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Original Article

Gene signatures and potential therapeutic targets of Middle East respiratory syndrome coronavirus (MERS-CoV)-infected human lung adenocarcinoma epithelial cells

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Abstract *Background:* Pathogenic coronaviruses include Middle East respiratory syndrome coronavirus (MERS-CoV), severe acute respiratory syndrome coronavirus (SARS-CoV), and SARS-CoV-2. These viruses have induced outbreaks worldwide, and there are currently no effective medications against them. Therefore, there is an urgent need to develop potential drugs against coronaviruses.
Methods: High-throughput technology is widely used to explore differences in messenger (m) RNA and micro (mi)RNA expression profiles, especially to investigate protein—protein interactions and search for new therapeutic compounds. We integrated miRNA and mRNA expression profiles in MERS-CoV-infected cells and compared them to mock-infected controls from public databases.
Results: Through the bioinformatics analysis, there were 251 upregulated genes and eight highly differentiated miRNAs that overlapped in the two datasets. External validation verified that these genes had high expression in MERS-CoV-infected cells, including RC3H1, NF-κB, CD69, TNFAIP3, LEAP-2, DUSP10, CREB5, CXCL2, etc. We revealed that immune, olfactory or sensory system-related, and signal-transduction networks were discovered from upregulated mRNAs in MERS-CoV-infected cells. In total, 115 genes were predicted to be related to miRNAs, with the intersection of upregulated mRNAs and miRNA-targeting prediction genes such as TCF4, NR3C1, and POU2F2. Through the Connectivity Map (CMap) platform, we suggested potential compounds to use against MERS-CoV infection, including diethylcarbamazine, harpagoside, bumetanide, enalapril, and valproic acid.
Conclusions: The present study illustrates the crucial roles of miRNA-mRNA interacting networks in MERS-CoV-infected cells. The genes we identified are potential targets for treating MERS-CoV infection; however, these could possibly be extended to other coronavirus infections.

Introduction

Coronaviruses (CoVs) are a category of enveloped positive-sense single-stranded RNA viruses composed of club-like spikes that project from their surfaces; these CoVs considerably infect the human respiratory system and central nervous system (CNS). To the present, at least six strains of coronavirus have been identified. Four human coronaviruses (HCoVs), including HCoVHKU1, HCoV-OC43, HCoV-NL63, and HCoV-229E, are not highly morbific and have only slight effects on the respiratory system.[1] However, two other strains of coronaviruses, Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV) are highly pathogenic. MERS-CoV induced 858 deaths and about 2494 cases at the end of November 2019.[2] SARS-CoV caused two large-scale pandemics and resulted in more than 8422 cases and 916 deaths in 2003.[3] Coronavirus outbreaks indicate that some coronaviruses can become highly pathogenic when they are transmitted from animals to humans. Therefore, it is important to explore antiviral treatments and vaccines targeting highly pathogenic coronaviruses.[4] In December 2019, a third pathogenic HCoV, called the 2019 novel coronavirus (abbreviated as SARS-CoV-2) was detected. According to statistical reports from the World Health Organization (WHO) (https://www.who.int/emergencies/diseases/novel-coronavirus-2019) to March 22, 2021, there had been over 122,822,505 confirmed cases worldwide with over 2,709,041 deaths. Human-to-human droplet and contact transmission were reported. However, there are currently no effective medications against coronaviruses; nor are effective vaccines available. Therefore, there is an urgent need to develop therapeutic drugs for coronavirus diseases.[5,6]

High-throughput technology extensively applies a holistic approach from expressions of thousands of genes to functional genomics and biological systems. The interactions of microRNAs (miRNAs) with their corresponding mRNA targets play a major role in the wide field of epigenetic gene regulation, and the mRNA-miRNA network also provides numerous possible results.[7,8] In addition, a combination and analysis of multiple databases may reveal the universal regulation network. miRNAs inhibit translation of proteins or enhance degradation of transcripts by binding to the 3' untranslated region (UTR) of mRNAs. miRNAs bind to the viral genome or through virus-mediated changes in the host transcriptome, affect replication and pathogenesis of RNA viruses. Meanwhile, since miRNAs are stable during long-term storage in blood,[9] they are potential candidates as biomarkers for the screening, diagnosis, and prediction of viral infections. There are several high-throughput datasets that contain mRNAs and miRNAs after a coronavirus infection, and recent studies have demonstrated that some of these downstream genes are potential targets for therapy or prediction.[10,11] In addition, the Calu-3 cell is an ideal model for investigating and studying coronavirus infection.[12] Therefore, we selected Calu-3 cells as a MERS-CoV infection model for the present study.[7,8,13] A previous

study used MERS-CoV to infect Calu-3 epithelial cells and identified 3.4% of differences in the expression of circRNAs, 5.3% of differences in the expression of miRNAs, and 29.9% of differences in the expression of mRNAs.[13] However, integrated analyses of mRNA and miRNA sequencing from coronavirus-infected human cell models are limited. In the current study, we hypothesize that investigating mRNA/miRNA interactions may contribute evidence to decrease the translational gap between laboratory research and clinical applications, which will be worthwhile in the development of efficient treatment strategies for coronavirus outbreaks. Therefore, we chose both RNA and miRNA high throughput database for further investigation.

Effective treatments for coronavirus infections urgently need to be developed; however, discovering new drugs costs considerable time and effort. Drug repurposing is an effective strategy to shorten the time and lessen the costs of development. The Gene Expression Omnibus (GEO) database combined with the Connectivity Map (CMap) platform provides comprehensive testing of US Food and Drug Administration (FDA)-approved compounds. Recent research also used these databases to obtain drug-gene signatures for the novel coronavirus SARS-CoV-2. In the present study, we used a bioinformatics approach to study MERS-CoV-infected human lung adenocarcinoma epithelial cells. Databases of miRNA and mRNA expression were integrated to explore the miRNA-mRNA interactive network. MERS-CoV-associated miRNAs and their target genes are potential candidates for therapy, and FDA-approved compounds were searched using the CMap platform. From results of the present study, we provide sufficient evidence of miRNA-mRNA interactive networks associated with MERS-CoV infection with their possible extension to other strains of coronavirus infections.

