



Deciphering molecular mechanisms of SARS-CoV-2 pathogenesis and drug repurposing through GRN motifs: a comprehensive systems biology study

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Abstract

The world has recently been plagued by a new coronavirus infection called SARS-CoV-2. This virus may lead to severe acute respiratory syndrome followed by multiple organ failure. SARS-CoV-2 has approximately 80–90% genetic similarity to SARS-CoV. Given the limited omics data available for host response to the viruses (more limited data for SARS-CoV-2), we attempted to unveil the crucial molecular mechanisms underlying the SARS-CoV-2 pathogenesis by comparing its regulatory network motifs with SARS-CoV. We also attempted to identify the non-shared crucial molecules and their functions to predict the specific mechanisms for each infection and the processes responsible for their different manifestations. Deciphering the crucial shared and non-shared mechanisms at the molecular level and signaling pathways underlying both diseases may help shed light on their pathogenesis and pave the way for other new drug repurposing against COVID-19. We constructed the GRNs for host response to SARS-CoV and SARS-CoV-2 pathogens (in vitro) and identified the significant 3-node regulatory motifs by analyzing them topologically and functionally. We attempted to identify the shared and non-shared regulatory elements and signaling pathways between their host responses. Interestingly, our findings indicated that *NFKB1*, *JUN*, *STAT1*, *FOS*, *KLF4*, and *EGRI* were the critical shared TFs between motif-related subnetworks in both SARS and COVID-19, which are considered genes with specific functions in the immune response. Enrichment analysis revealed that the NOD-like receptor signaling, TNF signaling, and influenza A pathway were among the first significant pathways shared between SARS and COVID-19 up-regulated DEGs networks, and the term “metabolic pathways” (hsa01100) among the down-regulated DEGs networks. *WEE1*, *PMAIP1*, and *TSC22D2* were identified as the top three hubs specific to SARS. However, *MYPN*, *SPRY4*, and *APOL6* were the tops specific to COVID-19 in vitro. The term “Complement and coagulation cascades” pathway was identified as the first top non-shared pathway for COVID-19 and the MAPK signaling pathway for SARS. We used the identified crucial DEGs to construct a drug–gene interaction network to propose some drug candidates. Zinc chloride, Fostamatinib, Copper, Tirofiban, Tretinoin, and Levocarnitine were the six drugs with higher scores in our drug–gene network analysis.

Keywords COVID-19 · SARS · Regulatory network motifs · Host response · DEGs

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Abbreviations

GRN	Gene regulatory network
DEGs	Differentially expressed genes
KEGG	Kyoto Encyclopedia of Genes and Genomes
GO	Gene ontology
BP	Biological process
SARS-CoV	Severe acute respiratory syndrome coronavirus
TF	Transcription factor
ACE2	Angiotensin-converting enzyme 2

Introduction

COVID-19, SARS, and MERS are all frightening diseases caused by viral agents from a large family of viruses called coronaviruses (Munster et al. 2020). Although dozens of coronaviruses have been reported to infect various living organisms, including mammals, rodents, and birds, only seven have been able to infect humans. In 2020, Chen et al. listed six important pathogenic coronaviruses, including (HKU1, 229E, OC43, NL63, MERS, and SARS-CoV) (Chen et al. 2020). SARS-CoV-2 is considered the seventh member of the coronavirus family, which can infect humans and cause deadly pneumonia even in the young population (Paules et al. 2020). One of the predominant symptoms of patients can be severe acute respiratory syndrome (SARS), a respiratory condition with approximately 10% mortality (Organization 2003).

Analysis of the SARS-CoV-2 genome showed that this new virus shares about 80–90% sequence identity with the original SARS-CoV (Zhou et al. 2020). SARS-CoV is a positive-stranded ribonucleic acid (RNA) virus of the family of Coronaviridae (Guan et al. 2004; Peiris et al. 2004). Their genome is surrounded by a membrane that has various parts, such as the transmembrane (M) glycoprotein, the spike (S) glycoprotein, and the envelope (E) protein (Neuman et al. 2006). The S protein, an essential protein available on the SARS-CoV membrane, is crucial in penetrating host cells and initiating infection. The S protein interacts with the type 2 enzyme of humans converting angiotensin (ACE2) (Li et al. 2003). Thus, ACE2 is a functional SARS-CoV receptor in vivo (Imai et al. 2005; Kuba et al. 2005). Likewise, the SARS-CoV-2 spike S protein binds to its human receptor ACE2 (angiotensin-converting enzyme 2) through its receptor-binding domain (RBD), and ACE2 is recognized as a receptor in SARS-CoV-2 entry (Zhang et al. 2020).

Given their phylogenetic proximity, deciphering each of these two evolutionary cousins' molecular mechanisms will likely help us predict another viral pathology underlying mechanisms. However, more information is available on the SARS-CoV molecular mechanism than SARS-CoV-2; there are still many gaps in our knowledge of the host response

mechanisms to SARS-CoV. For example, although some studies have tried approximating both illnesses' mechanisms (Delgado-Chaves et al. 2020; Guzzi et al. 2020), no study has yet deciphered the regulatory motif elements in SARS-CoV host response GRN. Although both diseases were soon known for their pathogenesis in leading to acute respiratory distress, a recent single-cell sequencing study predicted that SARS-CoV-2 could harm most other organs in the body, such as the heart, kidney, and brain (Zaim et al. 2020). Their results proved that our understanding of the virus mechanisms has been insufficient over the past few months.

COVID-19 still kills some youth with no history of physical weakness daily and can potentially harm several vital organs, including the lung, brain, and digestive system (Renu et al. 2020). So far, no significantly effective drug has been discovered, perhaps due to its complex mechanism (Organization 2020). Unfortunately, many patients with severe forms of COVID-19 still die worldwide each day, and it highlights the importance of the immediate discovery of effective medications against the infection, and many research centers are working hard to find an effective drug against it. However, many common approaches to identifying disease mechanisms and drug design are reductionist in scope or at least time-consuming.

A relatively new and holistic study approach in biology, called systems biology, improved our understanding of various diseases' molecular mechanisms in the last twenty years. It has helped boost our knowledge of the regulatory interactions mediating many diseases (Dix et al. 2016; Sakata and Winzeler 2007). Various network analyses, including GRNs (gene regulatory networks), have been the main pivots in several studies (Noori et al. 2020; Sun et al. 2012). They have been used to decipher the mechanisms underlying drug effects or other treating interferences (Dehghan et al. 2022; Farahani et al. 2018; Khazaei-Poul et al. 2022). Previous studies have shown that transcription factors and miRNAs are the main pillars of GRNs and play crucial roles in regulating their target genes (Zhou et al. 2007). It has been shown that TF-mediated transcriptional and posttranscriptional miRNA-mediated regulation is usually associated with each other in networks (Chen et al. 2011). GRNs have contributed to predicting the mechanisms underlying various diseases and even repurposing drugs (Zickenrott et al. 2016). New omics techniques have provided high-throughput transcript-omics data, paving the way to gain a more in-depth holistic insight into various processes mediated in disease pathogenesis. Shared crucial regulatory elements among some relevant diseases have recently been applied to heighten our perception and insights into the disease's molecular mechanisms in more detail (Sun and Hu 2016). The new insights may clarify the crucial molecules that may presumably be applied to identify new prophylactic treatments, diagnosis methods, and even new drug designs.

Drug design methods use different *in silico* approaches to facilitate and accelerate the finding of proper medications. One common approach is applying docking methods and molecular dynamics. Several studies have attempted to introduce drug candidates against the crucial proteins of SARS-CoV-2 in a relatively short time after its emergence. SARS-CoV-2 3C₁pro (Molavi et al. 2021), main protease (Singh et al. 2022a), helicase (Borgio et al. 2020), RdRp (RNA-dependent RNA polymerase) (Singh et al. 2021), the non-structural and protein 15 (Singh et al. 2022b), have been targeted using these methods by several studies. They have also been applied in investigations on SARS-CoV-2 entry through the affinity of spike protein and its receptor (Aljindan et al. 2021). Another *in silico* approach to introducing drug candidates is applying drug-gene network analysis, and several studies have applied it to propose drug candidates against diseases (Dehghan et al. 2021; Huang et al. 2020).

In this study, we pursue three goals. First, we focus on analyzing the molecular mechanism of the host response to SARS-CoV. To do this, we first identify the molecular mechanisms of the SARS-CoV host respiratory system response by deciphering the SARS regulatory network motifs. As the second aim, we investigated the molecular relationships between SARS and COVID-19 regulatory networks, thereby identifying shared processes that are likely the crucial components of the molecular pathogenesis in both diseases. We also aimed to identify the non-shared crucial molecules and their functions to predict the specific mechanisms of each infection. It helps unravel the molecular mechanisms responsible for the two infections' manifestations. For example, we identified six transcription factors as probably shared master regulators of SARS-CoV-2 and SARS-CoV pathogenesis, introduced in subsequent sections. Besides, we identified some crucial nodes in the SARS-specific networks, such as WEE1, PMAIP1, and TSC22D2. However, others were identified as crucial and specific to COVID-19 *in vitro*, like *MYPN*, *SPRY4*, and *APOL6*. Third, we aimed to repurpose new candidate drugs against COVID-19 or its symptoms based on our identified shared crucial molecules underlying both diseases' pathogenesis. We utilize a drug-gene interaction network for the selected crucial genes to recommend new candidate drugs against COVID-19.

Methods

Overview of the study design

Initially, to retrieve DEGs (differentially expressed genes), three different microarray datasets were found for respiratory cell lines infected with SARS-CoV. For data related to COVID-19, the analyzed RNA-seq data from two different

respiratory cell lines were used. The up- and down-regulated DEGs were filtered by *P* value and fold change (*p* value < 0.05 and |Log FCI| > 0.5).

Several experimental databases were used to obtain four relationships between our DEGs, their regulating TFs, and miRNAs, and the TFs regulating the miRNAs to construct the GRNs (gene regulatory networks) for each disease. FANMOD software was used to identify the significant regulatory motifs for each network separately. Criteria of *Z*-score > 2.0 and *p* value < 0.05 were then applied to identify the significant motifs and motif-related subnetworks constructed using Cytoscape. The intersecting network between the motif-related subnetworks of each disease was constructed and analyzed to obtain the crucial nodes shared between the two diseases. The shared nodes with higher degree scores were identified. The non-shared nodes for each disease were also determined. We also constructed a drug-gene interaction network for the crucial shared DEGs between the two infections and the crucial DEGs specifically for COVID-19 using the dgidb database to propose possible candidate drugs and verified some using literature. The shared and non-shared pathways and gene ontology terms for the two infections were also identified and discussed as the possible mechanisms of the two diseases.

