structure, many aspects of virus-host interactions are still unknown during infection. In recent years, the relationship has been reported between small RNA molecule pathways, especially microRNA (miRNA) pathways, and diseases including cancer, cardiovascular disease, neurodegenerative disease and diseases caused by viruses and bacteria (Backes et al., 2012; Femminella et al., 2015; Ha, 2011; Jopling, 2005; Qi et al., 2016).

miRNAs are ~19–24 nt non-coding RNA molecules that post-transcriptionally regulate gene expression by binding to target messenger RNAs (mRNAs) (Bartel, 2009). First identified in *Caenorhabditis elegans*, miRNAs are expressed by all metazoans and plants, as well as by several DNA and RNA viruses, and function as regulators of cellular processes such as development, differentiation, growth, homeostasis, stress responses, apoptosis and immune activation (Beckham and Parker, 2008; Friedman et al., 2009; Skalsky and Cullen, 2010; Xiao and Rajewsky, 2009).

Results obtained in the studies conducted after 2003 SARS and 2014 Ebola virus outbreaks showed that viral small non-coding RNA molecules are involved in pathogenesis of the disease (Morales et al., 2017; Prasad et al., 2019; Teng et al., 2015). Discovering these RNA molecules may help to understand virus-host interactions during infection that could speed up effective treatment of SARS-CoV-2 caused COVID-19. In this study bioinformatics-driven prediction tools were used to find the presence of putative miRNAs encoding by SARS-CoV-2 genome. The identified 40 different putative miRNAs encoded from different regions of SARS-Cov-2 viral RNA genome were found to be targeting different human genes involved in host biological processes such as apoptosis, immune system, cell cycle and regulation of transcription signaling pathways. Our results may help to understand virus-host interaction and the role of viral miRNAs during SARS-CoV-2 infection. As there is no current drug and effective treatment available for COVID-19, it may also help to develop new treatment strategies.

2. Materials and methods

2.1. Bioinformatics prediction of SARS CoV2 miRNAs

We retrieved full SARS-CoV-2 viral genome with NC_045512 accession number from the genome browser at NCBI Database.

A flowchart describing the computational prediction of the putative SARS-CoV-2 miRNAs is shown in Fig. 1.

Precursor miRNA (pre-miRNA) candidates assumed to be encoded from the viral genome were predicted by the VMir program (Grundhoff et al., 2006). This program identifies possible stem-loop structures using the RNAFold database. VMir predictions were carried out using the default parameters. The putative pre-miRNAs that satisfied the filter parameters of a VMir score ≥ 150 and a window count ≥35 and stringency = 30 were selected for further assessment. Then, MiPred analysis was carried out in the R package program to remove pseudo miRNAs from the obtained sequences to identify candidates with appropriate secondary structure folds and free energy values (Jiang et al., 2007). MatureBayes program was used settings to obtain mature 3′ and 5′ stem sequences from pre-miRNA sequences, with default settings (Gkirtzou et al., 2010). The BLASTn option in the miRBase database was used to investigate the similarity of identified mature viral miRNAs to previously identified human miRNAs (Griffiths-Jones et al., 2008). To provide a more accurate estimate, the secondary and tertiary structures of each candidate pre-miRNA sequences were determined using RNAFold program in Vienna format (dot-bracket) and then visualized using the VARNA program (Darty et al., 2009). Finally, the tertiary structures of candidate premiRNAs were visualized by the RNA FRABASE database (Popenda et al., 2010).

2.2. Prediction of SARS CoV-2 miRNA targets

Human target genes of novel the SARS-CoV-2 miRNAs were investigating using three miRNA prediction methods: TargetScanHuman 7.2 custom with using miRNAs' 7-mer seed regions; miRDB custom with target score ≥ 95 option and miRanda 3.0 with Score Threshold≥140 filter option (Agarwal et al., 2015; Enright et al., 2003; Wong and Wang, 2015). The common target genes, which are obtained from these three programs, were used for subsequent KEGG and GO analysis.

2.3. Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis and constructing gene regulation network

We used KEGG (Kyoto Encyclopedia of Genes and Genomes) a database resource that integrates genomic, chemical and systemic functional information, for pathway analysis (Kanehisa, 2000). The PATHWAY database records networks of molecular interactions in the cell that includes organism-specific network maps (http://www.genome.jp/kegg/). Integration map of target genes were drawn using NetworkAnalyst program (G. Zhou et al., 2019).

2.4. Gene ontology (GO) analysis

Target genes with an interaction value of over 80 were filtered and used in GO and PANTHER analyzes (Ashburner et al., 2000; Carbon

et al., 2019; Mi et al., 2019). GO analysis of the significant probe list was performed using PANTHER (http://www.pantherdb.org/) text files containing the Gene ID list.

3. Results

3.1. Prediction of mature SARS-CoV-2 miRNAs

SARS-CoV-2 genome with the accession number of NC_045512 was used as a reference genome. Using VMir default parameters, we identified 1114 candidate precursor miRNA (Pre-miRNA) sequences between 50 and 220 nucleotides in length. When pseudo-pre-miRNA sequences were removed, 20 real pre- miRNA candidates were found in MiPred analysis. Sequence of each pre-miRNAs, their secondary structure in Vienna format and MFE values are given in Table 1.

MatureBayes tool was used to predict mature pre-miRNAs from the pre-miRNA stem loops and 40 mature miRNAs were identified and named as SARS-CoV-2 pre-miR-5p for forward and SARS-CoV-2 pre-miR-3p for reverse orientation (Table 2). Position of each SARS-CoV-2 pre-miRNA, their orientation and minimum free energy values (MFE) with forward or reverse orientation are shown on the genome of the virus (Fig. 2). Out of 20 pre-miRNAs 14 pre-miRNAs were encoded from viral *orf1ab* gene (SARS-CoV-2 pre-miR-D1 and pre-miR-D2 were from *nsp2*; SARS-CoV-2-pre-miR-D3, pre-miR-D4, pre-miR-D5, pre-miR-R1, and pre-miR-R2 were form *nsp3*; SARS-CoV-2-pre-miR-D6 was from *nsp4*; SARS-CoV-2-pre-miR-D7 and pre-miR-D8 were from *nsp6*; SARS-CoV-2-pre-miR-R3 was from *RdRP*; SARS-CoV-2-pre-miR-D9 was from exonuclease; SARS-CoV-2-pre-miR-D10 from *EndoRNase*; SARS-CoV-2-pre-miR-D11 was from *ribose methlytransferase*). Three miRNAs were encoded from M gene (SARS-CoV-2-pre-miR-D12, and pre-miR-D13, pre-miR-R5). SARS-CoV-2-pre-miR-R4, pre-miR-D14 and pre-miR-R6 were encoded from *orf3a*, *orf7a/b* and *orf10* respectively.

The BLASTn option was used in the miRBase database to investigate whether each mature SARS-CoV-2 premiRNA sequence shows any similarity to previously identified miRNAs. We found that the SARS-CoV-2-D1-5p, D4-3p, R3-3p and R6-5p pre-miRNAs exhibit similarity to human hsa-miR-4696-3p, hsa-miR-4502, hsa-miR-363-5p and hsa-miR-411-5p, respectively.

RNA fold prediction was used to verify the secondary and tertiary structures of each SARS-CoV-2 pre-miRNA. Folds of these pre-miRNAs, along with their flanking sequences, into the expected hairpin structures were successful in all candidates. The MFE value of each stable stem loop secondary structure varies between −15.6 and − 42.10 (Table 1). The secondary structures of each pre-miRNA sequence visualized with the VARNA program and the tertiary structures obtained computationally are given in Fig. 3. The positions of possible mature miRNAs were colored in secondary and tertiary structures (Fig. 3).