Materials and methods

Bioinformatics and high-throughput database analysis

We searched GEO databases for available datasets comparing MERS-CoV-infected and mock-infected epithelial cells of human lung adenocarcinomas. Two high-throughput datasets (GSE65574 and GSE139516) with differential expression data for mRNAs and miRNAs were acquired from National Center for Biotechnology Information (NCBI) GEO databases,[7,13] and the GSE56677 dataset was used for external validation.[8] The raw data were downloaded, and we used CLC Genomics Workbench v10.1 for analysis.[14,15] The Database for Annotation, Visualization and Integrated Discovery (DAVID, vers. 6.8) includes gene functional classifications, various processes and pathways in gene ontology (GO), and the Kyoto Encyclopedia of Genes and Genomes (KEGG). We used an agglomeration algorithm in DAVID to cluster genes into different groups according to their biological functions, signaling pathways, as well as the associated diseases. The top 5% of highly expressed mRNAs in MERS-CoV-infected cells were compared to mock-infected controls, and the P value was set at 0.05 according to previous methods.[16—21] Significantly differentially expressed genes were input to the GO database and gene set enrichment analysis (GSEA) software to construct biological networks or regulatory pathways, and the cutoff point of significant enrichment was set to $P < 0.05$.

Analyses using miRNA-targeting prediction databases

We used miRWalk 2.0 to predict potential miRNA-targeted genes; this program contains eight different software packages: mirWalk, MicroT4, miRanda, miRDB, miRmap, RNA22, RNAhybrid, and TargetScan.[22] The miRmap score is the repression strength of miRNA binding to its target mRNA. We set the cutoff of the miRmap score to >99, and the top 5% of significantly highly expressed miRNAs from MERS-CoV-infected cells compared to mock-infected controls were analyzed. The results were imported into miRtest, which evaluates both individual miRNAs and their target mRNAs in the same analysis for consistency.

Investigation of protein—protein interactions (PPIs)

The Search Tool for the Retrieval of Interacting Genes (STRING) software contains protein—protein interaction (PPI) networks from 5090 organisms, 24.6 million proteins, and more than 2000 million interactions.[23] We used STRING (vers. 11.0) to analyze PPIs based on the top 5% of genes with highly differential expression. The k-means clustering algorithm was selected to classify the proteins into different groups.

Connections between potential therapeutic compounds and miRNA-targeted genes via Connectivity Map

The miRNA-targeted genes from MERS-CoV-infected cells were compared to those from mock-infected controls through the CMap platform.[24,25] CMap applies a systematic approach to reveal interactions among diseases and to reveal FDA-approved compounds, based on alterations in the gene expressions of MERS-CoV-infected cells.

Results

The miRNA and mRNA profiles of MERS-CoV-infected human lung adenocarcinoma epithelial cells

We identified differential expressions of mRNAs and miRNAs from MERS-CoV-infected and mock-infected epithelial cells of human lung adenocarcinomas and analyzed them using miRtest. Upregulated mRNAs and miRNAs in MERS-CoV-infected cells were examined with an ingenuity pathway analysis (IPA) to find downstream networks, with STRING software to detect PPIs, and with DAVID to investigate associated pathways. We also used miRmap and Venn diagrams to screen miRNA-targeted genes and potential miRNA-mRNA interactions (Fig. 1).

The top 5% of highly expressed mRNAs in MERS-CoV-infected cells were compared to the mock-infected controls. There were 1636 mRNAs selected from the GSE65574

dataset and 2831 mRNAs from the GSE139516 dataset (Fig. 2A). We compared these data in a Venn diagram and found that there were 251 upregulated genes in MERS-CoV-infected cells that overlapped in these two datasets. These genes were uploaded into KEGG; the associated pathways are shown in Fig. 2B. We also used GO software to analyze pathways to connected biological processes (Fig. 2C), related molecular functions (Fig. 2D), and associated cellular components (Fig. 2E). The gene lists in each pathway are shown in Supplementary Tables 1—3. From the above analysis, we found that "viral carcinogenesis" may play a critical role in MERS-CoV-infected cells (Supplementary Figure 1). In order to confirm the significance of the 251 upregulated genes in Fig. 2A, we investigated their expression levels in MERS-infected Calu-3 cells in the GSE56677 dataset. Upregulated genes in MERS-infected cells included RC3H1, GPNMB, NFKBIA, GEM, CD69, TNFAIP3, LONRF3, LEAP2, C16orf72, ENO3, etc (Fig. 3, Supplementary Table 4).