Expression data

COVID-19 data collection

Since COVID-19 is a relatively new infection, the related omics data are still insufficient in omics databanks. A recently published article regarding respiratory infections by Daniel Blanco-Melo et al. was utilized to extract the analyzed data related to COVID-19. In this study, two epithelial cell lines of the lungs' respiratory system (NHBE and A549) were applied as *in vitro* study models (Blanco-Melo et al. 2020b). A549 cells were infected at a multiplicity of infection (MOI) of 0.2 virus particles per cell for 24 h with SARS-CoV-2. However, NHBE cells were infected with SARS-CoV-2 at MOI of 2 for 24 h. Total RNA from infected and mock cells was extracted, and RNA-Seq libraries of poly-adenylated RNA were prepared using the TruSeq RNA Library Prep Kit v2 (Illumina). The cDNA libraries were sequenced using an Illumina Next-Seq 500 platform (www.illumina.com). The raw data of each cell line were analyzed separately to obtain genes that were expressed differentially (DEGs), and then, up- and down DEGs were filtered. In this study, the criteria for filtering were *p* value < 0.05 and |Log FCI| > 0.5. Our study's results relate to respiratory epithelial cells, excluding immunological cells.

SARS data collection

Initially, to retrieve DEGs (differentially expressed gene data), we searched through NCBI Gene Expression Omnibus (GEO) and the European Array Express databank (Barrett et al. 2007). The SARS omics data was limited in databanks compared to data available for other diseases. A possible explanation might be that the SARS disease was controlled relatively soon and did not last long in populations compared with other diseases. Three different data sets were found and selected to be further analyzed (GSE33267, GSE37827, and GSE47960) (Aevermann et al. 2014; Mitchell et al. 2013; Sims et al. 2013b). In both GSE33267 and GSE37827, Calu-3 cells (a lung cancer cell line) were infected with icSARS-CoV (Mitchell et al. 2013). In GSE47960, HAE cells (human airway epithelium cell line) were infected by SARS-CoV (Sims et al. 2013b).

SARS data analysis of differentially expressed genes

The datasets relating to the 60 h early post-infection time-point, shared among the three SARS datasets, were analyzed. The three datasets were filtered, normalized, and analyzed (*t* Test) using the *ge-Workbench_2.6.0* software. DEGs were filtered by criteria for *P* value and fold change (*p* value < 0.05 and *I*Log FC > 0.5). We then selected the DEGs shared among the three dataset results using the online Venn diagram tool for the early time point (60 h post-infection) (<http://bioinformatics.psb.ugent.be>). We used the available raw data from the early 60-h post-infection time-point shared among the SARS datasets. We chose the 60 h post-infection data because first, it was available in all the three SARS-related datasets, and second, it had shown the most infection impact (among the shared time-points of the available datasets) and had the highest pick in the graph curves of the study by Sims et al. (Sims et al. 2013a). Note that, 60 and 24 h post-infection time-points are both considered as very early time-points in viral infection with SARS-CoV-2 among the three hypothetical stages and patients may show clinical manifestations even after 14 days post-infection and then continue in the first stage for five days on average (Molavi et al. 2021; Romagnoli et al. 2020).

Transcription factors and miRNAs regulating the DEGs

We used only the miRNAs whose interaction with their target gene was experimentally proven, so miRNAs regulating the DEGs were retrieved using two manually curated miRNA databases (miRTarBase and miRecords) (Chou et al. 2016; Xiao et al. 2009). To obtain transcription factors regulating genes, we used the TRRUST and TRANSFAC/JASPAR databases (<http://jaspar.genereg.net>) (Chekmenev et al. 2005; Han et al. 2018).

Identification of miRNAs regulating TFs

TFs were considered genes when identifying miRNA–TF interactions using the miRTarBase and the miRecords to find the regulatory relationships between miRNAs and TFs (Chou et al. 2016; Xiao et al. 2009).

Identification of TFs regulating miRNAs

The TransmiR database (<http://www.cuilab.cn/transmir>), an open-source database that identifies transcription factors regulating microRNAs, was used to find the regulatory relationship of TF–miRNA pairs (Tong et al. 2019; Wang et al. 2010).

Construction of gene regulatory networks (GRN) and network topological analysis

We combined the four types of relationships (miRNA_Gene, TF_Gene, miRNA_TF, and TF_miRNA) to construct a transcription factor–microRNA–target gene regulatory network for SARS and COVID-19 distinctly and visualized them using Cytoscape 3.8.0. The overall four regulatory networks for the up- and down-regulated DEGs in each disease were constructed separately.

The four regulatory networks were then analyzed topologically using the Cytoscape network analyzer tool (Shannon et al. 2003). Highly connected nodes were considered hubs (top 10% of nodes with the highest degree). Since bottleneck-ness is also a significant representative of essentiality in regulatory networks, we also identified the bottlenecks (Yu et al. 2007). The top 10% of nodes with the highest betweenness centrality were considered bottlenecks. The hub and bottlenecks were used to create the new subnetworks. The new intersection network was created using the shared nodes between hubs and bottlenecks (hub bottlenecks) in SARS GRN and COVID-19 GRNs for up- and down-regulated DEGs separately (Killcoyne et al. 2009).

Motif detection

FANMOD software was applied to identify the 3-node motifs. FANMOD is a tool for identifying motifs and uses recent algorithms to improve motif detection efficiency by some orders of magnitude compared to other software. It identifies motifs occurring significantly in the network more than detected in 1000 random networks using *Z*-score. We detected 3-node motifs based on *Z*-score > 2.0 and *p* value < 0.05 (Wernicke and Rasche 2006). The motifs with at least two colors in their edges (at least included two types of relationships) were selected as significant motifs in SARS and COVID-19 for up- and down-regulated genes separately. The nodes participating in the selected significant motifs

were then retrieved using the Venn diagram. Further, they were visualized separately using Cytoscape, and a new regulatory subnetwork was constructed for each separate motif ID number (FANMOD motif ID number) in Cytoscape. The participating nodes that played dual roles (shared between the TF and DEG lists) were also identified.

Identification of motifs intersection network

The motif-related subnetworks obtained in the previous step were then merged to construct a new subnetwork in each disease (for up and down DEGs separately). The nodes shared among all the motif-related subnetworks were subsequently identified for each disease (for up and down DEGs separately) and introduced as essential molecules underlying the molecular mechanisms of host response in each viral infection independently. Besides, the non-shared nodes of the significant motifs for each disease were identified separately to predict the specific mechanisms for each infection.

Pathway and gene ontology functional analysis for hub-bottleneck genes of the union of motif-related subnetworks

Gene set enrichment analysis (GSEA) is a common approach for functional analysis and interpretation of gene expression data based on DEGs functional annotation. We used two online tools: the DAVID functional annotation tool (DAVID; <http://david.abcc.ncifcrf.gov>) and the STRING database (Huang et al. 2007).

Three types of gene sets were selected for further enrichment analysis. Firstly, genes related to the intersection between the two disease GRN networks were enriched. Secondly, genes related to the merged union of motif-related subnetworks in each of the four GRN networks were enriched, and finally, the non-shared nodes of the significant motifs were enriched separately. The non-shared nodes can probably help us predict the specific mechanisms for each infection. It will probably contribute to identifying the molecular mechanisms responsible for partially different manifestations of the two infections.

Following the construction of union subnetworks, we analyzed each subnetwork topologically to select the four subnetworks' hub-bottleneck genes. The transcription factors were also selected if they were among the DEGs of the network disease. The four new gene sets were then enriched individually for the biochemical pathways of the Kyoto Encyclopedia of Genes and Genomes (KEGG) (KEGG; www.genome.ad.jp/KEGG) using both DAVID and STRING database, and the shared results between the two online tools were listed separately for each of the four sets of genes. The shared pathways and BPs terms were then sorted based on the p-value obtained from the STRING

tool. Finally, we determined the shared and non-shared biochemical pathways and Gene Ontology terms (GOs) between SARS and COVID-19 (in vitro). A workflow representing data analysis, GRN construction, and further steps of the survey is depicted in Fig. 1.

Drug–gene interaction network construction

The crucial TF/genes identified exclusively related to COVID-19 and the crucial shared TF/genes between the two infections were selected to construct the drug–target interaction network for further drug screenings against COVID-19. We used the drug–gene interaction database (dgidb) to retrieve the medications with possible interactions with the selected genes (Cotto et al. 2018). Cytoscape (3.8.0) was used for network construction and visualization. The network was analyzed, and the drugs with higher degree scores (two or higher) were nominated to construct a new drug–gene sub-network. The new repurposed drug candidates were then validated using the ClinicalTrials.gov databank for COVID-19 clinical trials and discussed for COVID-19 or similar pathogens.

Results

We investigated the molecular regulatory mechanism of the host response to SARS-CoV-2 by comparing its regulatory network motifs with SARS-CoV. We aimed to investigate the molecular relationships between the SARS and COVID-19 regulatory networks. We identified the crucial shared and non-shared nodes between the networks of SARS and COVID-19 (in vitro). Finally, based on drug–gene interaction network analysis, we used the identified crucial molecules to repurpose drugs against COVID-19 or its manifestations.

COVID-19 and SARS data collection

The omics data for SARS-CoV-2 was available only for 24 h post-infection. We analyzed the SARS data for 60 h post-infection (the available shared early point data) to compare the two disease networks in the two available early time points. First, we filtered, normalized, and analyzed the raw data of three SARS-related datasets using ge-Workbench software (p value < 0.05 and $|\text{Log FC}| > 0.5$). We searched for the shared DEGs between the three data analysis results and identified them as DEGs related to the SARS-CoV host response in vitro for the time points. We identified 329 up-regulated and 228 down-regulated DEGs as SARS-related DEGs in 60 h. (Data are shown in Supplementary Figure S1 and Supplementary Table S1C.) For two COVID-19 datasets, we identified 572 up-regulated and 291 down-regulated