3.2. Prediction of SARS-CoV-2 miRNA target genes

Based on the sequences of the 40 SARS-CoV-2 miRNAs, their target genes were searched by TargetScan and miRDB under custom prediction and miRanda with filtering option. In total 5615, 1235 and 265 human genes were targeted by 40 SARS-CoV-2 miRNAs with TargetScan, miRDB and miRanda programs, respectively. The results of the all target genes according to of each miRNA are given in Table S1. Sequences with network interaction scores <80 were removed and the high probability targets of each miRNA, determined by TargetScan, miRDB and miRanda programs, were used in further analysis.

Identified proteins were clustered into groups according to their biological process, molecular function, protein class, cellular compartment and signaling pathways using GO and PANTHER analysis. With such a grouping, multiple functions and protein interaction networks of a protein can be revealed. Biological process group included proteins involved in cellular process (22.10%), biological regulation (15%) and metabolic process (15.30%) (Fig. 4a). The cellular compartment group included proteins located in cell (23.80%), cell part (23.80%) and organelles (16.90%) (Fig. 4b). Molecular function group comprised proteins with binding (41.90%), catalytic activity (34.70%), and transcription regulator activity (9%) (Fig. 4c). Protein Class group included proteins with protein modifying enzyme (24.5%), gene specific transcriptional regulator (15.1%) and protein-binding activity modulator (13.2%) (Fig. 4d).

Our KEGG analysis revealed that while 89 different clusters are formed in terms of pathways, 31 of them are represented below 1%. The top represented pathways were 5.10% Gonadotropin-releasing hormone receptor (P06664), 5.10% Angiogenesis (P00005), 5.00% CCKR signaling map (P06959), 4.40% EGF receptor signaling pathway (P00018), 4.10% Inflammation mediated by chemokine and cytokine signaling (P00031), 3.20% T cell activation (P00053), 3.50% PDGF signaling (P06959) P00047) and 2.70% Interleukin signaling pathway (P00036) (Fig. 4e). These pathways are all important for host-virus interactions.

KEGG pathway enrichment annotations were also carried out using the NetworkAnalyst program in order to obtain target gene interaction map. Potential target genes involve in negative regulation of apoptotic processes, viral processes, protein phosphorylation and regulation of cell cycle (Fig. 5).

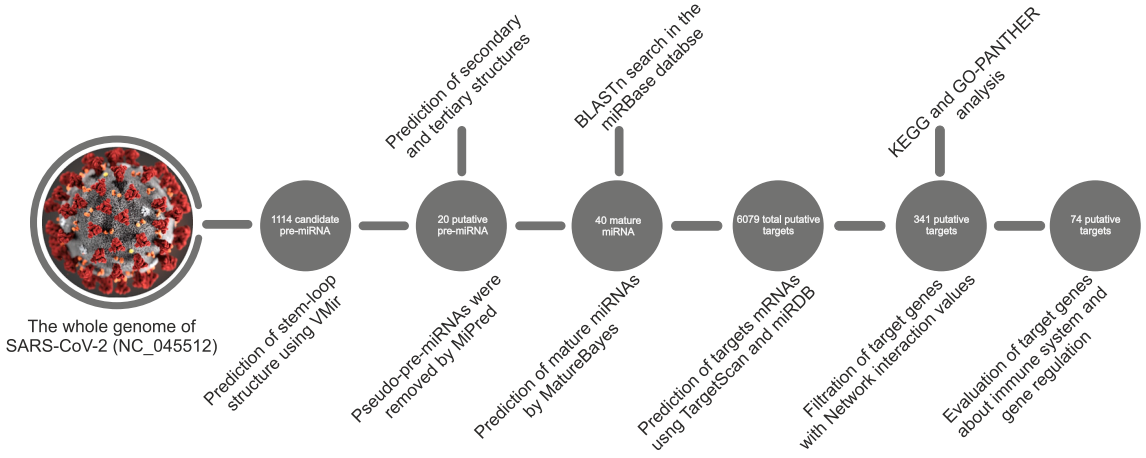

Fig. 1. Workflow of SARS-CoV2 encoded miRNA prediction. Flowchart describes the computational prediction steps. The MiPred algorithm was used to identify genuine pre-miRNAs, and the MatureBayes tool was used to predict the mature miRNA sequences.

Table 1
SARS-CoV-2 encoded pre-miRNAs.

Name	Orientation	Primary sequence	Secondary structure	MFE	Location	Gene	Size	Score
SARS-CoV-2-pre-miR-R6	Reverse	GAAUUCAUUCUGCACAAGAGUA GACUAUAUAUCGUAAACGGAAAAGCG AAAACGUUUAUAUAGCCCAUCUGCC UUGUGUGGUCUGCAUGAGUUU	((((((((.((((((((.((((((((((.(((…((……)) …))))..)))))))….))))))))))))…..))))))))	−29,00	29,532–29,625	gene N (structural nucleocapsid phosphoprotein) + ORF10	94	129.2
SARS-CoV-2-pre-miR-R5	Reverse	UAAUAUCUCUGCUAUAGUAAC CUGAAAGUCAACGAGAUGAAACAUCU GUUGUCACUUACUGUACAAG CAAAGCAAUAUUG	(((((((((.((((((((((..(((……..((((.((((((…)))))))))))).)))))))…)))).)).)..))))))	−16,20	27,167–27,246	gene M+ ORF6	80	120.7
SARS-CoV-2-pre-miR-R4	Reverse	AUUGUGUGAAUUUGGACAUGUU CUUCAGGCUCAUCAACAAUUUUAU UGUAGAUGAAGAAGGUAACAU GUUCAACACCAGU	((((.(((……..(((((((((((((((....(((((.(((((…))))).)))))..)))))..))))))))))))))))	−25,30	26,059–26,138	ORF3a	80	122.2
SARS-CoV-2-pre-miR-R3	Reverse	GGCAUACUUAAGAUUCAUUUGA GUUAUAGUAGGGAUGACAUUACGUUUU GUAUAUGCGAAAAGUGCAUCUUGAUCC UCAUAACUCAUUGAAUCAUAAUAAAGUCUAGCC	(((..((((..((((((..((((((((((..((((((.((((……..((((((….))))))))))))))))))….)).)))))))).)))))).……))))…)))	−28,80	14,973–15,081	ORF1ab-RdRP	109	129.5
SARS-CoV-2-pre-miR-R2	Reverse	GGUUUAUGUAACAAUUUAGCUCCUU UCUUAAAAGAGGGUGUGUAGUGUUUA UAAUCAAUAGCCACCACAUCACCAUUUAA GUCAGGGAAAAAUGUAACUUUAAGCU	((((((.((.(((.(((……..((((((.((((((….(((((((.(((.((((… ..)))).)))).)))).))))))..)))))))).)))..))))))	−25,10	6126–6231	ORF1ab- nsp3	106	134.4
SARS-CoV-2-pre-miR-R1	Reverse	GUGGUUAUAUAAUUGUCUGUUGG CACUUUUCUCAAAGCUUUCGCUAGCA UUUCAGUAGUGCCACCAGCCUU UUUAGUAGGUAUAACCAC	((((((((((.((((.(((.((((((……..((.((((((… …))).)))).)).)))))))).)))……))))..))))))))))	−28,70	4160–4248	ORF1ab- nsp3	89	119.1
SARS-CoV-2-pre-miR-D14	Direct	GGAAGUUCAAGAACUUUACUCUCC AAUUUUUCUUAUUGUUGCGGCAAU AGUGUUUAUAACACUUUGCUUCAC ACUCAAAAGAAAGACAGAAUGAUUGAACUUUC	((((((((((……..(((.(((….(((((((((.(((.((.(((((. ((((((…))))))))))).))).. ..))))))))))).)))..)))))))))))	−25,50	27,666–27,769	ORF7a + ORF7b	104	144.6
SARS-CoV-2-pre-miR-D13	Direct	GCUGUGACAUCAAGGACCUGCCUAAAGAA AUCACUGUUGCUACAUCACGAACGCUUU CUUAUUACAAAUUGGGAGCUUCGCAGCGU GUAGCAGGUGACUCAGGUUUUGCUGCAUACAGU	((((((……(((((((((……((…(((((..(((((((((..((((.((((((….. …….))))))))))….))))))))))))))))))))))……))))))	−36,60	26,995–27,113	gene M (structural membrane glycoprotein)	119	118.4
SARS-CoV-2-pre-miR-D12	Direct	ACCGCUUCUAGAAAGUGAACUCGUAAU CGGAGCUGUGAUCCUUCGUGGACAUCU UCGUAUUGCUGGACACCAUCUAGGACGCUGU	((.(((((((((..(((..((.((((((((((..(((.(((… ..))))))))))).)))))..)).)))..)))))))).)).))	−27,20	26,915–26,999	gene M (structural membrane glycoprotein)	85	120.8
SARS-CoV-2-pre-miR-D11	Direct	AACAAAAGCUAGCUCUUGGAGGUUCCGUGGCU AUAAAGAUAACAGAACAUUCUUGGAAUGCUG AUCUUUAUAAGCUCAUGGGACACUUCGC AUGGUGGACAGCCUUUGUU	((((((.(((.(((..(((((((((((((((((((((..(((..(((((.. .))))))))))))))).)))..)))))))).)))))…….)))….))).))))))	−38,40	21,131–21,240	ORF1ab- ribose methyltransferase	110	143.6
SARS-CoV-2-pre-miR-D10	Direct	UAUUUCAUAACAGAUGCGCAAACAGGUUC AUCUAAGUGUGUGUGUUCUGUUAUUGAUUUA	((..((((((((((.(((((.(((((…..)) ……))).)))))))))))).)))..))	−15,60	20,452–20,511	ORF1ab- endoRNase	60	120.6
SARS-CoV-2-pre-miR-D9	Direct	UAUAGAUUAUGUACCACUAAAGUCUGCU ACGUGUAUAACACGUUGCAAUUUA GGUGGUGCUGUCUGUA	(((((((…(((((((((.((((.(((.(((((.. …))))).)))))))).)))))))).)))))))	−30,40	19,425–19,492	ORF1ab- exonuclease	68	121.8
SARS-CoV-2-pre-miR-D8	Direct	UGACACUCGUUUAUAAAGUUU AUUAUGGUAAUGCUUUAGAUCAA GCCAUUUCCAUGUGGGCUCUUAU AAUCUCUGUUA	(((((.....(((((((((((((((.((((.(((((((… …))).)))))..)))))))))))).)))))))….)))))	−17,10	11,409–11,486	ORF1ab- nsp6	78	123.1
SARS-CoV-2-pre-miR-D7	Direct	GCUAGUUGGGUGAUGCGUAUUAUGA CAUGGUUGGAUAUGGUUGAUACU AGUUUGUCUGGUUUUAAGCUAAAAGAC UGUGUUAUGUAUGCAUCAGCUGUAGU	((((..(((.((((((((((.((((((((((.....(((((…(((((.....))))). …)))))……))))))))))))))))))))))))))	−36,60	11,234–11,334	ORF1ab- nsp6	101	148
SARS-CoV-2-pre-miR-D6	Direct	GCUGCGCUGUGCACCUUUUUGUUAAA UAAAGAAAUGUAUCUAAAGUUGCGUA	(((.(((((((((.....(((((((..((((((…(((((((..(((((((((.((.(((.. ..))).)))))))))))….)))))))…..)))))))))))))…..))))…….)))).)))	−35,00	9797–9924	ORF1ab- nsp4	128	125.4