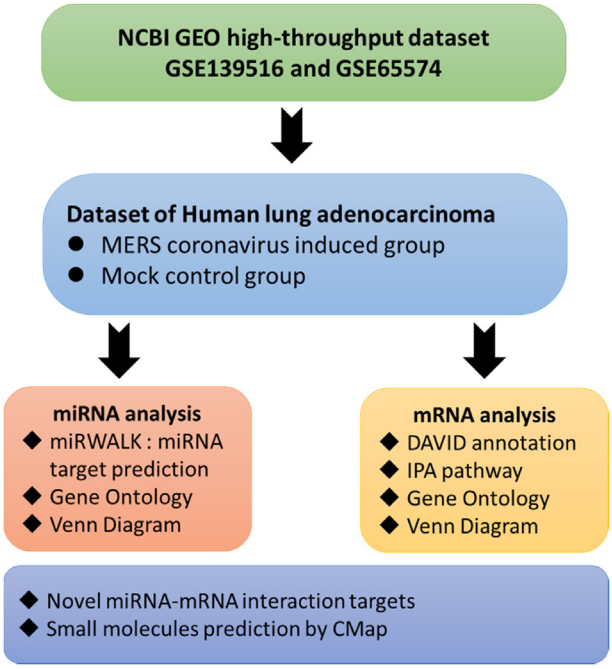


Figure 1. Schematic illustration of the study design. The high-throughput datasets of MERS-CoV-infected human lung adenocarcinoma epithelial cells and mock-infected controls were acquired from GEO. Differentially expressed miRNAs (>2 multiples of change) were analyzed with miRWalk and miRtest for targeting predictions. The top 5% of differentially expressed mRNAs were identified and analyzed with IPA for the pathway analysis and with DAVID for functional interpretations. Downstream signaling pathways were analyzed by GO software. miRNA-mRNA interactions were determined by a Venn diagram analysis. FDA-approved compounds were simulated by CMap. Abbreviations: CMap, Connectivity Map; CoV, coronavirus; DAVID, Database for Annotation, Visualization and Integrated Discovery; FDA, Food and Drug Administration; GEO, gene expression omnibus; GO, gene ontology; IPA, ingenuity pathway analysis; MERS, Middle East respiratory syndrome; miRNA, micro-ribonucleic acid; mRNA, messenger ribonucleic acid.

MERS-CoV-infection-related pathways in the GSEA

We utilized a public database to verify the importance of targeted pathways, as in Fig. 2. Differentially expressed genes (DEGs) in MERS-CoV-infected cells compared to mock-infected controls were analyzed using the KEGG, GO, BioCarta, and Hallmark databases. Upregulated genes were analyzed using a GSEA to identify potential regulated networks. Copious amounts of serum interferons (IFNs) are one of the clinical features of patients with MERS-CoV infection, and we detected positive correlations of IFN-α/γ responses in MERS-CoV-infected cells. There were several immune-related pathways enriched after MERS-CoV infection (Fig. 4A—I). Enriched pathways included olfactory and sensory systems, and olfactory neuropathy is one of the symptoms associated with SARS-CoV and SARS-CoV-2.[26—28] Enriched pathways also included cellular defense responses to viral infections (Fig. 4J—M). Some signaling pathways were also upregulated, including the Janus kinase (JAK)/signal transduction and activator of transcription (STAT), the neuroactive ligand receptor, and the electron transfer system (ETS) pathways (Fig. 4N—P).

Discovering miRNA-mRNA interactions in MERS-CoV-infected human lung adenocarcinoma epithelial cells

The top 5% of highly expressed miRNAs were identified in MERS-CoV-infected cells compared to the mock-infected controls. There were 139 miRNAs from the GSE65574 dataset and 228 miRNAs from the GSE139516 dataset. The intersection of these two groups of miRNAs contained hsa-miR-1247-3p, hsa-miR-127-5p, hsa-miR-376a-3p, hsa-miR-3180-3p, hsa-miR-3663-3p, hsa-miR-622, hsa-miR-3180, hsa-miR-4660, and hsa-miR-3173-3p. Hsa-miR-376a-3p was excluded, because this miRNA targeted fewer than 1000 genes, and it failed to establish intersections with other miRNAs. Therefore, eight miRNAs were uploaded to the miRWalk software to predict miRNA-targeted genes. In total, 115 genes were identified (Fig. 5A). Through a Venn diagram, we compared these miRNA-targeted genes with upregulated mRNAs from MERS-CoV-infected cells in the GSE65574 and GSE139516 datasets. The analytical results of miRNA-mRNA interactions detected three genes: transcription factor 4 (TCF4), nuclear receptor subfamily 3 group C member 1 (NR3C1), and POU class 2 homeobox 2 (POU2F2) (Fig. 5B). Potential PPI networks among the TCF4, NR3C1, and POU2F2 genes were drawn using STRING software (Fig. 5C).

Identification of potential inhibitory compounds from the Connectivity Map

We used CMap to investigate potential compounds that might inhibit upregulated genes in MERS-CoV-infected cells. In total, 1309 compounds were collected, and more than 7000 treatments with different dosages were found in different cell lines to obtain gene expression