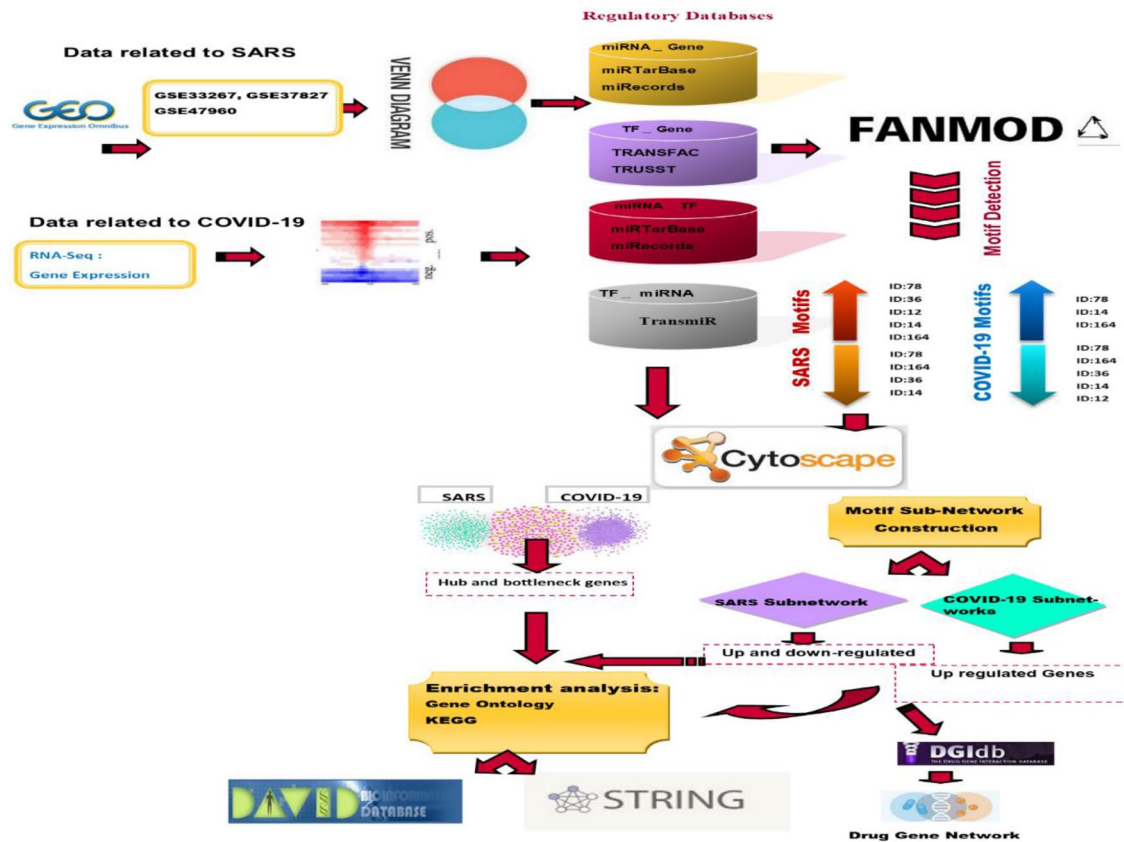


Fig. 1 This workflow represents the graphical overview of the study

genes as the COVID-19-related DEGs. (Data are presented in Supplementary Table S1D.) We searched for the shared DEGs among the two diseases. We also searched for the crucial shared DEGs between COVID-19 and DEGs related to SARS-related networks of 60 h. The results were related to respiratory epithelial cells, excluding immunological cells.

Transcription factors–miRNAs–DEGs relationships

Supplementary Tables S2, S3, S24, and S25 contain the regulatory elements of up-regulated and down-regulated SARS and COVID-19-related DEGs. miRecords and miRTarBase were searched to retrieve miRNAs that regulate the DEGs. Finally, 1512 microRNAs were retrieved for the up-regulated SARS DEGs, and 1406 miRNAs were obtained for the down-regulated DEGs. Moreover, 1569 miRNAs were extracted for the up-regulated COVID-19 DEGs and 1365 miRNAs for the down-regulated COVID-19 DEGs.

Transcription factors that regulate the DEGs were obtained from the TRUST and TRANSFAC/JASPAR databases. Finally, 306 TFs were extracted for the SARS

up-regulated DEGs and 211 TFs for the SARS down-regulated DEGs. Table S25 shows 319 and 242 transcription factors regulating the up- and down-regulated COVID-19 DEGs (Supplementary Table S3).

The subsequent regulatory relationships were the miRNAs regulating TFs. We detected 1493 unique miRNAs suppressing the TFs related to the SARS down-regulated DEGs and 1575 single miRNAs regulating the TFs related to the SARS up-regulated DEGs (Table S1D). TFs were considered genes, and two miRNA databases (miRTarBase and miRecord) were searched for TF-suppressing miRNAs in COVID-19. Table S25 shows that 1604 and 1526 microRNAs were obtained for the TFs related to the COVID-19 up- and down-regulated DEGs, respectively.

To construct the interaction of TFs regulating the miRNAs (TF → miRNA), we used the TransmiR database. We finally found 357 TFs regulating the miRNAs related to the SARS up- and down-regulated DEGs. The obtained miRNAs linked to the COVID-19 DEGs were also searched in the TransmiR database. Finally, 357 TFs were retrieved for the miRNAs regarding the up-regulated DEGs and 355 for the down-regulated (Tables S3 and S24).

Construction of gene regulatory networks and the intersection between SARS and COVID-19

Four separate regulatory networks of the TF-miRNA target gene were constructed by combining the four relationships (miRNA → gene, TF → gene, miRNA → TF, and TF → miRNA interactions) using the Cytoscape software for SARS and COVID-19 up- and down-regulated DEGs separately. A total of 2747 nodes and 24,903 edges for the SARS up-regulated DEGs network, and 2494 nodes and 18,138 edges for the SARS down-regulated DEGs regulatory network were identified. The COVID-19 up-regulated DEGs regulatory networks included 2755 nodes and 31,376 edges, and the down-regulated network included 2374 nodes and 19,163 edges.

Shared hub bottlenecks between SARS and COVID-19

To identify the shared hubs and bottlenecks between the SARS and COVID-19 regulatory networks, we first constructed four new subnetworks separately; each included the top 10% union of the hubs and the top 10% of the bottlenecks of its corresponding network (For COVID-19 and SARS up- and down-regulated separately). The four new subnetworks were then merged in Cytoscape (pairwise) using the intersectional merging analysis. Two new networks were created as intersection subnetworks between SARS and COVID-19 (for up and down networks separately). As visualized in Figure S2, the intersection between the two SARS and COVID-19 sub-networks of the top 10% hubs and bottlenecks was constructed (related to up-regulated DEGs). The new intersection subnetworks are included 138 nodes and 1344 edges, containing 64 TFs and 43 miRNAs, and 19 genes. Some crucial nodes played dual roles in the subnetwork as both

Gene and TF, including *STAT2*, *FOSL2*, *TNF*, *NFKB1*, *FOS*, *JUN*, *FOSL1*, *STAT1*, *IL6*, *PRDM1*, *KLF6*, and *HIF1A*. Figure S3 demonstrates a similar comparison between the two subnetworks related to the down-regulated DEGs between the two diseases. It included 137 nodes and 936 edges, 52 miRNAs, 78 TFs, and some genes, including *PPARGCIA*, *SPTLC3*, *TSC22D3*, and *VAV3*. The nodes with the dual role (gene/TF) in this intersection were *ZNF148* and *TCF4*.

Top shared biochemical pathways and GOs between the two diseases

Shared hub-bottleneck nodes in SARS and COVID-19 regulatory networks were used to determine the functional enrichments. The NOD-like receptor signaling pathway was the top shared pathway between SARS and COVID-19 up-regulated DEGs (Table S4 and Table 1). Besides, as shown in Table S5 and Table 2, defense response, response to other organisms, and innate immune response were the top three shared biological processes between SARS and COVID-19 up-regulated DEGs. However, it is apparent from Table 2 that the most significant biological process shared between SARS and COVID-19 in down-regulated DEGs was energy homeostasis. As shown in Table 1, the most significant pathway between SARS and COVID-19 in down-regulated DEGs was the term “Metabolic pathways” (hsa01100).

Motif detection

To decipher the regulatory 3-node motifs, the data of the four mentioned types of the relationships (miRNA_Gene, TF_Gene, miRNA_TF, and TF_miRNA) were incorporated together. They were inserted as an input file to the FANMOD software (TSV format) for each of the four regulatory networks separately (SARS up, SARS down, COVID up, COVID down) (Wernicke

Table 1 Shared biochemical pathways (KEGG) enrichment analysis results between SARS and COVID-19 for up- and down-regulated genes

	Term (KEGG pathway)	False-discovery rate	Matching proteins in the network
Up	NOD-like receptor signaling pathway	2.85E-21	BIRC3, CXCL1, CXCL2, GBP1, IFI16, IL6, IRF7, IRF9, JUN, NFKB1, NFKBIA, OAS1, OAS2, OAS3, STAT1, STAT2, TNF, TNFAIP3
	Herpes simplex infection	2.74E-19	C3, DDX58, HLA-B, IFIH1, IL6, IRF7, IRF9, JUN, NFKB1, NFKBIA, OAS1, OAS2, OAS3, SP100, STAT1, STAT2, TNF
	TNF signaling pathway	6.59E-16	BIRC3, CCL20, CXCL1, CXCL2, EDN1, IL6, JUN, MAP3K8, NFKB1, NFKBIA, PTGS2, TNF, TNFAIP3
	Influenza A	2.96E-15	DDX58, IFIH1, IL6, IRF7, IRF9, JUN, NFKB1, NFKBIA, OAS1, OAS2, OAS3, STAT1, STAT2, TNF
	Measles	4.86E-15	DDX58, IFIH1, IL6, IRF7, IRF9, NFKB1, NFKBIA, OAS1, OAS2, OAS3, STAT1, STAT2, TNFAIP3
Down	Metabolic pathways	0.0406	DH5, ALDH5A1, ALDH6A1, ALG1, BPNT1, CYCS, DHCR24, EPHX2, GALM, GALNT4, GATM, GLUD1, GPAM, HEXA, MAN1A2, MAN1C1, MAOB, POLD3, RDH10, SDHD, SHMT1, SPTLC3

The top 5 terms of KEGG biochemical pathways are reported after being sorted by significant *P* values < 0.05

Table 2 Shared Gene Ontology Biological Processes (GO-BP) enrichment analysis results between SARS and COVID-19 for up and down-regulated genes

	Term (biological process)	False-discovery rate	Matching proteins in the network
Up	Defense response	6.11E-32	BATF2, C3, CCL20, CXCL1, CXCL2, DDX58, DTX3L, EDN1, EGR1, FOSL1, GBP1, HLA-B
	Response to other organisms	6.11E-32	IFIH1, IFITM1, IFITM3, IL6, IRF1, IRF7, IRF9, ISG15, JUN, NFKB1, NFKB2, NFKBIA, OAS1, OAS2, OAS3, PARP9
	Innate immune response	3.33E-26	C3, CCL20, DDX58, DTX3L, EDN1, EGR1, GBP1, HLA-B, IFI16, IFIH1, IFITM1, IFITM3, IRF1, IRF7, IRF9
	Multi-organism process	1.25E-25	STAT1, STAT2, SYNE1, TNF, TNFAIP3, ZC3HAV1
	Response to virus	1.27E-23	IFITM3, IL6, IRF1, IRF7, IRF9, ISG15, OAS1, OAS2, OAS3, PARP9, STAT1, STAT2, TNF, ZC3HAV1
Down	Energy homeostasis	0.0266	CD36, FLCN, FOXO1, NR1D2, PPARGC1A

The top 5 terms of Biological Processes are reported after being sorted by significant P values < 0.05

and Rasche 2006). FANMOD software parameters were set to detect the significant 3-node size motifs. The FANMOD detected 13 significant motifs in the SARS up-regulated DEGs network and 12 in the SARS down-regulated DEGs (Supplementary Table S6A). Supplementary Table S6B shows nine significant motifs in the COVID-19 up-regulated DEGs network, and 12 significant motifs in the COVID-19 down-regulated DEGs were detected.

Motifs detected for the SARS DEGs

The significant motifs with edges with at least two colors were selected to construct the new motif-related sub-networks of the SARS up-regulated DEGs. The selected motifs identification codes are available in Table S24, and the motif nodes are depicted in Supplementary Table S7. The motifs with the same identity number were merged and considered unique motifs (Table S24). For example, we combined the subnetworks of the two motifs with the same identity number of 78. In this motif, 57 TFs and 91 unique microRNAs participated. We checked to see which of these TFs were among our related DEGs. Interestingly, we found that some TFs played a role as a gene and were available among the related DEGs; therefore, they were also considered dual TF/DEGs in the SARS network, including *NFKB1*, *FOS*, *JUN*, *STAT1*, *EGR1*, *PRDM1*, and *KLF4*. More detailed explanations of the other SARS Motif-related nodes are represented in Supplementary text S1.