(*continued on next page*)

Table 1 (*continued*)

Name	Orientation	Primary sequence	Secondary structure	MFE	Location	Gene	Size	Score
		GUGAUGUGCUAUUACCUCUUACGCAAU						
		AUAAUAGAUACUUAGCUCUUUAUAAU						
		AAGUACAAGUAUUUUAGUGGAGC						
SARS-CoV-2-pre-miR-D5	Direct	GACAAUGUCUUAUCUACUUUUAUUUC	((((((((((.(((((.((...(((((((....))).))))))))	−23,80	8144–8221	ORF1ab- nsp3	78	119.2
		AGCAGCUCGGCAAGGGUUUGUUGAUUC	.....))).)))).....))))))))))					
		AGAUGUAGAAACUAAAGAUGUUGUU						
SARS-CoV-2-pre-miR-D4	Direct	UGUCAACCUAUACUGUUACUAG	((.(((((((.(((((((((((((((..	−26,80	7961–8026	ORF1ab- nsp3	66	117.4
		AUCAGGCAUUAGUGUCUGAUGUU	..)))))))).))))))))))).)..))))))					
		GGUGAUAGUGCGGAAGUUGCA						
SARS-CoV-2-pre-miR-D3	Direct	GAUGAAAGGAUUGAUAAAGUA	((((...((.((.(((((((.(((((..(((((.((((((.(((((((.	−42,10	2801–2925	ORF1ab- nsp3	125	132.5
		CUUAAUGAGAAGUGCUCUGCCUAU	(((((((((.(((...))).)).)))))))).))))).....					
		ACAGUUGAACUCGGUACAGAAGUA	))))))))).)))))..)))))))))..))))					
		AAUGAGUUCGCCUGUGUUGUGGCAG						
		AUGCUGUCAUAAAAACUUUGCAACCAGUAUC						
SARS-CoV-2-pre-miR-D2	Direct	UAACAUCUUUGGCACUGUUUAUGA	(((((.((...(((((((.	−29,30	2107–2213	ORF1ab- nsp2	107	126.3
		AAAACUCAAACCCGUCCUUGAUUG	(((.(((((((..(((..(((((((.....(((((.....))))))))))).))).					
		GCUUGAAGAGAAGUUUAAGGAAGG	..))))))).)))))))...)).))))					
		UGUAGAGUUUCUUAGAGACGGUUGGGAAAUUGUUA						
SARS-CoV-2-pre-miR-D1	Direct	CAAUACUAGAUGGAAUUUCACAGU	(((.....(((((((((((.(((.(((((((.((.(..	−18,70	1968–2035	ORF1ab- nsp2	68	116.8
		AUUCACUGAGACUCAUUGAUGCU	...)).)))))))).))).)))))))..)))					
		AUGAUGUUCACAUCUGAUUUG						

3.3. Analysis of SARS-COV-2 miRNA targeted genes

In order to narrate gene targets of SARS-CoV-2 miRNAs, we further filtered the genes according to cytotoxin and gene regulation-association using PANTHER analysis. Main gene targets are shown in Table 3.

Our analysis showed that proteins that have critical roles in NFKB, JAK/STAT and TGFB pathways were targets of SARS-CoV-2 miRNAs. In addition, epigenetic factors such as JARIDs and HDACs are targeted by SARS-CoV-2 miRNAs. Tumor suppressor genes including *RB1*, *PTEN*, *AKT1, CTNNB1* and different serine threonine and tyrosine kinases are among the viral miRNA targets.

4. Discussion

The COVID-19 outbreak caused by SARS-CoV-2, which started in December 2019 in Wuhan, China, spread all over the world. >60 million people were infected and >1,410,000 people were killed to date (Valencia, 2020; Wu et al., 2020). The epidemic declared as a pandemic continues to threaten the whole world. Despite the intensive studies of the scientific world, an effective treatment against the disease has not been developed yet. Although SARS-CoV-2 transmission method, entry into cells, and genome structure were determined, virus-host interaction is still unknown.

Viruses, despite their small genomes, have evolved to use the cellular mechanisms of the host they infect (Domingo and Perales, 2019; Holmes, 2009). Most DNA viruses encode miRNAs to hijack vital cellular functions of hosts to maintain viral pathogenesis, but RNA-virus encoded miRNAs remain controversial. Several miRNAs have been experimentally identified including West Nile virus (WNV), Dengue virus (DENV), hepatitis A virus (HAV) and Ebola virus. Additionally viral miRNAs in numerous viruses have been identified using computational prediction followed by experimental validation (Hussain and Asgari, 2014; Hussain et al., 2012; Liang et al., 2014).