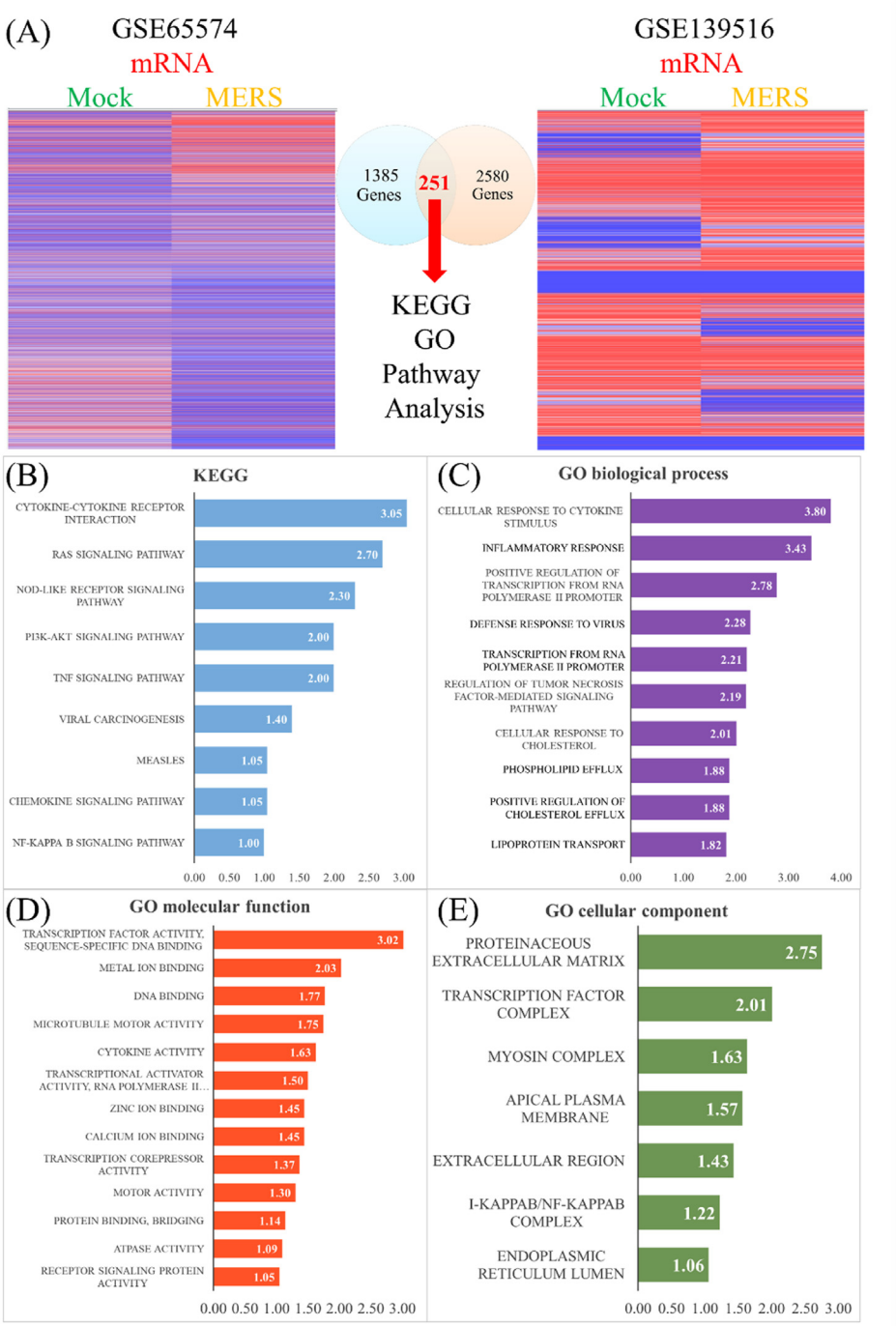


Figure 2. Upregulated genes in MERS-CoV-infected cells and associated pathways. (A) Upregulated genes in the GSE65574 and GSE139516 datasets were merged in a Venn diagram. MERS-CoV-infected cells were compared to mock-infected controls. In total, 251 overlapping genes were obtained. (B) Potential regulatory networks from analyzing these 251 upregulated genes in KEGG databases. (C) Biological processes in the GO analysis of 251 upregulated genes. (D) Molecular function in the GO analysis of 251 upregulated genes. (E) Cellular components in the GO analysis of 251 upregulated genes. The pathways with a *P* value of <0.05 were considered significant. The area and number of each pathway reflect -log10 values of the significance level. Abbreviations: CoV, coronavirus; DAVID, Database for Annotation, Visualization and Integrated Discovery; DNA, deoxyribonucleic acid; GO, gene ontology; IPA, ingenuity pathway analysis; KEGG, Kyoto Encyclopedia of Genes and Genomes; MERS, Middle East respiratory syndrome; mRNA, messenger ribonucleic acid; NF-κB, nuclear factor kappa B; NOD, nucleotide-binding oligomerization domain; PI3K/Akt, phosphoinositide 3-kinase/protein kinase B; TNF, tumor necrosis factor.