Motifs detected for COVID-19 DEGs

As represented in Supplementary Table S8, nine motifs were identified as significant by FANMOD in the COVID-19 up-regulated DEGs networks and 12 motifs in the down-regulated. The selected motifs (with edges with at least two colors) and the number of participating nodes in each motif-related subnetwork are introduced in Table S25. In

brief, three motif-related subnetworks were selected in the COVID-19 up-regulated DEG networks. For example, motif No.14 had 76 TFs and 300 microRNAs, of which *HIF1A*, *RELB*, *FOSL1*, *STAT1*, and *PRDM1* were considered TF/Gene. More detailed explanations of the other COVID-19 Motif-related nodes are represented in Supplementary text S1.

Motifs intersection networks

The nodes participating in regulatory motifs are essential in disease molecular biology. Therefore, we attempted to identify the shared nodes among the selected motifs separately in each of the four networks. We first made a separate sub-network for each motif to construct the intersectional sub-networks. We then merged them (the Cytoscape command) to create intersectional subnetworks. These intersections for SARS are shown in Fig. 2 for up-regulated DEGs, in which *FOS*, *JUN*, *NFKB1*, *STAT1*, *KLF4*, and *EGR1* considered TF/DEGs, and Figure S4 depicts the junction between the significant motifs in down-regulated DEGs in SARS.

Furthermore, the top ten shared nodes between the selected motifs related to SARS up- and down-regulated DEGs are shown in Table 3. The shared nodes between the selected motifs related to COVID-19 up-regulated DEGs are shown in Fig. 3. Some nodes matched to this intersection played dual roles as TF and DEGs, including *STAT1*, *NFKB1*, *HIF1A*, and *PRDM1*.

Moreover, *FOS*, *KLF4*, *EGR1*, *JUN*, and *ATF3* were the other shared gene/TFs between motif-related subnetworks. (They had dual regulatory roles). Moreover, the top ten shared nodes between the selected motifs related to COVID-19 up- and down-regulated DEGs are demonstrated in Table 4. All crucial nodes in SARS and COVID-19 are shown in Tables S1A and S1B.

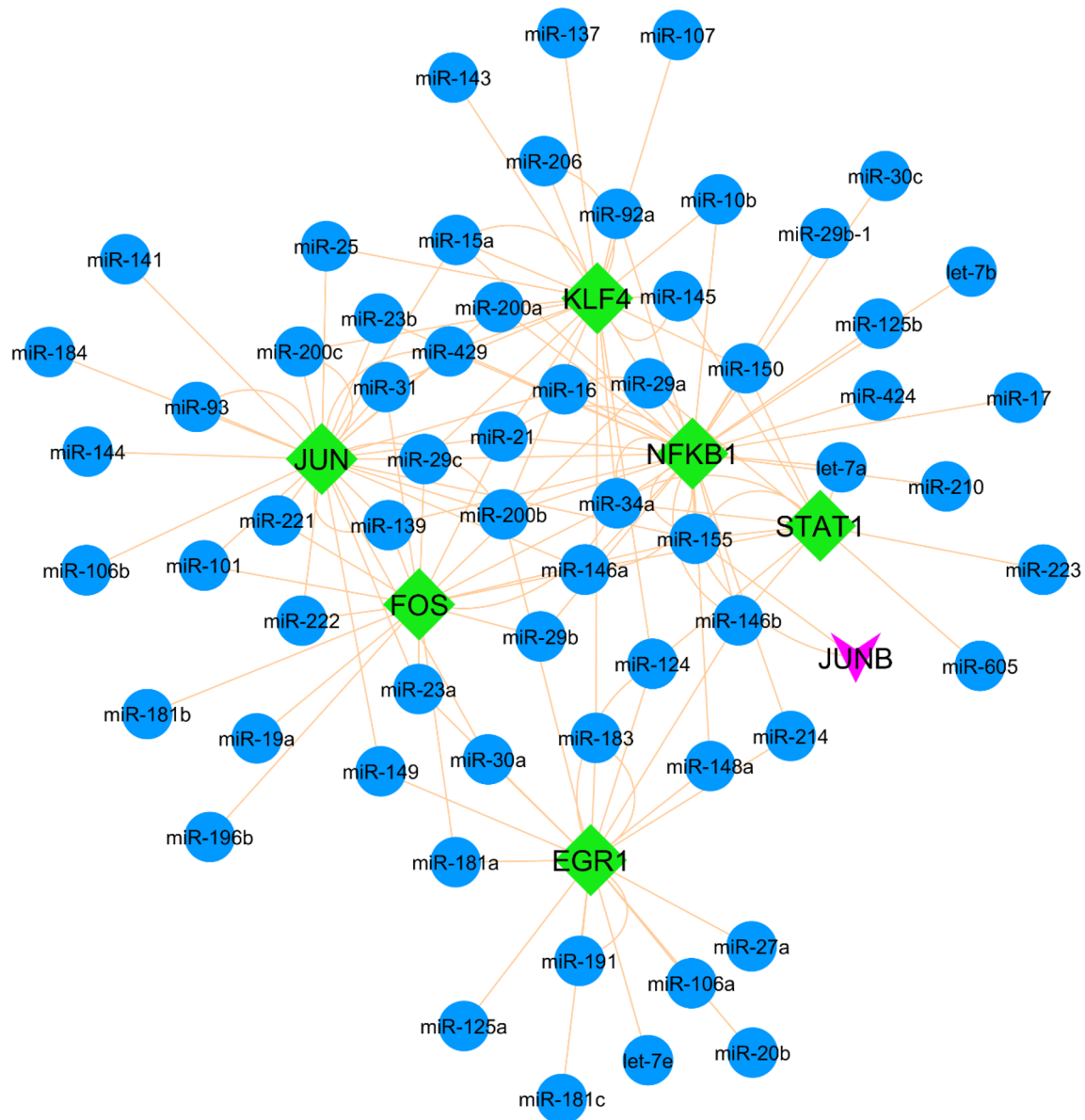


Fig. 2 The intersection among motif-related subnetworks of SARS up-regulated DEGs network. The V-shape nodes show only TFs; circle nodes represent miRNAs; diamond nodes depict only genes. The regulatory nodes modulate the shared DEGs between all five signifi-

cant motif-related subnetworks of the SARS up-regulated DEGs network. All motifs are represented by their identification motif number in the FANMOD software encyclopedia. Z-scores (> 2) and P values (< 0.05) are reported for each motif

We also identified 414 genes in COVID-19 exclusively and 192 nodes in SARS as up-regulated DEGs of the significant motif-related subnetworks for each disease (Supplementary Table S9). As shown in Table S9, 63 nodes in COVID-19 and 50 nodes in SARS were considered motifs down-regulated DEGs in each disease separately.

Topological analysis of hub-bottleneck genes of union motif networks

To obtain hub bottlenecks in the SARS and COVID-19 up- and down-regulated DEG networks, we merged the motif subnetworks in each of the four networks separately and

Table 3 The top ten hubs and bottlenecks available at the intersection of the SARS motif-related sub-networks

	Gene name (hubs)	Degree	Gene name (bottlenecks)	Betweenness centrality
Up-regulated	SOD2	250	SOD2	0.056841
	NFIC	236	NFIC	0.046579
	GATA6	192	TP53	0.030677
	MYC	178	WEE1	0.027972
Down-regulated	NFIC	228	NFIC	0.057847
	GATA6	195	GATA6	0.043001
	TP53	192	TP53	0.039817
	MYC	177	VAV3	0.036697
	SP1	163	SP1	0.035191

The sub-network from significant motifs in up and down DEGs in SARS were constructed and merged. The top 10% of hub bottlenecks were selected for enrichment analysis

created four new union subnetworks. Then, we analyzed each of these four subnetworks to identify their hub bottlenecks (the shared nodes between the top 10% of values of the degree and betweenness centrality), introduced in Supplementary Tables S10 and S11. The top five hubs and bottlenecks for each of the four networks are represented in Tables 3 and 4. Fifty-three nodes were considered the hub bottlenecks in the COVID-19 and 192 SARS up-regulated DEGs subnetwork and 166 nodes in COVID-19 and also 156 nodes in SARS as down-regulated nodes.

Overall, as represented in Tables S1A and S1B, we identified that *NFKB1*, *JUN*, *STAT1*, *FOS*, *KLF4*, and *EGR1* were the critical shared TFs between motif-related subnetworks in both SARS and COVID-19. The crucial TF/DEGs shared among the two infections included *NFKB1* and *STAT1*. Besides *JUN*, *FOS*, *KLF4*, and *EGR1* were the shared TF/genes between motif-related subnetworks in SARS and COVID-19. Moreover, *NFKB1*, *JUN*, *STAT1*, and *FOS* also were identified as the shared hub bottlenecks. *WEE1*, *PMAIP1*, and *TSC22D2* were identified as the top three DEGs with the highest degree scores specific to SARS. Besides, *MYPN*, *SPRY4*, and *APOL6* were the crucial nodes specific to COVID-19 in vitro.

Functional analysis results of hub bottlenecks of union motif networks

The four lists of genes were prepared according to the method section details to perform the functional enrichment analysis. We then used DAVID and STRING tools for enrichment analysis and identified their shared pathways and GOs.

The top results of enrichment analysis for SARS-related biochemical pathways are available in Table 6, and other

significant results are listed in Table S14 (based on *p* value). The most significant pathway term for the top 10% hub bottlenecks of the SARS network motifs (related to up-regulated DEGs) appeared to be apoptosis. The most significant biological process term (BP) was regulating the macromolecule metabolic process. (Top BP results for BPs are listed in Table 5 and others in Table S13.) As demonstrated in Table No.5 and Table S15, the top BP related to the SARS down-regulated DEGs subnetwork was the lipid metabolic process. The two top significant pathways related to its down-regulated subnetwork appeared to be the term “Metabolic pathways and Carbon metabolism” (Table 6).

The nodes exclusively for each disease were enriched separately to obtain the BPs and pathways not shared between the two diseases, and the BPs and pathways for each disease were identified. Afterward, we determined the shared and non-shared BPs and pathways using the Venn diagram. We reported the non-shared BPs and pathways specific to each disease separately (Supplementary Tables S16–S19).

As available in Tables S16–17, the most significant BP for COVID-19 up-regulated DEGs was the pattern recognition receptor signaling (GO: 0002221), and the most significant pathway was complement and coagulation cascades. Furthermore, the most significant BP in SARS was the regulation of response to the stimulus, and the first pathway was the MAPK signaling pathway.

The top enrichment analysis results for COVID-19-related gene ontology terms are available in Table 7, and other significant results are listed in Table S20. The top GO term (in COVID-19 up-regulated DEGs subnetwork) was transcription by RNA polymerase II. Noteworthy, the Hepatitis B pathway had the first rank among the COVID-19 up-regulated DEGs subnetwork results (Table S21).