Here, we applied a computational method to predict miRNAs encoded by SARS-CoV-2 along with their putative targets in humans. Targets of predicted miRNAs were clustered into groups according to their biological processes, molecular function, and cellular compartments using GO and PANTHER databases. By using KEGG pathway enrichment analysis top pathways were identified. Finally, we have constructed an integrative pathway network analysis with target genes.

The computational method used in this study was very similar to one applied previously to predict Ebola virus miRNAs (Liang et al., 2014). We identified 40 mature miRNAs encoded from SARS-CoV-2-genome. Considering the results, we obtained, it was seen that SARS-COV-2 miRNAs, targeted many human genes with different functions as expected.

miRNAs are potentially ideal tools for viruses to modulate gene expression because a single miRNA could modulate many genes. They act to achieve latent and lytic life cycle balance for persistence of virus, immune evasion, prevention of apoptosis, viral replication regulation and host cell cycle regulation (Takane and Kanai, 2011).

Our target gene analysis showed that the identified 40 SARS-CoV-2 miRNAs target genes in signaling pathways including NFKB, JAK/STAT and TGFB signaling pathways. All of these pathways are vital for the cell to be protected from viral infections. The NFKB pathway is often targeted by viral pathogens to enhance viral replication, host cell survival and host immune evasion. Viruses may activate or suppress NFKB (Santoro et al., 2003). In our study *NFKB1* and *NFKBIE* genes were targeted by SARS-COV-2 miRNAs; SARS-CoV-mir-D8-5p, SARS-CoV-mir-R1-5p respectively. The *NFKB1* gene encodes the protein p105, which can undergo proteosomal processing to p50. The protein p50 most commonly binds to p65 and together constitute the classic canonical NFKB heterodimer that drives expression of pro-inflammatory genes (Schmitz et al., 2014). *NFKBIE* encodes Iκ B epsilon (Iκ Bε) and it inhibits NFKB by complexing with and trapping it in the cytoplasm (Rahman and McFadden, 2011). Another gene in this pathway targeted

Table 2
MatureBayes analysis of SARS-CoV-2 miRNAs.

MatureBayes results				
Name	5′ stem		3′ stem	
	Position	Sequence	Position	Sequence
SARS-CoV-2-pre-miR-R6	15–36	AAGAGUAGACUAUAUAUCGUAA	54–75	UUUAUAUAGCCCAUCUGCCUUG
SARS-CoV-2-pre-miR-R5	34–55	AGAUGAAACAUCUGUUGUCACU	44–65	UCUGUUGUCACUUACUGUACAA
SARS-CoV-2-pre-miR-R4	32–53	AUCAACAAUUUUAUUGUAGAUG	54–75	AAGAAGGUAACAUGUUCAACAC
SARS-CoV-2-pre-miR-R3	15–36	CAUUUGAGUUAUAGUAGGGAUG	58–79	AAAAGUGCAUCUUGAUCCUCAU
SARS-CoV-2-pre-miR-R2	24–45	UUCUUAAAAGAGGGUGUGUAGU	61–82	CCACCACAUCACCAUUUAAGUC
SARS-CoV-2-pre-miR-R1	23–44	CACUUUUCUCAAAGCUUUCGCU	48–69	AUUUCAGUAGUGCCACCAGCCU
SARS-CoV-2-pre-miR-D14	46–67	AUAGUGUUUAUAACACUUUGCU	81–102	AAAGACAGAAUGAUUGAACUUU
SARS-CoV-2-pre-miR-D13	32–53	ACUGUUGCUACAUCACGAACGC	72–93	GAGCUUCGCAGCGUGUAGCAGG
SARS-CoV-2-pre-miR-D12	35–56	UGAUCCUUCGUGGACAUCUUCG	52–73	CUUCGUAUUGCUGGACACCAUC
SARS-CoV-2-pre-miR-D11	15–36	UUGGAGGUUCCGUGGCUAUAAA	61–82	UGAUCUUUAUAAGCUCAUGGGA
SARS-CoV-2-pre-miR-D10	3–24	UUCAUAACAGAUGCGCAAACAG	37–58	GUGUGUGUUCUGUUAUUGAUUU
SARS-CoV-2-pre-miR-D9	7–28	UAUGUACCACUAAAGUCUGCUA	42–63	UUGCAAUUUAGGUGGUGCUGUC
SARS-CoV-2-pre-miR-D8	12–33	AUAAAGUUUAUUAUGGUAAUGC	44–65	GCCAUUUCCAUGUGGGCUCUUA
SARS-CoV-2-pre-miR-D7	43–64	AUACUAGUUUGUCUGGUUUUAA	75–96	UGUGUUAUGUAUGCAUCAGCUG
SARS-CoV-2-pre-miR-D6	33–54	AUGUAUCUAAAGUUGCGUAGUG	99–120	UAUAAUAAGUACAAGUAUUUUA
SARS-CoV-2-pre-miR-D5	26–47	AGCAGCUCGGCAAGGGUUUGUU	42–63	UUUGUUGAUUCAGAUGUAGAAA
SARS-CoV-2-pre-miR-D4	5–26	ACCUAUACUGUUACUAGAUCAG	39–60	GAUGUUGGUGAUAGUGCGGAAG
SARS-CoV-2-pre-miR-D3	37–58	CUGCCUAUACAGUUGAACUCGG	69–90	AAUGAGUUCGCCUGUGUUGUGG
SARS-CoV-2-pre-miR-D2	27–48	ACUCAAACCCGUCCUUGAUUGG	64–85	AAGGAAGGUGUAGAGUUUCUUA
SARS-CoV-2-pre-miR-D1	7–28	AGAUGGAAUUUCACAGUAUUCA	41–62	GAUGCUAUGAUGUUCACAUCUG

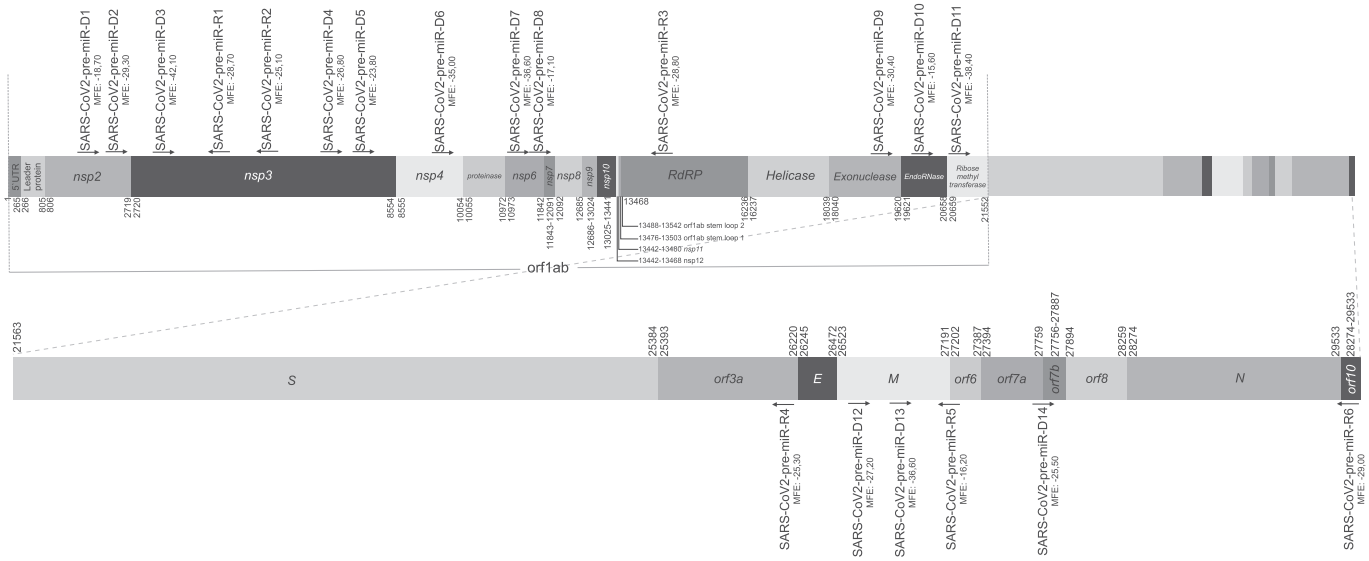

Fig. 2. SARS-CoV-2 encoded pre-miRNAs. Position, orientation and minimum free energy values (MFE) of each SARS-CoV-2 encoded pre-miRNA are shown on viral genome.