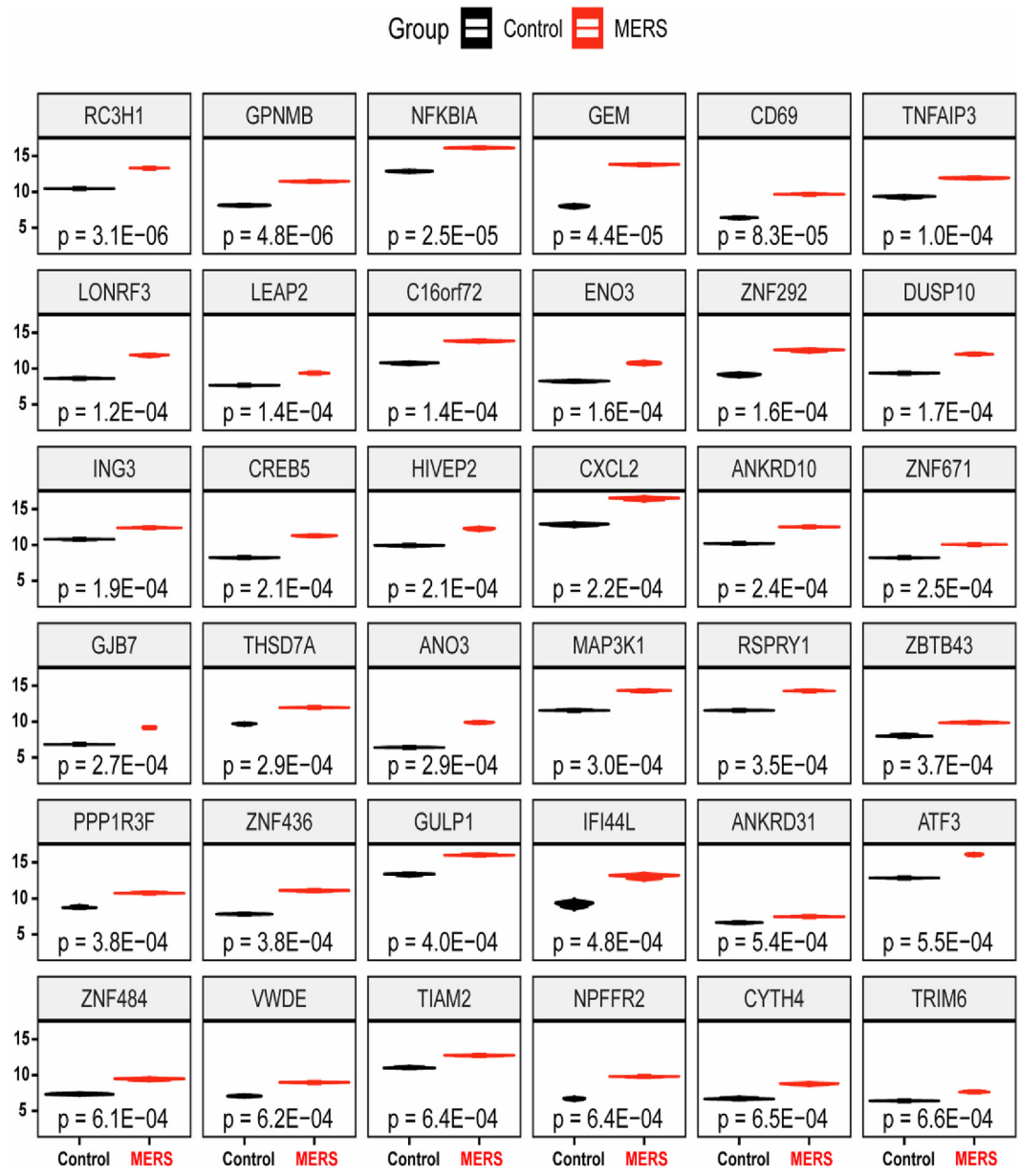


Figure 3. External validation of MERS-CoV-regulated genes by MERS-CoV-infected Calu-3 cells. The 251 MERS-CoV-regulated genes obtained from the GSE65574 and GSE139516 datasets were further validated with the GSE56677 dataset. Gene expressions in individual MERS-CoV-infected Calu-3 cells are shown. Violin plots show expression levels of each gene from the 36-gene differentiation signature in individual cells. The *P* values for each gene are shown in Supplementary Table 4. Abbreviations: CoV, coronavirus; MERS, Middle East respiratory syndrome.

profiles. The compounds with the most negative correlations, which may have the highest potential of suppressive effects against MERS-CoV-infected cells (Fig. 6A). The top 30 negatively correlated compounds in MERS-CoV-infected cells from the GSE65574 dataset were compared to those from the GSE139516 dataset. Only compounds in the intersection of the two datasets are listed in Fig. 6B, and 50 potential therapeutic drugs are listed in Supplementary Table 5. Further investigation is necessary to confirm the therapeutic potential of these compounds against MERS-CoV infection and other coronavirus infections.

Discussion

Although significant improvements in biomedical and pharmaceutical research have occurred in recent decades, the annual number of new drugs approved by the US FDA is limited. Drug repurposing shortens the time and reduces the costs of drug development.[29–31] During the ongoing SARS-CoV-2 pandemic, the search for therapeutic drugs is extremely urgent. Studying previous coronavirus infections due to SARS-CoV and MERS-CoV is a readily available scheme.[32,33] The present study used comprehensive bioinformatics to investigate miRNA-mRNA interactions in MERS-