The top three GOs are energy homeostasis, regulation of the developmental process, and multicellular organismal homeostasis (Tables S22 and 7). Glucagon signaling was the most significant signaling pathway in COVID-19 down-regulated DEGs network (Tables S23 and 8).

Drug–target interaction network results

As available in Supplementary Table S12, 51 unique nodes were considered the selected genes, and 83 unique medicines were nominated as the related drugs to the selected genes in the drug–target interaction network. Among the 83 drugs, 31 with higher degrees than two (based on the results between two and nine) were selected to create a new sub-network, as visualized in Fig. 4. The new repurposed drug candidates were then validated using the ClinicalTrials.gov databank for COVID-19 clinical trials and discussed for COVID-19 or similar pathogens. As shown in Table 9, five drugs were previously registered in ClinicalTrials.gov to be evaluated as possible treatments for COVID-19 and were found as

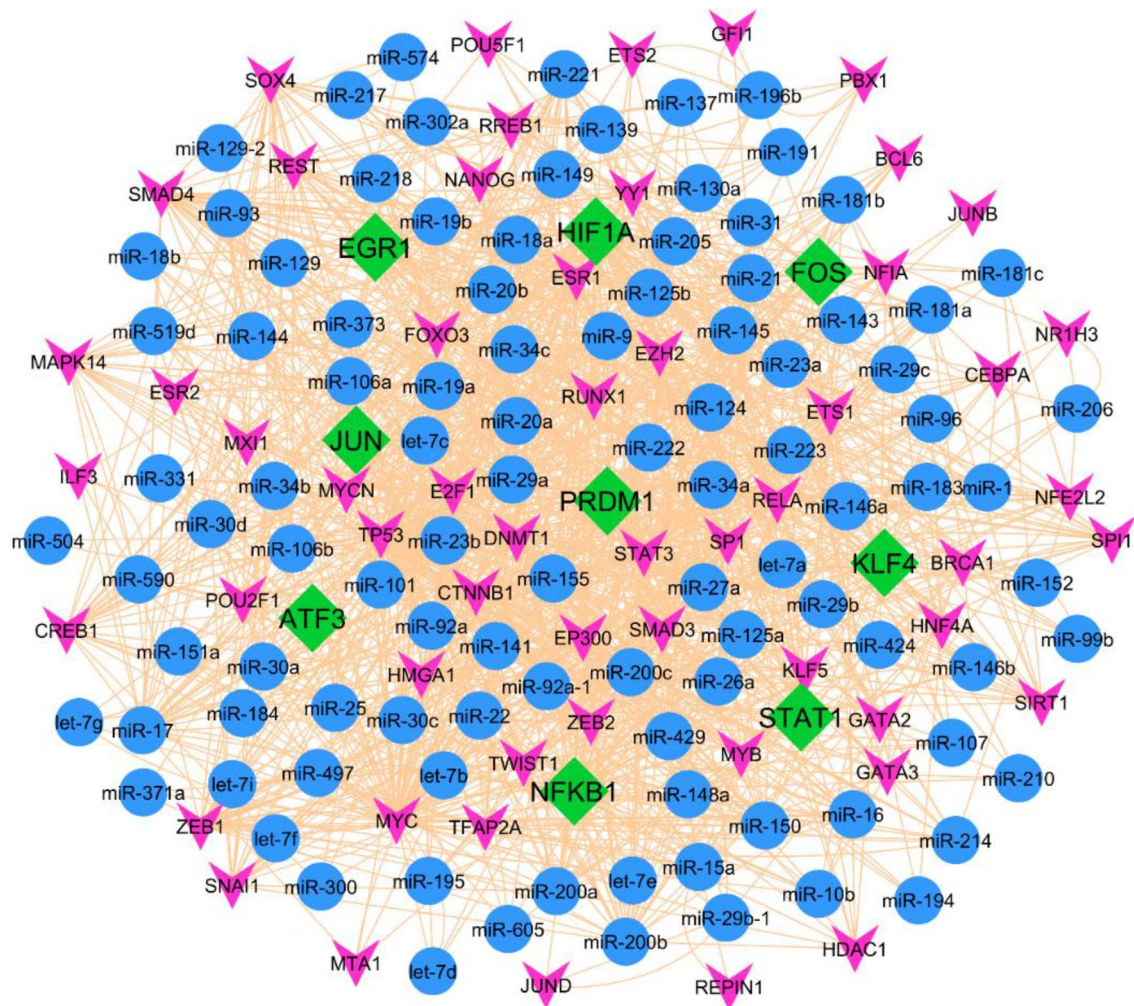


Fig. 3 The intersection among motif-related subnetworks of COVID-19 up-regulated DEGs network. The V-shape nodes show only TFs; circle nodes represent miRNAs; diamond nodes depict TF/Genes (Dual role). The regulatory nodes modulate the shared DEGs between all three (three motif IDs) significant motif-related subnetworks of the

COVID-19 up-regulated DEGs network. All motifs are represented by their identification motif number in the FANMOD software encyclopedia. Z-scores (>2) and P values (<0.05) are reported for each motif

possible logical choices for investigation against COVID-19 treatment by other researchers.

Discussion

Regulatory networks are comprised of a set of recurrence regulatory patterns named motifs. They are re-selected based on their biological functions in evolution. They are found in various organisms, showing that they are the basic building blocks of biological networks (Alon 2007). These repeating circuit elements usually appear much higher frequency than randomized networks (Milo et al. 2004). Here, we have investigated these regulatory motifs to decipher the host response complex mechanism to COVID-19 and SARS-CoV pathogenesis (in vitro). The present study is the first

system biology regulatory network study for COVID-19 (in vitro) that has attempted to decipher the regulatory network motifs to identify the molecular mechanism of COVID-19 pathogenesis. We have finally applied the identified targets in repurposing new drug candidates against the deadly COVID-19, hoping our recommended medications appear effective in further in vitro and in vivo investigations.

This study compared the motif-related subnetworks of differentially expressed genes between respiratory cells treated with SARS-CoV-2 and SARS-CoV. We revealed the shared crucial nodes, molecular mechanisms, and signaling pathways underlying both viral infections. Our findings indicated that NFKB1 and STAT1 were shared DEG/TFs between motif-related subnetworks in SARS and COVID-19 up-regulated networks. They also were identified as the shared hub bottlenecks. Enrichment analysis revealed that

Table 4 The top ten hubs and bottlenecks available at the intersection of the COVID-19 motif-related sub-networks

	Gene name (hubs)	Degree	Gene name (bottle-necks)	Between-ness centrality
Up-regulated	MYC	139	HIF1A	0.083288
	TP53	129	TP53	0.076228
	HIF1A	112	MYC	0.071981
	STAT3	83	miR-21	0.054524
	NFKB1	78	miR-155	0.050347
Down-regulated	NFIC	268	NFIC	0.058479
	TP53	223	VAV3	0.039508
	FOXC1	211	FOXC1	0.037026
	YY1	206	YY1	0.032442
	GATA6	206	TP53	0.032431

The sub-network from significant motifs in up and down DEGs in COVID-19 were constructed and merged. The top 10% of hub bottlenecks were selected for enrichment analysis

the NOD-like receptor signaling pathway was the first significant pathway shared between SARS and COVID-19 up-regulated DEGs networks. However, the term “metabolic pathways” (hsa01100) was identified as the most significant pathway term shared among the down-regulated DEGs networks. The infected cell almost always experiences metabolic changes when viruses infect it. Deepak Sumbria et al. recently reviewed several examples, including how viruses can affect the metabolism of fatty acids, glutaminolysis, and energy (Sumbria et al. 2021). Some viruses only infect the already metabolically active cells. Their infection usually downregulates some metabolic processes. It happens, for instance, with HIV, which prefers to infect cells in an activated metabolic state (Datta et al. 2016; Sumbria et al. 2021).

The SARS-CoV-2 infection has also been linked to metabolic changes. During COVID-19, this metabolic dysregulation affects multiple organs. Therefore, its early detection may serve as a prognosis marker (Datta et al. 2016).

WEE1, *PMAIP1*, and *TSC22D2* were identified as the top three DEGs with the highest degree scores specific to SARS.

Table 5 The functional enrichment analysis was performed on the set of up and down-regulated genes of the SARS regulatory network for Gene Ontology Biological Processes (GO-BP)

	Term (biological process)	False-discovery rate	Matching proteins in the network
Up-regulated network	Regulation of macromolecule metabolic process	7.67E-10	PTPN14, SEMA7A, SERTAD2, SIK1, SLC6A4, SMAD7, SOD2, SP110, STAT2, THBS1, TNFAIP3, TNFRSF10B, TRAF1, TRIB1, TSC22D2, ZBTB10, ZNFX1
	Regulation of primary metabolic process	9.01E-10	PTPN14, SEMA7A, SERTAD2, SIK1, SMAD7, SOD2, SP110, STAT2, THBS1, TNFAIP3, TNFRSF10B, TRAF1, TRIB1, TSC22D2, ZBTB10, ZNFX1
	Regulation of cellular metabolic process	1.32E-09	SMAD7, SOD2, SP110, STAT2, THBS1, TNFAIP3, TNFRSF10B, TRAF1, TRIB1, TSC22D2, ZBTB10, ZNFX1
	Response to abiotic stimulus	7.19E-09	NFKB1, PARP1, PER1, PMAIP1, SIK1, SLC6A4, THBS1, TNFRSF10B, TSC22D2
	Regulation of gene expression	1.88E-08	SOD2, SP110, STAT2, THBS1, TNFAIP3, TRAF1, TRIB1, TSC22D2, ZBTB10, ZNFX1
Down-regulated network	Lipid metabolic process	0.0255	ATF3, BACH2, BBC3, BCL3, BMP2, BRD2, C3, CCL5, CD55, CREB5, CREBBP, CXCL10, CYR61, DOT1L, DTX3L, DUSP10, DUSP4, DUSP8, DYRK1A, EDN1
	Protein targeting to membrane	0.0274	ADH5, ALDH5A1, ALG1, ANG, BPNT1, DHCR24, ERBB3, HEXA, NR2F2, PPM1L, RDH10, SPTLC3, ZADH2
	Peptidyl-cysteine modification	0.0274	GOLGA7, RPL14, RPL22, RPL23A, SSR1
	Cellular lipid metabolic process	0.0274	ADH5, GOLGA7, ZDHHC20
	Protein localization to membrane	0.0274	ADH5, ALDH5A1, ALG1, ANG, BPNT1, ERBB3, HEXA, PPM1L, RDH10, SPTLC3, ZADH2

The top 5 terms of Biological Processes are reported after being sorted by significant *P* values < 0.05

Table 6 The functional enrichment analysis was performed on the set of up and down-regulated genes of the SARS regulatory network for the Kyoto Encyclopedia of Genes and Genomes database (KEGG) biochemical pathways