by SARS-CoV-2 miRNA is *IKBKE*. It is a serine/threonine kinase that plays an essential role in regulating inflammatory responses to viral infection, through the activation of the type I IFN, NFKB and JAK/STAT signaling (Collins and Mossman, 2014; Rahman and McFadden, 2011). MERS-CoV and SARS-CoV proteins have also been shown to promote NFKB activation. Canton et al. reported that MERS-CoV 4b protein is necessary for the inhibition of NFKB activation in the context of MERS-CoV infection (Canton et al., 2018). Treatment with drugs that inhibited NFKB activation led to a reduction in inflammation and lung pathology in both SARS-CoV-infected cultured cells and mice and significantly increased mouse survival after SARS-CoV infection (Vitiello et al., 2012). So, the role of *NFKB1* and *NFKBIE* targeted by SARS-CoV2 miRNAs can be studied as a potential therapeutic target in COVID-19 (Vitiello et al., 2012), as there are >700 NFKB inhibitors described (Gilmore and Herscovitch, 2006).

CXCL proteins are pro-inflammatory proteins induced during viral infections. CXCL9 and CXCL10 stimulate the activation and migration of immune cells to the infected sites, especially activated T cells. Previous studies with human immunodeficiency virus (HIV), human cytomegalovirus, hepatitis C virus (HCV) and avian H5N1 and human pdmH1N1 and H3N2 viruses showed that CXCL8, CXCL9, and CXCL10 are associated with pathogenicity and may serve as clinical indicator for clinical disease severity and progression and antiviral therapy outcome (Betakova et al., 2017; Stacey et al., 2009). In our study, *CXCL1*, *CXCL9*, *CXCL10*, *CXCL11* and *CXCL16* were targeted by 6 different SARS-CoV-2 miRNAs (Table 3). Analysis the level of these proteins in COVID-19 patients may help to understand the severity of the disease.

JAK-STAT pathway is another important pathway for cells to fight against viral infections. As this pathway is an important regulator of host immune responses against invading viruses, it has been the target of many viral proteins (Shuai and Liu, 2003). It has also been implicated in many autoimmune diseases (Salas et al., 2020). Therefore, the JAK/STAT pathway should work effectively, when the immune system is activated, and the effect of this pathway should be reduced when the

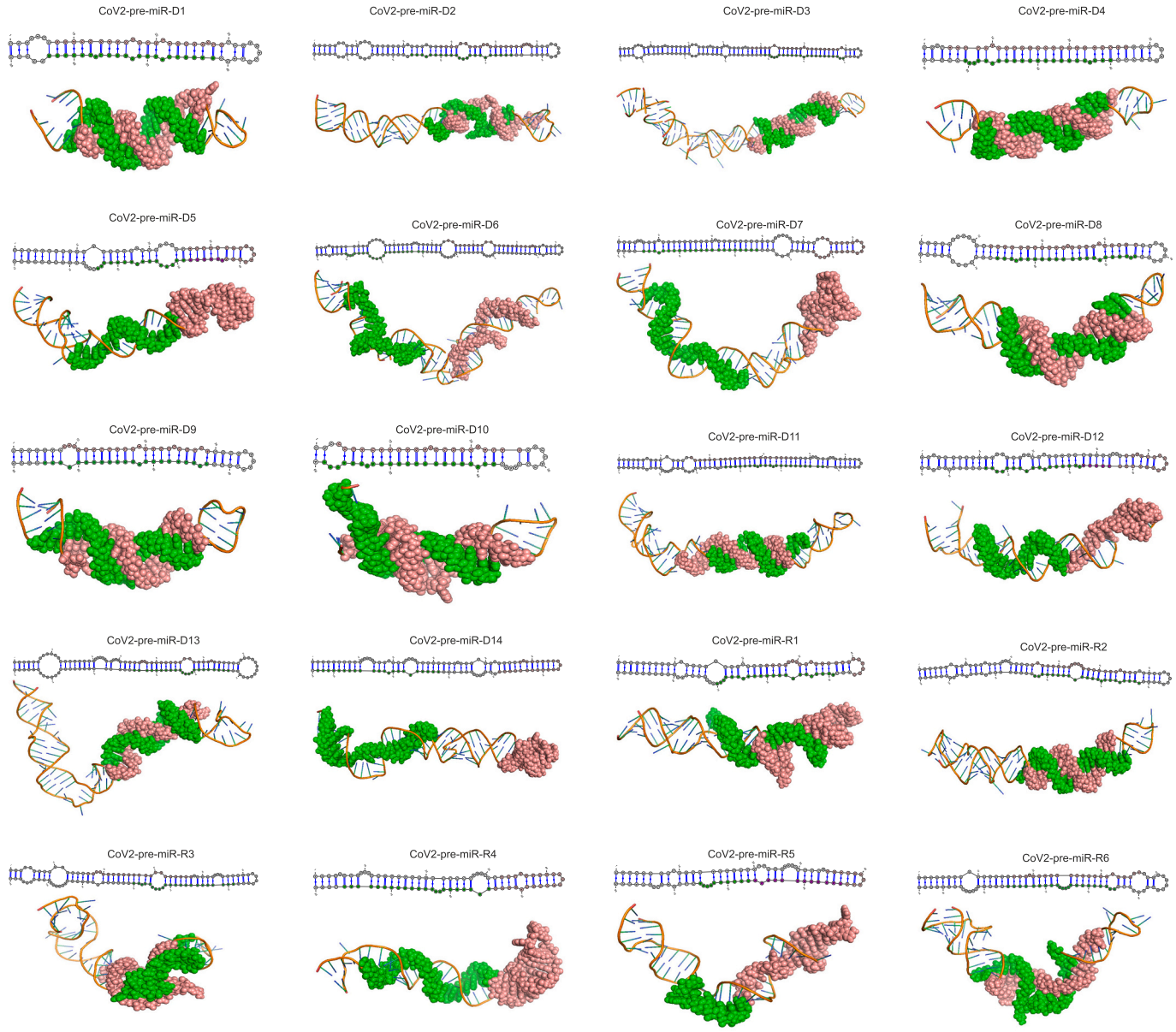

Fig. 3. Predicted hairpin structures of SARS-CoV-2 encoded potential pre-miRNAs. The secondary and tertiary structures of each SARS-CoV-2 encoded pre-miRNAs are shown. While regions with pink color indicate the mature 5' miRNAs of each pre-miRNA, the green color indicate the mature 3' miRNAs of each pre-miRNA. Nucleotides shared between 5′ and 3′ mature miRNAs are represented in purple. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

virus is eliminated (O'Shea and Plenge, 2012). This pathway is activated by membrane receptor-associated Janus kinases (JAK1, JAK2, JAK3). The binding of cytokines, such as interferons and interleukins, causes activation of JAKs which in turn activate STATS. These activated STATs form hetero- or homodimers, then translocate to the cell nucleus to induce transcription of target genes including immune response genes (Harrison, 2012). In our study *JAK1* and *JAK2* and *STAT3*, *STAT4*, *STAT5B* and *STAT6* were targeted by SARS-CoV-2 miRNAs (Table 3). This pathway has been targets for the majority of viruses including coronaviruses, SARS-CoV and MERS-CoV. In addition, many viruses target more than one factor in this pathway (Fleming, 2016; Kuchipudi, 2015; Laurent-Rolle et al., 2010; Parisien et al., 2001; Simmons et al., 2010). In some cases, the virus induces the upregulation of suppressor of cytokine signaling cellular genes (*SOCS*) that regulate this pathway (Starr et al., 1997). Indeed, in our study SARS-CoV-2 miRNAs SARS-CoV-mir-D6-3p, SARS-CoV-mir-D8-5p, SARS-CoV-mir-D10-5p, SARS-CoV-2-mir-D5-3p, SARS-CoV-2-mir-D11-5p, SARS-CoV-2-mir-D14-3p, SARS-CoV-2-mir-D6-3p, SARS-CoV-2-mir-D8-5p, SARS-CoV-2-mir-D2-3p, SARS-CoV-2-mir-D14-5p, SARS-CoV-mir-D6-5p, SARS-CoV-mir-R3-3p, SARS-CoV-mir-R5-5p targeted *SOCS1–6* genes. It seems that in order to control this pathway SARS CoV2 targets many proteins in this pathway with miRNAs encoded from its genome.