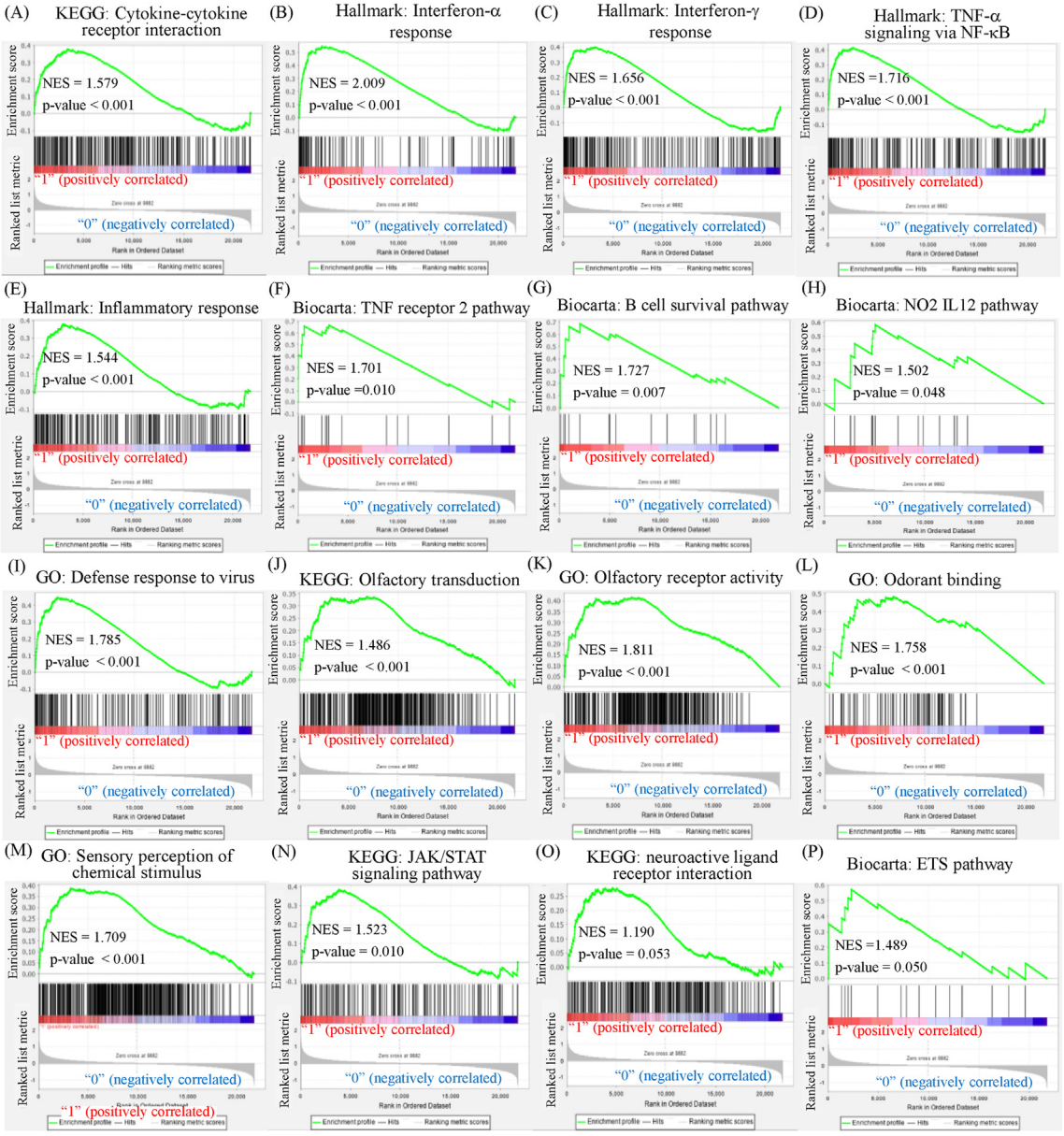


Figure 4. Enriched pathways in MERS-CoV-infected cells were associated with immune, olfactory, and sensory systems and cellular response-related pathways. (A) KEGG analysis of cytokine–cytokine receptor interactions. (B) Hallmark analysis of IFN-α responses. (C) Hallmark analysis of IFN-γ responses. (D) Hallmark analysis of TNF-α signaling via NF-κB. (E) Hallmark analysis of inflammatory responses. (F) Biocarta analysis of TNF receptor 2 pathways. (G) Biocarta analysis of B cell survival pathways. (H) Biocarta analysis of NO_2 IL-12 pathways. (I) GO analysis of dense responses to the virus. (J) KEGG analysis of olfactory transduction. (K) GO analysis of olfactory receptor activity. (L) GO analysis of odorant binding. (M) GO analysis of sensory perception of chemical stimuli. (N) KEGG analysis of the JAK/STAT signaling pathway. (O) KEGG analysis of neuroactive ligand receptor interaction. (P) Biocarta analysis of the ETS pathway. The 251 upregulated genes from the GSE65574 and GSE139516 datasets in Fig. 2 were imported for analysis. MERS-CoV-infected cells were compared to mock-infected controls. An enrichment score (upper third of each graph) of >0 was defined as upregulation. Each bar over the middle third indicates one gene located in the ranking. Meanwhile, he red represents a positive correlation, whereas the blue indicates a negative correlation. The distribution of the ranked list along with the gene list is shown as the gray part in the lower third of each graph. Abbreviation: CoV, coronavirus; ETS, electron transfer system; IFN, interferon; JAK, Janus kinase; KEGG, Kyoto Encyclopedia of Genes and Genomes; IL-12, interleukin-12; MERS, Middle East respiratory syndrome; NF-κB, nuclear factor kappa B; NO_2, nitrogen dioxide; STAT, signal transducer and activator of transcription; TNF, tumor necrosis factor.

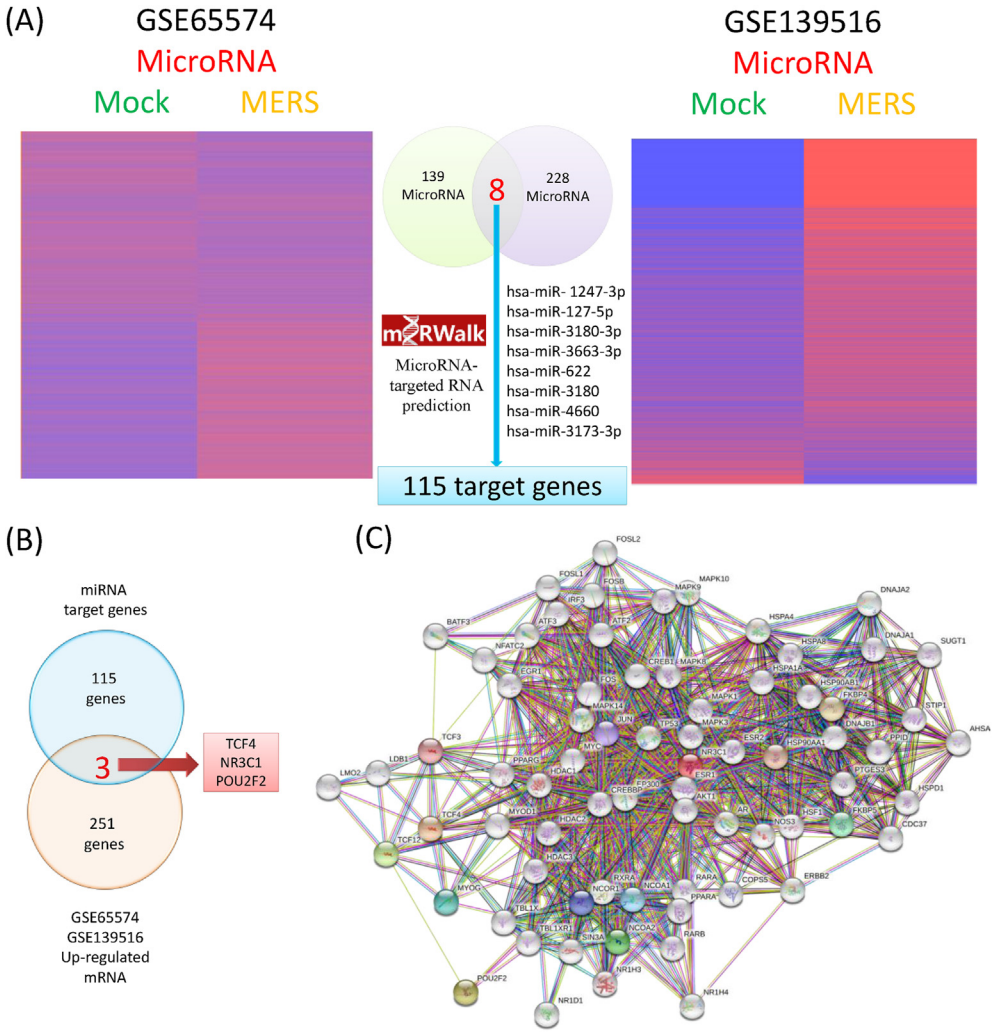
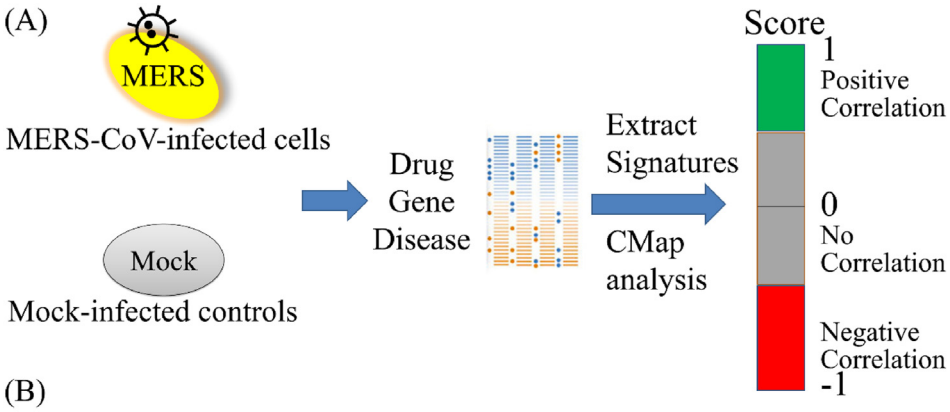