	Term (KEGG pathway)	False-discovery rate	Matching proteins in the network
Up-regulated network	Apoptosis	8.92E-08	ATM, BBC3, FOS, JUN, NFKB1, PARP1, PMAIP1, TNFRSF10B, TRAF1
	Herpes simplex infection	8.92E-08	C3, CLOCK, CREBBP, FOS, HLA-C, JUN, NFKB1, PER1, STAT2, TRAF1
	Pathways in cancer	6.01E-06	ABL1, BBC3, CREBBP, FOS, HHIP, IGF2, JUN, NFKB1, PIM1, PMAIP1, STAT2, TRAF1
	TNF signaling pathway	6.93E-05	EDN1, FOS, JUN, NFKB1, TNFAIP3, TRAF1
	HTLV-I infection	6.93E-05	ATM, CREBBP, EGR1, FOS, FOSL1, HLA-C, JUN, NFKB1
Down-regulated network	Metabolic pathways	3.04E-05	ADH5, ALDH5A1, ALDH6A1, ALG1, BPNT1, CYCS, DHCR24, EPHX2, GALM, GALNT4, GATM, GLUD1, GPAM, HEXA, MAN1A2, POLD3, RDH10, SDHD, SHMT1, SPTLC3
	Carbon metabolism	0.0108	ADH5, ALDH6A1, GLUD1, SDHD, SHMT1

The top 5 terms of KEGG biochemical pathways are reported after being sorted by significant *P* values < 0.05

Table 7 The functional enrichment analysis was performed on the up-and down-regulated genes of the COVID-19 regulatory network for Gene Ontology Biological Processes (GO-BP)

	Term (biological process)	False-discovery rate	Matching proteins in the network
Up-regulated network	Transcription by RNA polymerase II	3.67E-23	CREB1, EGR1, ESR1, ETS1, FOXO3, HIF1A, HMGA1, HNF4A, JUN, KLF4, MYC, MYCN, NFKB1, POU2F1, REL
	Negative regulation of transcription, DNA-templated	1.26E-22	SMAD3, SMAD4, STAT1, STAT3, TNF, TP53, TWIST1, YY1, ZEB1
	Transcription, DNA-templated	2.38E-22	HMGA1, HNF4A, JUN, KLF4, MYC, MYCN, NFKB1, POU2F1, PRDM1, REL, RELA, REST, SMAD3, SMAD4
	Negative regulation of gene expression	2.58E-22	REL, RELA, REST, SMAD3, SMAD4, STAT1, STAT3, TNF, TP53, TWIST1, YY1, ZEB1
	Negative regulation of transcription by RNA polymerase II	1.80E-21	PRDM1, REL, RELA, REST, SMAD3, SMAD4, STAT1, STAT3, TNF, TP53, TWIST1, YY1, ZEB1
Down-regulated network	Energy homeostasis	0.00033	CD36, FLCN, FOXO1, NR1D2, PPARGC1A
	Regulation of developmental process	0.0065	CD36, CD86, COL1A1, DUSP10, EPHA4, FGF1, FLCN, FOXO1, IL6R, MAF, NR1D2, PALM2, PDE5A, PPARGC1A, PRKACB, SEMA3E, SEMA6D, SOX6, STX1B, TCF4, TP53INP1, TSC22D3
	Multicellular organismal homeostasis	0.0305	CD36, FLCN, FOXO1, NANOS1, NR1D2, PPARGC1A, PRKACB
	Negative regulation of developmental process	0.0305	DUSP10, EPHA4, FLCN, FOXO1, PPARGC1A, PRKACB, SEMA3E, SEMA6D, SOX6, STX1B, TP53INP1, TSC22D3
	Regulation of cell differentiation	0.0461	CD36, CD86, COL1A1, DUSP10, EPHA4, FLCN, FOXO1, IL6R, MAF, NR1D2, PDE5A, SEMA6D, SOX6, STX1B, TCF4, TP53INP1

The top 5 terms of Biological Processes are reported after being sorted by significant *P* values < 0.05

The "Complement and coagulation cascades" pathway was identified as the first top non-shared pathway for COVID-19, and the MAPK signaling pathway was identified as the

first for SARS. Here, we discuss and verify that some of our identified crucial molecules and pathways proposed in this network model study are previously experimentally proven

Table 8 The functional enrichment analysis was performed on the set of up and down-regulated genes of the COVID-19 regulatory network for the Kyoto Encyclopedia of Genes and Genomes database (KEGG) biochemical pathways

	Term (KEGG pathway)	False discovery rate	Matching proteins in the network
Up-regulated network	Hepatitis B	1.26E-17	CREB1, E2F1, IL6, JUN, MYC, NFKB1, RELA, SMAD3, SMAD4, STAT1, STAT3, TNF, TP53
	HTLV-I infection	6.87E-15	CREB1, E2F1, EGR1, ETS1, IL6, JUN, MYC, NFKB1, RELA, SMAD3, SMAD4, TNF, TP53
	Pathways in cancer	4.49E-14	E2F1, ESR1, ETS1, HIF1A, IL6, JUN, MYC, NFKB1, RELA, SMAD3, SMAD4, SP1, STAT1, STAT3, TP53
	Inflammatory bowel disease (IBD)	3.59E-12	IL6, JUN, NFKB1, RELA, SMAD3, STAT1, STAT3, TNF
	Pancreatic cancer	1.17E-11	E2F1, NFKB1, RELA, SMAD3, SMAD4, STAT1, STAT3, TP53
Down-regulated network	Glucagon signaling pathway	0.05327601	FOXO1, PRKACB, PPARGC1A
	Insulin resistance	0.062166115	CD36, FOXO1, PPARGC1A
	AMPK signaling pathway	0.078014596	CD36, FOXO1, PPARGC1A
	Axon guidance	0.082439875	EPHA4, SEMA6D, SEMA3E
	Platelet activation	0.085809923	PRKACB, COL1A1, MYLK

The top 5 terms of KEGG biochemical pathways are reported after being sorted by significant *P* values < 0.05

in other similar viral pathologies and recommend them for further experimental studies for COVID-19.

NF-κB1 (Nuclear Factor Kappa B Subunit 1) was shared between the two diseases and identified as a node with dual roles (gene/TF) that were up-regulated in both SARS and COVID-19 in vitro. The *NF-κB1* plays a role in some immune diseases and is associated with some immunodeficiency diseases based on the GeneCards databank (Stelzer et al. 2016). The regulation of *NF-κB* is linked to inflammatory and autoimmune diseases and some viral infections. The *NF-κB* has been reported in many animal cell types. It is an essential intermediate in various biochemical pathways related to cell life, such as apoptosis, cellular responses to cytokines, and viral antigens.

Regarding apoptosis regulation, *NF-κB* acts pro-apoptotic and antiapoptotic, depending on cell type. However, it serves more as an apoptosis blocker. Some studies have suggested that finding more in-depth insight into the *NF-κB* mechanism might help develop suitable therapeutics for some immune diseases (Baichwal and Baeuerle 1997; Barkett and Gilmore 1999; Gilmore 2006). *NF-κB* has been proposed as an essential signaling hub. It integrates the intracellular and intercellular signaling pathways, therefore facilitating the host response without inflammatory injuries (Quinton and Mizgerd 2011).

Some studies have reported the activation of *NF-κB1* in lung alveolar epithelial cells in patients infected with other respiratory pathogens (Quinton et al. 2007; Xu et al. 2008). In line with this, Frieman et al. revealed that the SARS-CoV papain-like protease (PLP) could block the *NF-κB* signaling pathway and affect the host immune system. Indeed, viral PLP antagonizes the host IFN induction and *NF-κB* signaling pathways. Using the PLP, the virus regulates the host's

innate immune responses against itself (Frieman et al. 2009). Thus, SARS-CoV probably uses PLP as a strategy against *NF-κB* signaling and IFN secretion to overcome the host immune response. Moreover, an in silico docking screening study has recently attempted to identify some candidate drugs against SARS-CoV-2 PLP to overcome the COVID-19 dilemma (Kumar et al. 2020). These findings confirm that *NF-κB1* is a crucial regulatory element in the host immune response to SARS-CoV-2, regulates apoptosis and cell life, and targets its viral antagonists (like PLP). It may help to develop suitable medicines against SARS-CoV-2.

STAT1 (The signal transducer and activator of transcription 1) was another shared crucial gene. It mediates cellular responses to different cytokines and growth factors, including cytokine KITLG/SCF and interferons (IFNs). Liang; et al. reported that *STAT1* activation plays a vital role in IFN α signaling for the NK cell cytotoxicity function (Liang et al. 2003). The role of *STAT1* in various viral infections, including the Ebola virus (EBOV)(Reid et al. 2006), Hepatitis C virus (HCV) (Lin et al. 2005), Nipah virus (NiV) (Rodriguez et al. 2002), and influenza A (Melen et al. 2003), has been investigated in several studies. They reported that the expression of some viral proteins suppresses type-I interferon signaling by reducing phosphorylated *STAT1*, and also, some of them could block the nuclear accumulation of *STAT1* (Mu et al. 2020). A study on knocked-out mice (*STAT^{-/-}*) showed that lack of *STAT* might contribute to wound healing loss and fibrosis induction. It led to a fatal condition after SARS-CoV infection (Frieman et al. 2010). Lung injuries such as fibrosis are considered a severe health problem in patients with severe COVID-19. These findings suggest that *STAT1* plays a crucial role in the immune response to various