The transforming growth factor beta (TGFB) signaling pathway is involved in many cellular processes including cell survival, apoptosis and immunity. Although TGFB pathway has important roles in many cellular processes, it is often manipulated by viruses as it is a simple pathway. In our study, proteins that play crucial role in almost every step of this pathway are targeted by SARS-CoV-2 miRNAs. *TGFBR1* and *TGFBR2*, *SMAD2, SMAD3* and *SMAD4* were all found to be targeted by SARS-CoV-2 miRNAs (Table 3). SARS-CoV nucleocapsid (N) protein was shown to inhibit the formation of SMAD3/4 complex, resulting in blocked TGFB-induced apoptosis and tissue fibrosis in SARS-CoV-infected host cells (Zhao et al., 2008). In addition, most of the pro-fibrotic activities was reported to cause by TGFB pathway modulation

Fig. 4. GO and PANTHER analysis of mature SARS-CoV-2 miRNAs. The predicted target gene of potential mature SARS-CoV-2 miRNAs were classified by the GO and PANTHER databases based on biological process (a), cellular component (b), molecular function (c), protein class (d) and he pathway enrichment analysis of candidate genes (e). Top enriched pathways are listed (p *value* < 0.01) (e).

(Flanders, 2004). SARS-CoV-2 has also been reported to cause lung fibrosis in some COVID-19 patients. Investigation on these miRNAs could help to understand mechanism of fibrosis caused by SARS-CoV-2 in COVID-19 patients.

Interleukins play essential roles in the activation and differentiation of immune cells, as well as proliferation, maturation, migration, and adhesion. They also have pro-inflammatory and anti-inflammatory properties (Akdis et al., 2016). In our study *IL2*, *IL5*, *IL7*, *IL8*, *IL10*, *IL13*, *IL15*, *IL16*, *IL17*, *IL21*, *IL22*, *IL24*, *IL25* and *IL33* were targeted by SARS-CoV-2 miRNAs (Table 3). It has been shown that interleukin levels change during COVID-19. By targeting these ILs, the virus may control systemic inflammation. IL10 and IL17 for example are critical cytokines to protect host from tissue damage and autoimmunity during acute phase of immune responses. Inhibition of these cytokines could cause excessive immune activation seen in COVID-19 (Conti et al., 2020; Ouyang et al., 2011; Veldhoen, 2017). In addition, it has been reported that intensive care unit COVID-19 patients had higher plasma levels of IL2, IL7, IL10, suggesting a possible cytokine storm associated with these cytokines (Huang et al., 2020).

Histone demethylases and histone deacetylates play important roles in epigenetic regulatory pathway of immune responses. In our study we found that *JARID* histone demethylase genes including *JARID1A- JARID1C* and *JARID2* and histone deacetylase genes including *HDAC1- HDAC2- HDAC3* were targeted by SARS-CoV2 miRNAs (Table 3). It has previously been shown that JARID1 and JARID2 play regulatory role in human cytomegalovirus and Ebola virus infection. On the other hand, HDACs are involved in the regulation of the replication of numerous viruses (Bayarsaihan, 2011; Blair et al., 2011; Herbein and Wendling, 2010; Teng et al., 2015).

It has been reported that cleavage of SARS coronavirus spike protein by protease TMPRSS11A enhances virus entry into human bronchial epithelial cells. It was also shown that it activates influenza A virus hemagglutinin and MERS coronavirus spike proteins (Kam et al., 2009; Zamora et al., 2017). *TMPRSS11A* was also found to be targeted by SARS-CoV-2 miRNA in our study (Table 3).

The infection with RNA viruses leads to the induction of signaling

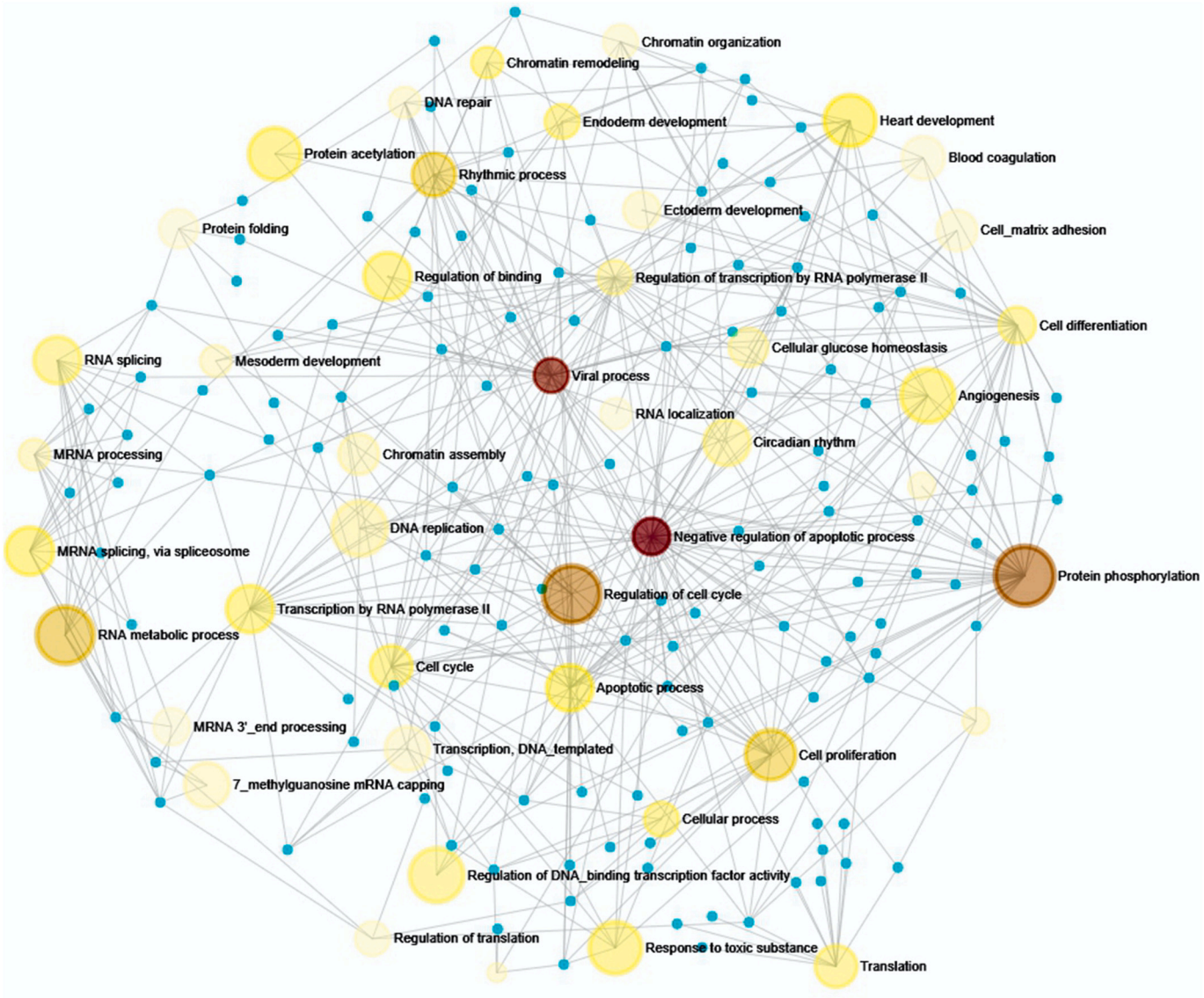

Fig. 5. Signaling pathway network analysis. SARS-CoV-2 miRNA targeted genes were filtered according to their roles in immune system and gene regulation network and analyzed with NetworkAnalyst program.