Figure 5. Interacting network of miRNAs and mRNAs in MERS-CoV-infected cells. (A) Highly differential expressions of miRNAs in the GSE65574 and GSE139516 datasets were merged in a Venn diagram. MERS-CoV-infected cells were compared to mock-infected controls. In total, eight overlapping miRNAs were obtained and uploaded to miRWalk, and 115 targeted genes were obtained. (B) miRNA-targeted genes and upregulated mRNAs were merged in a Venn diagram. Three overlapping genes were obtained. (C) The TCF4, NR3C1, and POU2F2 genes were input into STRING software to predict protein—protein interactions. Using k-means clustering, the network was further clustered into different subclusters. Abbreviation: CoV, coronavirus; MERS, Middle East respiratory syndrome; miRNA, micro-ribonucleic acid; mRNA, messenger ribonucleic acid; NR3C1, nuclear receptor subfamily 3 group C member 1; POU2F2, POU class 2 homeobox 2; TCF4, transcription factor 4.

CoV-infected models. We also explored MERS infection-related pathways to explain clinical manifestations of patients with a coronavirus infection. Conceivable drugs for treating MERS-CoV infection were predicted by CMap. The current study provides therapeutic advice for patients with MERS infection, and the results can potentially be extended to SARS-CoV-2 infections.

High-throughput methods with RNA sequencing have improved accuracy and sensitivity in studying miRNAs, which have become an important point in virus studies, because they regulate viral replication and gene expressions, which also control expressions of essential genes for viral proliferation. However, there are only a few studies about miRNAs in coronavirus-infected human cells, and integrated research into miRNA and mRNA databases in MERS-CoV-infected cells is currently lacking. Previous studies have demonstrated that coronavirus infection is correlated with cytokine storms and inflammation.[34,35] The coronavirus E protein/mitogen-activated protein kinase (MAPK)/phosphoinositide 3-kinase (PI3K)/protein kinase B/nuclear factor (NF)-κB signaling pathways respond to MERS-CoV infection and may serve as potential drug targets for therapeutic intervention strategies.[36,37] Clinical studies have shown that SARS-CoV and SARS-CoV-2 cause olfactory dysfunction.[38—40] The present study produced similar results, and the enrichment of multiple immune-related pathways in MERS-CoV-infected cells was shown by the KEGG, Hallmark, and Biocarta software analyses (Fig. 4). The PI3K/AKT pathway was activated in the KEGG analysis (Fig. 2B) and the NF-κB pathway in the KEGG and GO analyses of MERS-CoV-infected cells (Fig. 2B, E). External validation data in Fig. 3 and Supplementary Table 4 also



(B)

Rank (-1 → +1) GSE65574	Rank (-1 → +1) GSE139516	CMap name	Dose	Cell	Score GSE65574	Score GSE139516
1	24	DL-PPMP	2 μM	MCF7	-1	-0.883
2	2	BCB000040	10 μM	MCF7	-0.906	-0.983
4	11	diethylcarbamazine	10 μM	MCF7	-0.901	-0.910
5	12	acemetacin	10 μM	MCF7	-0.887	-0.902
9	6	CP-863187	10 μM	MCF7	-0.879	-0.929
10	13	harpagoside	8 μM	MCF7	-0.873	-0.901
11	14	isoflupredone	10 μM	PC3	-0.871	-0.901
12	4	adiphenine	11 μM	MCF7	-0.871	-0.958
13	1	homatropine	11 μM	MCF7	-0.871	-1
15	5	diclofenamide	13 μM	MCF7	-0.86	-0.939
20	8	Naftidrofuryl	8 μM	MCF7	-0.842	-0.914
21	3	bumetanide	11 μM	MCF7	-0.842	-0.966
22	21	enalapril	8 μM	MCF7	-0.841	-0.888
25	23	meteneprost	10 μM	MCF7	-0.83	-0.883
26	16	valproic acid	500 μM	MCF7	-0.829	-0.895
27	25	fluticasone	8 μM	MCF7	-0.828	-0.879
30	15	merbromin	5 μM	MCF7	-0.825	-0.901

Figure 6. Connectivity Map (CMap) analysis of potential compounds for MERS-CoV-infected cells. (A) We uploaded up- and downregulated genes identified in MERS-CoV-infected cells into CMap software to predict potential compounds compared to mock-infected controls. (B) The 30 bottom compounds from the GSE65574 dataset were compared to those in the GSE139516 dataset. The 17 common compounds in both datasets are listed. Compounds with negative correlations were those with the most powerful ability against MERS-CoV-infected cells. Abbreviations: CoV, coronavirus; MERS, Middle East respiratory syndrome.

indicate that these genes are upregulated during MERS-CoV infection. These data are consistent with previous research. For example, RNA-binding protein Roquin (RC3H1) is essential for human cytomegalovirus (HCMV) lytic production,[41] NF-κB was correlated with SARS-CoV progression,[42] cluster of differentiation 69 (CD69) serves as an activation marker of blood iNKT and MAIT cells in COVID-19 patients.[43] TNFAIP3 responded to duck Tembusu viral infection,[44] LEAP-2 plays a crucial role in immune activation as well as cytokine production for human immunodeficiency virus (HIV)/hepatitis C virus (HCV) infections.[45] DUSP10 is an important regulator of rhinovirus infections,[46] miR-449a enhances hepatitis B virus (HBV) replication via cAMP-responsive element binding protein 5 (CREB5).[47] HIV enhancer binding protein 2 (HIVEP2) regulates neuronally mediated neuroinflammation,[48] and CXCL2 serves as a pro-viral factor in early infection models of SARS-CoV-2.[49] Alteration of olfactory or sensory gene signaling was detected in MERS-CoV-infected cells from the KEGG and GO analyses (Fig. 4). Our study used an enrichment analysis from gene sets of NCBI GEO datasets to explain clinical manifestations of patients and host cellular responses after viral infection.