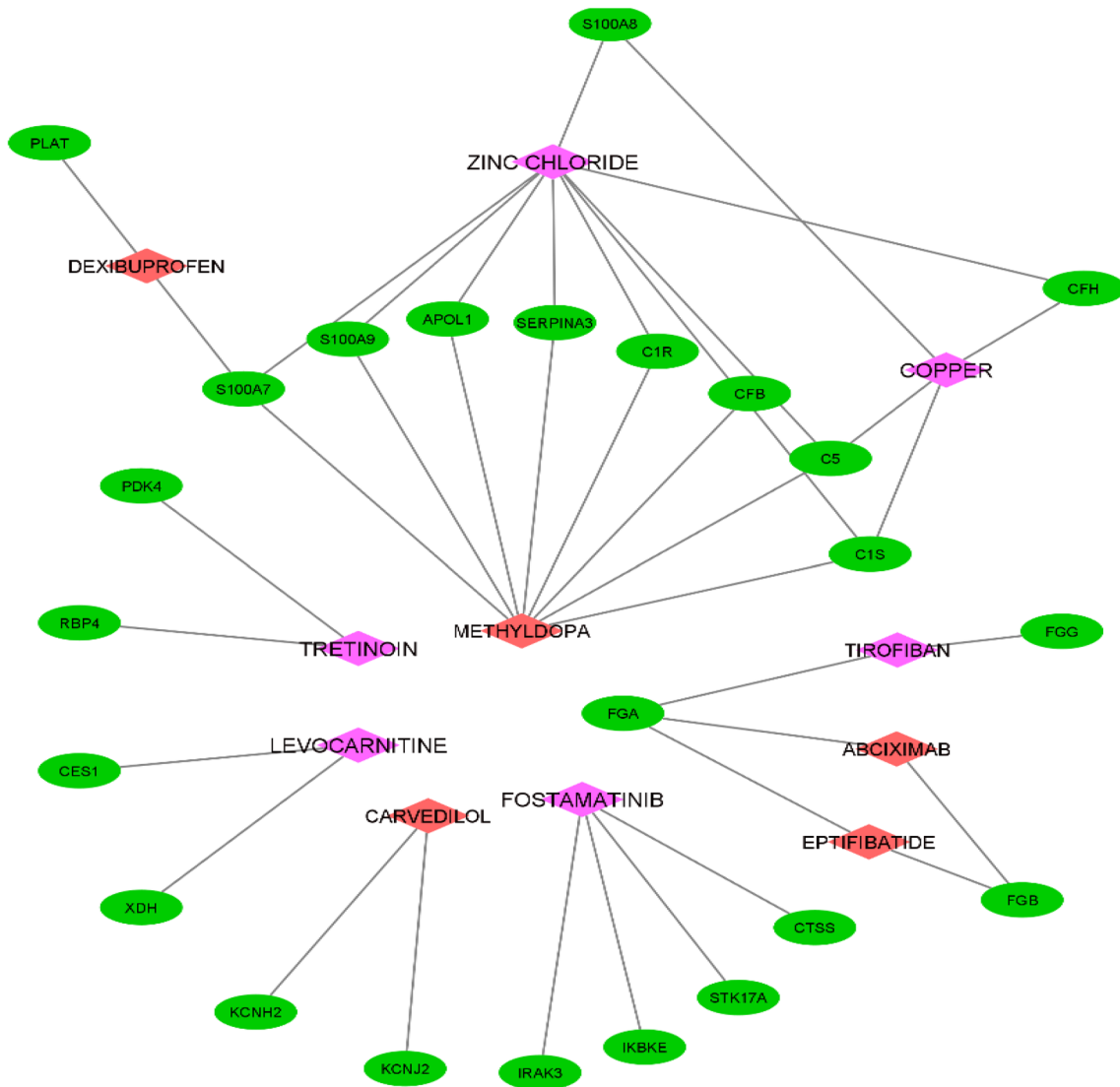


Fig. 4 The drug–gene interaction network. Green ovals show the crucial identified Genes, and diamonds depict our repurposed medications. The medicines previously registered for clinical trials against COVID-19 are distinct, using violet color

Table 9 The table represents the top six repurposed drugs interacting with the identified crucial genes in the Drug-Gene interaction network sorted by degree value

Drug name	Accession number	Clinical trial identifier (COVID-19)	Interacting gene	Degree
Zinc chloride	DB14533	CTRI/2020/08/027005 ACTRN12620000454976	C5, S100A9, S100A7, C1S, C1R SERPINA3, S100A8, APOL1, CFH	9
Fostamatinib	DB12010	EUCTR2020-001750-22-GB	CTSS, IRAK3, STK17A, IKBKE	4
Copper	DB09130	CTRI/2020/07/026515 CTRI/2020/07/026514 CTRI/2020/06/026256	C5, C1S, s100A8, CFH	4
Tirofiban	DB00775	NCT04368377	FGA, FGG	2
Tretinoin	DB00755	NCT04396067 NCT04568096	RBP4, PDK4	2
Levocarnitine	DB00583	NCT04573153	CES1, XDH	2

pathogens; however, it could also intermediate in other aspects of the COVID-19 pathogenesis, such as lung fibrosis. These findings support the hypothesis that the *STAT1* gene is likely a critical mediating element in the SARS-CoV-2 pathogenesis. It may affect the severity of COVID-19 symptoms and the body's response. However, further experimental studies are required to accurately determine the details of its molecular response in the body against SARS-CoV-2.

Interestingly, Lei et al. recently uncovered that SARS-CoV-2 initiated an unusual type-I IFN response in vitro. The expressions of *IFN-β* and *ISG56* were scarcely initiated early amid viral disease, whereas they surged at late time points. The postponed antiviral response could give a window for viral replication. The lack of an on-time and sufficient antiviral response could be pivotal to the COVID-19 pathogenesis. The finding of Lei et al. also seems consonant with our hypothesis since the data used in this study were related to an early time-point of infection for COVID-19 (Lei et al. 2020). Blanco-Melo et al. also compared SARS-CoV-2 with other respiratory infections. It appeared that SARS-CoV-2 disease drives a lower antiviral transcriptional response stamped by low IFN-I and IFN-III protein levels and lifted chemokine expression, clarifying the COVID-19 pro-inflammatory state in line with this speculation (Blanco-Melo et al. 2020a).

Our findings indicated that FOS, KLF4, EGR1, and JUN were four shared Gene/TFs between motif-related subnetworks in SARS and COVID-19. (They had dual regulatory roles). FOS and JUN also were identified as the shared hub bottlenecks. These four genes were as DEGs up-regulated in SARS-infected samples but not in COVID-19. However, they were predicted as TFs in our network with a crucial regulatory role in both disease networks.

Our results predicted FOS as a critical shared TF between the networks. The *FOS* gene family consists of four members: *FOS*, *FOSB*, *FOSL1*, and *FOSL2*. These genes encode leucine zipper proteins that can dimerize with JUN family proteins, forming the AP-1 transcription factor complex regulating TGF-beta-mediated signaling (Zhang et al. 1998). It has been reported that the expression of the N protein in SARS-CoV activates the AP-1 signaling pathway (He et al. 2003). Another study confirmed that augmentation of AP-1 due to SARS-CoV 3b protein causes pro-inflammatory cytokines and is responsible for the cytokine storm during SARS-CoV (Varshney and Lal 2011). FOS was predicted as an essential TF shared between the two diseases' up-regulated networks. It can be concluded that FOS probably plays a role as an intermediate in the COVID-19 pathogenesis by activating the AP-1 signaling pathway, ultimately leading to the cytokine storm in severe COVID-19 cases. Therefore, using it as a target in the drug-gene interaction network seems reasonable in the path to candidate medications.

We also reported *KLF4* (Kruppel-like factor 4) as another essential shared TF between the networks. *KLF4* is a transcription factor that mediates the proliferation, epithelial cells' differentiation, and apoptosis (Zhang et al. 2010). It also modifies the JAK-STAT3 signaling in some progenitor cells (McConnell and Yang 2010; Qin et al. 2013). Luo et al. indicated that *KLF4* negatively regulates cellular antiviral response and inhibits virus-triggered type I IFN signaling. In this study, *KLF4* was reported to bind to the *IFNB* promoter. Therefore, it inhibited the recruitment of IRF3 to the promoter of the *IFNB* gene.

They also showed that the knockdown of *KLF4* increases the induction of *IFNB1*, thus reducing viral replication (Luo et al. 2016). *KLF4* appeared to be overexpressed in our estimation following SARS-CoV entry and has a critical regulatory role in the SARS-CoV-2 GRN. It could be hypothesized that the *KLF4* inhibits the *IFNB* gene promoter and indirectly contributes to further viral replication in SARS and probably in COVID-19. Therefore, it plays a crucial role in the host response to SARS-CoV and SARS-CoV-2 pathogenesis. Further empirical investigations are required to confirm these assumptions.

EGR1 (early growth response 1) was another critical shared TF between the up-regulated networks of SARS and COVID-19. SARS-CoV PLpro has been reported to up-regulate *EGR1*, which affects the activation of the *TGF-β1* promoter. Therefore, it induces the *TGF-β1*-mediated pro-fibrotic responses in SARS-CoV pathogenesis (Li et al. 2016). Nishi et al. showed that *EGR1* could activate the basal transcriptional activity of the *EGFR* promoter (Nishi et al. 2002). The excessive activation of EGFR signaling leads to increased fibrosis after SARS-CoV infection (Venkataraman et al. 2017). Another study reported that *EGR1* is a gene of the pro-coagulation pathway affected by SARS-CoV infection in vitro. The study used a microarray technique. Its findings were confirmed by reverse transcription-quantitative PCR and immunoassays for some encoded proteins (Tang et al. 2005). This finding would probably explain the critical regulatory role of *EGR1* in thrombolytic problems in a subset of COVID-19 patients. The *EGFR1* is probably mediated in at least two ways in the molecular pathogenesis of COVID-19. The first way is through coagulopathy, and the other is to induce fibrosis.

JUN, another shared node between COVID-19 and SARS networks, is a transcription factor that plays a critical role in the CCR5 signaling pathway in macrophages (based on the GeneCard data for *JUN*). The CCR5 raises macrophage activation and survival during parainfluenza and influenza infection in mice by activating MAPKs and PI3K signaling (Tyner et al. 2005). MAPKs (mitogen-activated protein kinases) are well-known as signal transducers that respond to extracellular stimulation by cytokines, viral infections, growth factors, and stress. They play crucial roles in

regulating cell differentiation, proliferation, survival, and apoptosis (Garrington and Johnson 1999; Kyriakis and Avruch 2001; Whitmarsh and Davis 2000). In vitro and in vivo studies regarding SARS have clarified that activation of MAPKs is essential in regulating cytokine expression (Mizutani 2007). Cytokine storm and excessive cytokine secretion are severely life-threatening in patients with SARS and COVID-19 (Henderson et al. 2020; Huang et al. 2005). In vitro study by Mizutani et al. demonstrated that SARS-CoV infection induced the p38 MAPK signaling pathway in permissive Vero E6 cells (Mizutani et al. 2004). Therefore, targeting p38 MAPK or its downstream molecules could be a drug target. These findings also confirm that JUN molecular functions are related to the host response to the SARS-CoV-2 pathogenesis. It mediates macrophage activation and survival and probably affects the cytokine storm in COVID-19 patients. These findings further support our result in JUN emerging as an essential molecule at the intersection of networks among hundreds of other regulatory nodes.

HIF1A and PRDM1 were identified as two crucial genes exclusively in COVID-19 (up-regulated) in this study. *HIF1A* (hypoxia-inducible factor 1 subunit alpha) is a master transcriptional regulator in the adaptive response to hypoxia. It can activate genes capable of increasing oxygen delivery or facilitating metabolic adaptation to hypoxia (Li et al. 2009; Masson et al. 2001). Philip et al. have investigated the role of *HIF1A* in a fatal chronic lung disease called idiopathic pulmonary fibrosis (IPF). They have shown that HIF1A leads to pulmonary fibrosis.

Furthermore, they demonstrated that hypoxia, through HIF1A, leads to pulmonary fibrosis by up-regulating the ADORA2B receptors on AAMs (alternatively activated macrophages) and producing pro-fibrotic mediators (Philip et al. 2017). Since the lungs are the main target organ in SARS-CoV-2 pathogenesis, HIF1A probably mediates pulmonary events in severe COVID-19. It may also be a critical regulatory element influencing the host response to SARS-CoV-2 and needs further investigation.

PRDM1 (positive regulatory domain I), the other up-regulated crucial transcription factor in COVID-19, plays roles in different adaptive and innate tissue-resident T cell types. It also drives B-cell differentiation into terminal Ig-secreting plasma cells (Shapiro-Shelef and Calame 2005). Besides, it mediates the Ag presentation by MHC class I. An evolutionary study has identified the PRDM1 as a conserved critical regulator with a unique role in Ag presentation by MHC class I (Doody et al. 2007). These findings may explain that different aspects of host immune response in COVID-19 patients are related to *PRDM1*.