cascades in the infected host cell. The RNA viruses are recognized by specialized host cell proteins, thus triggering the activation of kinases and transcription factors which in turn mount antiviral response (Garcia-Sastre, 2006). In our study many kinases (*MAPK1*, *MAPK3*, *MAPK4*, *MAPK6*, *MAPK7*, *PIK3CA*, *CAMK*), transcription factors such as *E2F1*, *SP1*, *EIF4A1*, *TBP* and tumor suppressor genes including, *PTEN*, *AKT1*, *RB1* were all targeted by SARS-CoV-2 miRNAs.

Our integrative network analysis carried out with all SARS-CoV-2 miRNA targeted genes identified with miRDB database showed that genes of central nervous system and genes involved in regulation of transcription are the most two groups of genes targeted by viral miRNAs (data not shown). It was recently documented that, in addition to systemic and respiratory symptoms, 36.4% of patients with COVID-19 develop neurological symptoms, including headache, disturbed consciousness, and paresthesia (Mao et al., 2020).

5. Conclusions

40 SARS-CoV-2 miRNAs and their regulated targets were computationally predicted and reported. Our analysis showed that targeted genes play important roles in NFKB, JAK/STAT and TGFB signaling pathways, all of which have a great importance in different aspects of the host immune system. Some of these genes can be downregulated by miRNAs in order the virus to escape from immune system, some may be used to facilitate viral persistence within the cell. Following experimental validation and confirmation of our results, new therapeutic strategies could be developed to treat the COVID-19. Moreover, our results could help to understand virus-host interaction during SARS-CoV-2 infection.

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CRediT authorship contribution statement

Merve Nur Aydemir: Conceptualization, Methodology, Project administration, Data curation, Formal analysis, Validation, Writing – original draft, Writing – review & editing. **Habes Bilal Aydemir:** Data curation, Formal analysis, Methodology, Visualization, Validation, Software. **Ertan Mahir Korkmaz:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Mahir Budak:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Nilgun Cekin:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Ergun Pinarbasi:**

Table 3
Cytotoxin and gene regulation associated genes targeted by SARS-CoV-2 encoded miRNAs.

Gene	Gene name	SARS-CoV-2-miRNAs
NFKB1	nuclear factor kappa B subunit 1	SARS-CoV-2-pre-miR-R2-3p, SARS-CoV-2-mir-D8-5p, SARS-CoV-2-mir-R1-5p
NFKBIE	NFKB inhibitor epsilon	SARS-CoV-2-mir-D8-5p, SARS-CoV-2-mir-R1-5p, SARS-CoV-2-pre-miR-D10-3p, SARS-CoV-2-pre-miR-D10-3p,
IKBKE	inhibitor of nuclear factor kappa B kinase subunit epsilon	SARS-CoV-2-mir-D2-3p, SARS-CoV-2-pre-miR-D2-5p
CXCL9	C-X-C motif chemokine ligand 9	SARS-CoV-2-mir-R5-5p, SARS-CoV-2-mir-R5-3p, SARS-CoV-2-pre-miR-D9-3p, SARS-CoV-2-pre-miR-D1-5p, SARS-CoV-2-pre-miR-D1-5p
CXCL1	C-X-C motif chemokine ligand 1	SARS-CoV-2-mir-R2-5p, SARS-CoV-2-pre-miR-R6-5p
CXCL10	C-X-C motif chemokine ligand 10	SARS-CoV-2-mir-R1-3p, SARS-CoV-2-pre-miR-D10-3p, SARS-CoV-2-pre-miR-D11-5p
CXCL11	C-X-C motif chemokine ligand 11	SARS-CoV-2-mir-R4-3p, SARS-CoV-2-pre-miR-D2-3p
CXCL16	C-X-C motif chemokine ligand 16	SARS-CoV-2-mir-D6-3p, SARS-CoV-2-pre-miR-D2-3p, SARS-CoV-2-pre-miR-D8-3p, SARS-CoV-2-pre-miR-R5-3p
IFNGR2	Interferon gamma receptor 2	SARS-CoV-2-mir-D7-5p, SARS-CoV-2-mir-D14-5p
SOCS1	Suppressor of cytokine signaling 1	SARS-CoV-2-mir-D6-3p, SARS-CoV-2-mir-D8-5p, SARS-CoV-2-mir-D10-5p
SOCS3	Suppressor of cytokine signaling 3	SARS-CoV-2-mir-D5-3p, SARS-CoV-2-mir-D11-5p, SARS-CoV-2-mir-D14-3p
SOCS4	Suppressor of cytokine signaling 4	SARS-CoV-2-mir-D6-3p
SOCS5	Suppressor of cytokine signaling 5	SARS-CoV-2-mir-D8-5p, SARS-CoV-2-mir-D2-3p, SARS-CoV-2-mir-D14-5p, SARS-CoV-2-pre-miR-D9-3p
SOCS6	Suppressor of cytokine signaling 6	SARS-CoV-2-mir-D6-5p, SARS-CoV-2-mir-R3-3p, SARS-CoV-2-mir-R5-5p, SARS-CoV-2-pre-miR-R1-3p
STAT6	Signal transducer and activator of transcription 6	SARS-CoV-2-mir-D10-3p
STAT4	Signal transducer and activator of transcription 4	SARS-CoV-2-mir-R2-5p
STAT3	Signal transducer and activator of transcription 3	SARS-CoV-2-mir-D8-5p, SARS-CoV-2-mir-R2-5p, SARS-CoV-2-mir-R3-3p
STAT5B	Signal transducer and activator of transcription 5B	SARS-CoV-2-mir-D1-5p, SARS-CoV-2-mir-D3-3p, SARS-CoV-2-mir-D6-5p, SARS-CoV-2-mir-R1-5p, SARS-CoV-2-pre-miR-D5-5p, SARS-CoV-2-pre-miR-D13-5p
TGFBR1	Transforming growth factor beta receptor 1	SARS-CoV-2-mir-D9-5p, SARS-CoV-2-mir-D14-5p, SARS-CoV-2-mir-R2-5p, SARS-CoV-2-mir-R6-3p
TGFBR2	Transforming growth factor beta receptor 2	SARS-CoV-2-mir-R3-3p, SARS-CoV-2-mir-R6-3p
JAK1	Janus kinase 1	SARS-CoV-2-mir-R3-3p
JAK2	Janus kinase 2	SARS-CoV-2-mir-D4-5p
SMAD2	SMAD family member 2	SARS-CoV-2-mir-D8-3p, SARS-CoV-2-mir-R2-5p, SARS-CoV-2-mir-D3-5p, SARS-CoV-2-mir-R4-5p, SARS-CoV-2-mir-R1-5p, SARS-CoV-2-mir-R6-3p
SMAD3	SMAD family member 3	SARS-CoV-2-mir-D10-5p, SARS-CoV-2-mir-R6-5p, SARS-CoV-2-mir-R4-3p
SMAD4	SMAD family member 4	SARS-CoV-2-mir-R4-3p, SARS-CoV-2-mir-D10-5p, SARS-CoV-2-mir-D14-5p, SARS-CoV-2-mir-R1-5p, SARS-CoV-2-mir-D8-5p
SUMO1	Small ubiquitin like modifier 1	SARS-CoV-2-mir-D11-3p, SARS-CoV-2-mir-D6-5p, SARS-CoV-2-mir-D1-3p, SARS-CoV-2-mir-R6-3p SARS-CoV-2-mir-R2-3p, SARS-CoV-2-mir-D10-5p, SARS-CoV-2-mir-D1-5p, SARS-CoV-2-mir-R2-5p, SARS-CoV-2-mir-R3-3p
SUMO2	small ubiquitin like modifier 2	SARS-CoV-2-mir-D14-3p, SARS-CoV-2-mir-D9-5p
IL2	Interleukin 2	SARS-CoV-2-mir-D6-3p
IL5	Interleukin 5	SARS-CoV-2-mir-R2-5p, SARS-CoV-2-pre-miR-D5-5p
IL7	Interleukin 7	SARS-CoV-2-mir-D7-3p, SARS-CoV-2-mir-D11-5p, SARS-CoV-2-mir-R6-3p
IL8	Interleukin 8	SARS-CoV-2-mir-R3-5p, SARS-CoV-2-mir-R3-3p, SARS-CoV-2-mir-D6-3p
IL10	Interleukin 10	SARS-CoV-2-mir-D14-3p, SARS-CoV-2-mir-D6-3p
IL13	Interleukin 13	SARS-CoV-2-mir-R1-3p, SARS-CoV-2-mir-D6-3p
IL15	Interleukin 15	SARS-CoV-2-mir-D6-3p
IL16	Interleukin 16	SARS-CoV-2-mir-R4-3p
IL17A	Interleukin 17 A	SARS-CoV-2-mir-D6-3p, SARS-CoV-2-mir-R1-5p
IL21	Interleukin 21	SARS-CoV-2-mir-R6-5p
IL22	Interleukin 22	SARS-CoV-2-mir-D6-3p
IL24	Interleukin 24	SARS-CoV-2-mir-D14-3p, SARS-CoV-2-pre-miR-D6-3p, SARS-CoV-2-pre-miR-D10-3p
IL25	Interleukin 25	SARS-CoV-2-mir-R3-3p, SARS-CoV-2-pre-miR-D5-5p
IL33	Interleukin 33	SARS-CoV-2-mir-D14-5p
HDAC1	Histone deacetylase 1	SARS-CoV-2-mir-R6-3p, SARS-CoV-2-pre-miR-D2-5p, SARS-CoV-2-pre-miR-D2-3p,
HDAC2	Histone deacetylase 2	SARS-CoV-2-mir-D10-5p, SARS-CoV-2-mir-D6-3p,
HDAC3	Histone deacetylase 3	SARS-CoV-2-mir-R6-3p
JARID1A	Lysine demethylase 5A	SARS-CoV-2-mir-D2-3p, SARS-CoV-2-mir-D2-5p, SARS-CoV-2-mir-D7-5p, SARS-CoV-2-mir-R1-5p, SARS-CoV-2-mir-R2-5p, SARS-CoV-2-mir-R6-3p
JARID1C	Lysine demethylase 5C	SARS-CoV-2-mir-D12-5p, SARS-CoV-2-mir-D6-3p
JARID2	Jumonji and AT-rich interaction domain containing 2	SARS-CoV-2-mir-D1-5p, SARS-CoV-2-mir-D6-3p, SARS-CoV-2-mir-D14-3p
JARID1B	Lysine demethylase 5B	SARS-CoV-2-mir-D2-5p, SARS-CoV-2-mir-R2-5p, SARS-CoV-2-mir-R4-5p
E2F1	E2F transcription factor 1	SARS-CoV-2-mir-R3-3p, SARS-CoV-2-pre-miR-D5-5p
SOS1	SOS Ras/Rac guanine nucleotide exchange factor 1	SARS-CoV-2-mir-D14-5p, SARS-CoV-2-mir-R3-3p, SARS-CoV-2-mir-R4-5p, SARS-CoV-2-mir-D6-5p, SARS-CoV-2-mir-D6-3p
RHOA	ras homolog family member A	SARS-CoV-2-mir-D11-3p
TBP	TATA-box binding protein	SARS-CoV-2-mir-R1-5p
SP1	Sp1 transcription factor	SARS-CoV-2-mir-D4-5p, SARS-CoV-2-mir-R6-3p, SARS-CoV-2-mir-D6-3p, SARS-CoV-2-mir-D10-3p, SARS-CoV-2-mir-D14-3p, SARS-CoV-2-mir-R3-5p