There are several miRNAs involved in viral infection; for example, expressions of miR-1247-3p and its targeted genes increase in canine parvovirus-infected cells.[50] miR-622 is upregulated in mosquitoes with dengue virus infection.[51] Circulating miR-3180 was detected in patients with acute-phase HBV infection, which decreased during chronic infection.[52] Other miRNAs are related to signal transduction, such as miR-127-5p activates PI3K/Akt signaling.[53] The present study used a bioinformatics analysis and Venn diagrams to investigate miRNA expressions in MERS-CoV-infected cells from the GSE65574 and GSE139516 datasets. Common upregulated miRNAs included miR-1247-3p, miR-127-5p, miR-622, and miR-3180; miR-4660 is a serum marker of patients with oxalosis, but it is not involved in infection or immune regulation.[54] We also found miRNAs without available data as to their molecular function, including miR-3180-3p and miR-3173-3p. These miRNAs

were highly expressed in MERS-CoV-infected cells and may play important roles in disease development (Fig. 5A). We combined miRNA-targeted genes with upregulated mRNAs from the two datasets, and the intersection showed overexpression of the TCF4, NR3C1, and POU2F2 genes in MERS-CoV-infected cells (Fig. 5B). These three genes are reported in the viral infection-associated literature; for instance, Kaposi's sarcoma-associated herpesvirus reduces the binding affinity of the TCF4 protein to inhibit WNT signaling.[55,56] Human immunodeficiency virus (HIV) is one RNA virus associated with TCF-4 and regulated by canonical Wnt/β-catenin signaling.[57,58] Expression of NR3C1 is correlated with the serostatus of HIV.[59,60] The OFT-2 and NF-κB genes were firmly upregulated after influenza A virus infection in a monocytic cell line.[61] A nuclear antigen of the Epstein—Barr virus coincides with the POU2F2 protein to regulate transcription.[62] Interacting networks of TCF4, NR3C1, and POU2F2 proteins in MERS-CoV-infected cells were established by STRING software (Fig. 5C). Our study discovered major altered miRNAs, and their downstream target genes are potential targets for new therapeutic agents against MERS-CoV infection; this study also sheds light on how cells respond to viral infection.

Through the CMap analysis, we revealed that compounds with highly negative correlations might serve as potential treatments for MERS-CoV-infected cells (Fig. 6). Some of these data were confirmed by previous research. Diethylcarbamazine (DEC) is an antihelmintic drug for filariasis. For patients coinfected with the filarial parasite, *Wuchereria bancrofti*, and HIV, DEC helps anti-HIV drugs suppress the viral load and increase the CD4 level.[63,64] There is also some proposed evidence for using DEC in COVID-19.[65] Harpagoside has potent anti-inflammatory effects and is active against the vesicular stomatitis virus *in vitro*.[66] Ambroxol inhibits type 14 rhinovirus (RV14) infection in human tracheal epithelial cells and may serve as a potential therapeutic drug for severe influenza-associated complications.[67,68] Bumetanide is a loop diuretic; it inhibited interactions of HIV and human protein lens epithelium-derived growth factor (LEDGF/p75) in a cellular study.[69] Enalapril slows down the progression of HIV-associated kidney disease, and drug—drug interactions between enalapril and anti-HIV agents are possible.[70] Valproic acid is an anticonvulsive drug and inhibits the replication of enveloped viruses in cultured cells. Valproic acid suppresses uptake of virus particles during herpes virus infection,[71] and it induces antiviral action in SARS-CoV-2-infected lung epithelial cells.[72] Fluticasone may be a potentially effective drug for SARS-CoV-2.[73] Amitriptyline increased survival rates in an influenza A H5N1 virus-infected model.[74] Streptomycin may demonstrate some inhibitory effects against SARS-CoV-2[75] and could significantly reduce coxsackievirus B3 (CVB3) shedding and pathogenesis.[76] HIV-1 protease activity was inhibited by pepstatin.[77] Estradiol may protect women from the most serious complications of COVID-19,[78] and treatment with diphenhydramine in combination with ibuprofen provided therapeutic benefits in influenza A infection.[79] Myricetin inhibited HIV-1 as well as coronavirus disease progression.[80,81] Other compounds are also listed by CMap in Supplementary Table 5. These compounds might be potential therapeutic drugs against MERS-CoV infection.

Conclusions

The present study focused on MERS-CoV-infection-associated pathways. Although further studies are required to extend these results to SARS-CoV and SARS-CoV-2 infections, we demonstrated crucial roles of miRNAs and mRNAs in MERS-CoV-infected cells. These miRNA- and miRNA-targeted genes and upregulated mRNAs are potential targets for the treatment and prevention of MERS-CoV infection. These novel genes and drugs may guide experimental efforts towards developing vaccines against MERS-CoV and may further combat the coronavirus pandemic.

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Author contributions

Conceptualization, Y.H.W., H.P.H., C.Y.W., and M.D.L.; methodology and formal analysis, M.C.Y., I.J.Y., and J.H.H..; investigations, C.C.C. and C.F.C.; resources, N.N.P. and Z.S.; data curation, M.C.Y., I.J.Y., and J.H.H.; writing-original draft preparation Y.H.W and I.J.Y.; writing-review and editing, H.P.H., C.Y.W., and M.D.L.; project administration, H.P.H., C.Y.W., and M.D.L.; funding acquisition, M.C.Y., H.P.H., M.D.L., and C.Y.W. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2021.03.007.