(*continued on next page*)

Table 3 (*continued*)

Gene	Gene name	SARS-CoV-2-miRNAs
GRB2	growth factor receptor bound protein 2	SARS-CoV-2-mir-R2-3p
CDKL2	Cyclin dependent kinase like 2	SARS-CoV-2-mir-D11-3p
EIF4A1	Eukaryotic translation initiation factor 4A1	SARS-CoV-2-mir-D14-5p
CDK1	cyclin dependent kinase 1	SARS-CoV-2-mir-D14-3p
HIF1A	Hypoxia inducible factor 1 subunit alpha	SARS-CoV-2-mir-R5-5p, SARS-CoV-2-mir-D14-5p, SARS-CoV-2-mir-R2-5p, SARS-CoV-2-mir-R3-3p
CTNNB1	Catenin beta 1	SARS-CoV-2-mir-D14-5p
MAPK1	Mitogen-activated protein kinase 1	SARS-CoV-2-mir-D2-5p, SARS-CoV-2-mir-R2-3p, SARS-CoV-2-mir-R5-5p, SARS-CoV-2-mir-R6-3p, SARS-CoV-2-mir-R3-3p, SARS-CoV-2-mir-D8-5p, SARS-CoV-2-mir-R2-5p
MAPK3	Mitogen-activated protein kinase 3	SARS-CoV-2-mir-R6-3p
MAPK4	Mitogen-activated protein kinase 4	SARS-CoV-2-mir-D8-3p, SARS-CoV-2-mir-R3-3p, SARS-CoV-2-pre-miR-D11-5p
MAPK6	Mitogen-activated protein kinase 6	SARS-CoV-2-mir-D4-5p, SARS-CoV-2-mir-R1-3p, SARS-CoV-2-mir-D6-3p, SARS-CoV-2-pre-miR-R2-3p
MAPK7	Mitogen-activated protein kinase 7	SARS-CoV-2-mir-D6-3p
NOS3	Nitric oxide synthase 3	SARS-CoV-2-mir-D6-3p
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha	SARS-CoV-2-mir-D9-3p
TMPRSS11A	Transmembrane serine protease 11A	SARS-CoV-2-mir-D10-3p
TMPRSS4	Transmembrane serine protease 4	SARS-CoV-2-mir-D11-3p
AKT1	AKT serine/threonine kinase 1	SARS-CoV-2-mir-D11-5p, SARS-CoV-2-mir-D6-3p, SARS-CoV-2-mir-R5-5p
PTEN	Phosphatase and tensin homolog	SARS-CoV-2-mir-D8-3p, SARS-CoV-2-mir-D13-5p, SARS-CoV-2-mir-R1-3p, SARS-CoV-2-mir-D8-5p, SARS-CoV-2-mir-R6-3p, SARS-CoV-2-mir-R3-3p
RB1	RB transcriptional corepressor 1	SARS-CoV-2-mir-R3-3p, SARS-CoV-2-pre-miR-D14-5p
BCL2	BCL2 apoptosis regulator	SARS-CoV-2-mir-R2-5p, SARS-CoV-2-mir-R6-3p, SARS-CoV-2-mir-D10-5p
TNFRSF21	TNF receptor superfamily member 21	SARS-CoV-2-mir-R3-3p, SARS-CoV-2-mir-R2-3p

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Declaration of competing interest

There is no conflict of interest.

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