In addition to identifying crucial nodes, we also recognized the shared biochemical pathways between the two diseases in this study. The NOD-like receptor (NLR) signaling pathway was a shared significant pathway between

SARS and COVID-19 up-regulated DEGs networks. NLR signaling plays an essential role in host antiviral immunity pro-inflammatory mediators (Allen et al. 2011; Schneider et al. 2012). These (nucleotide-binding oligomerization domain-like) receptors are intracellular sensors for PAMPs (pathogen-associated molecular patterns). Each mediates in various signaling pathways, including NF- κ B and MAPK, IFN type I response, autophagy, and the generation of reactive oxygen species (ROS) (Lei et al. 2013; Moore et al. 2008; Tattoli et al. 2007). Animal studies have shown that the NLRP3 inflammasome is a vital component of the host immune response to viral infections (Allen et al. 2009).

The top shared biochemical pathway between the two diseases' down-regulated DEG networks was the "metabolic pathways" term. Metabolism is a major factor that regulates the function and differentiation of immune cells and influences the immune response process (Pearce and Pearce 2013; van der Windt et al. 2012; Wang and Green 2012). Our enrichment analysis results related to down-regulated DEG networks revealed that the Glucagon signaling pathway, one of the metabolic-related signaling, was the most significant in the COVID-19 network. These findings suggest that metabolic studies likely need more attention to discover the molecular aspects of host response in the pathogenesis of SARS-CoV and SARS-CoV-2.

VAV3 and *SPTLC3* were the two other shared crucial nodes participating in down-regulated DEG motif sub-networks. *VAV3* is one of the GEFs (guanine nucleotide exchange factors) that activates some pathways leading to transcriptional changes. *Vav3* is reported to participate in B cell antigen receptor (BCR) signaling. In this way, *VAV3* regulates different B cell receptor responses (Inabe et al. 2002). In this area, Zhivaki et al. have explained that Neonates Regulatory B Cells (nBreg) are infected by Respiratory Syncytial Virus (RSV) via the BCR. RSV-treated nBreg cells secreted anti-inflammatory cytokines (IL-10) in response to HRSV. Still, nBreg cells could not secrete cytokine IL-10 when treated with other viruses, including coronavirus 229E, influenza A (IAV), and HIV (Zhivaki et al. 2017). These findings highlight the importance of *VAV3* in different possible cell responses to various viruses via BCR. Therefore, our results indicate that SARS-CoV and SARS-CoV-2 entry to some cells may also be affected by *VAV3*, affecting BCR signaling in host response. Our analysis was performed only on respiratory epithelial cells, not immune cells like macrophages. Considering that *VAV3* has appeared as a crucial node in our results, we propose that its related immune signaling had better be experimentally investigated in further studies for COVID-19.

SPTLC3 (Serine Palmitoyltransferase Long Chain Base Subunit 3) was another down-regulated. It participates in the metabolism of some lipids and lipoproteins, and Ziv et al. investigated the role of Serine Palmitoyltransferase in

a marine viral infection. They showed that Serine Palmitoyltransferase is required for the viral infection. It activates sphingolipid biosynthesis. They explained that the EhV virus utilizes the host sphingolipid to produce some virus-specific lipids. These unique sphingolipids were vital for the life cycle of the EhV virus. Sphingolipids have a role in viral assembly and infectivity. They might have an evolutionary role in the pathogenesis of other viral infections, such as HIV and HCV (Ziv et al. 2016). SARS-CoV-2 is a positive-sense single-stranded RNA virus similar to HIV and HCV. SPTLC3 possibly mediates in SARS-CoV-2 pathogenesis through its effect on sphingolipid metabolism. The sphingolipid is crucial for viral components. The host cell probably has down-regulated *SPTLC3* to reduce the viral access to the sphingolipid.

Our study identified some shared crucial molecules responsible for the immune response to COVID-19 and SARS in vitro (Table S1A and S1B) and crucial molecules specific in response to COVID-19 and SARS. (Supplementary Table S9).

We have represented the overview of our crucial identified molecules and pathways in an integrative diagram in Fig. 5. Among several critical immune system players, our

study found some responsible for response against SARS-CoV-2 and SARS-CoV pathogens; some were identified as a specific response in one disease. SARS-CoV and SARS-CoV-2 pathogens are sensed by the mammalian Toll-like and NOD-like receptors. It induces pro-inflammatory cytokines (TGF- β and type I interferons). They are induced through three pathways (NF κ B, MAPK (*c-Jun*, *c-Fos*), and IRF). The pro-inflammatory cytokines act via the JAK-STAT (*STAT1*, *KLF4*) pathway. We found that some of the crucial identified TFs are related to these pathways and can be considered the leading players in the immune response to SARS-CoV-2 and SARS-CoV pathogens. We also found that some biochemical pathways, including Complement and coagulation cascade and Phagosome, and crucial molecules such as *WEE1*, *PMAIP1*, and *TSC22D2* were related to SARS in vitro. Besides *MYPN*, *SPRY4* and *APOL6* were the top crucial nodes specific to COVID-19 in vitro.

This study constructed a gene–drug interaction network and applied network analysis to propose several candidate drugs (Table S12). We applied the identified shared genes and crucial genes specific to COVID-19 to candidate new drugs interacting with a higher number of the crucial genes. The Drugs with higher degree scores

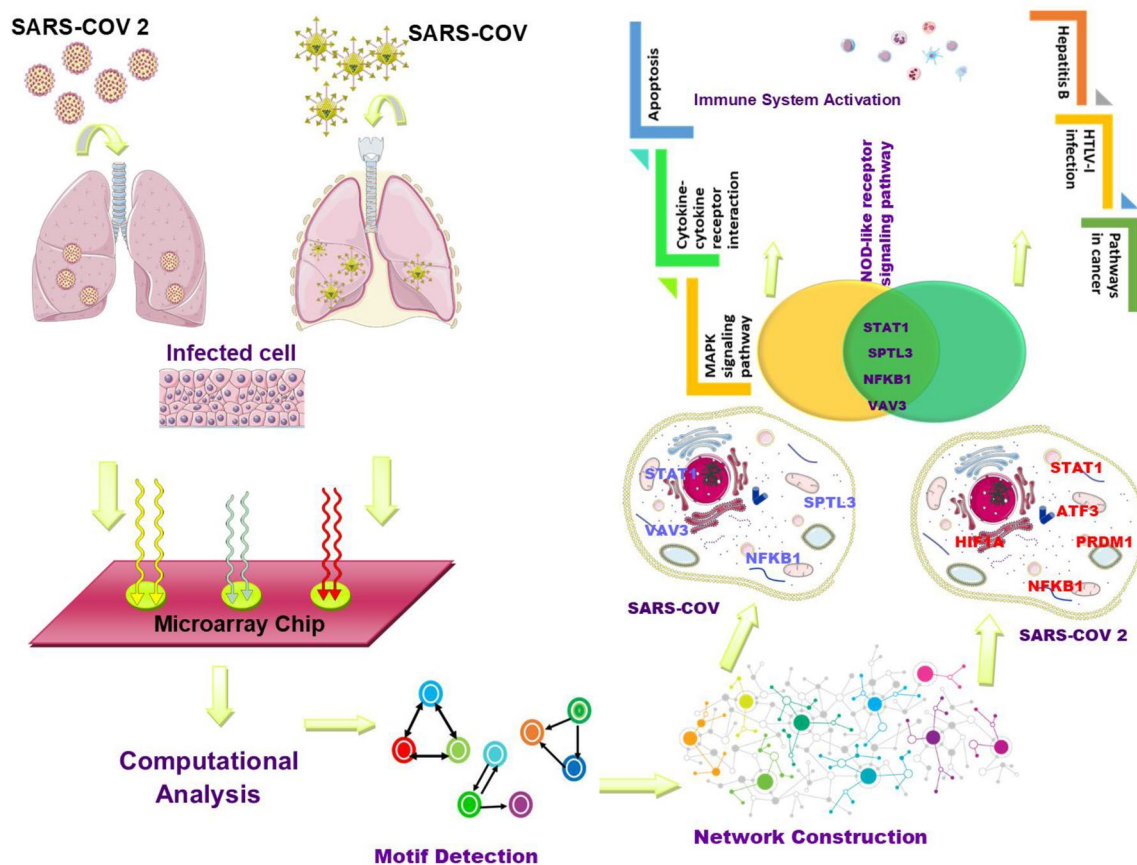


Fig. 5 The integrative diagram summarizes the crucial molecules and mechanisms

than two (based on the results between two and nine) were selected to create a new subnetwork (Fig. 4). Six previously FDA-approved drugs interacting with COVID-19 target genes included Fostamatinib, Tretinoin, Tirofiban, Zinc chloride, copper, and Levocarnitine. They were previously registered in ClinicalTrials.gov to be evaluated as possible treatments for COVID-19 patients. They were found as possible logical choices for investigation against COVID-19 treatment by other researchers. Their clinical trial registration codes are represented in Table 9. Herein, we have briefly verified them, representing them proposed by other investigations against COVID-19.

Treatment with Fostamatinib might be beneficial. It guards against COVID-19 patients' neutrophil extracellular traps (Strich et al. 2021). Thirty synergistic drug combinations, including Tretinoin and Fostamatinib, were suggested by Yang Liu et al. using a complementary exposure model to treat COVID-19 (Liu et al. 2021). Hypercoagulability may be life-threatening in patients with severe COVID-19. Tirofiban enhanced oxygenation in patients with elevated D-dimers who had already received antiplatelet therapy.

There are more elderly victims of COVID-19. A combination of micronutrients, especially zinc, and copper, can boost the immunity of aged people. They have a significant role in promoting public health (Chandra 1997). An ideal zinc level may increase host resistance to COVID-19 (Razzaque 2020).

Poor outcomes have been recorded in COVID-19 individuals with inadequate zinc. Hypozincemia has been linked to COVID-19 exacerbation (Jothimani et al. 2020; Yasui et al. 2020). Interestingly, low zinc levels have been linked to immune cell malfunction. Zinc's immunomodulatory effects as a COVID-19 preventive tool and supportive strategy have been proposed (Mossink 2020; Tayyib et al. 2020). Zinc supplementation is also indicated as a COVID-19 adjunct therapy. It has even been recommended with high doses for treatment investigations against COVID-19; however, achieving its therapeutic potential is considered challenging (Chinni et al. 2021).

Copper is also suggested as a possible adjunct therapy for COVID-19 patients who are critically ill (Fooladi et al. 2020). Copper and N-acetylcysteine (NAC) may have a role in a combination of prospective antiviral therapies against SARS-CoV-2, according to Andreou et al. Their findings supported the combination. They suggested that copper, NAC, colchicine, NO, and Remdesivir/EIDD-2801 be studied as a potential SARS-CoV-2 treatment scheme (Andreou et al. 2020). Overall, reports about some of the proposed drugs verify the rationale behind the network model and show that our other proposed medications are worth further experimentally investigating against SARS-CoV-2 in vitro and in vivo. Besides, since our data was related to the early time points of the infection with SARS-CoV-2, it probably

is better to first investigate the candidate medications in the early stages of the disease.

Conclusion

Designing suitable therapeutic drugs demands elucidation of the mechanisms underlying the disease. System biology approaches have recently contributed to predicting a faster and more in-depth insight into diseases' molecular pathology. This study provides an exciting opportunity to advance our knowledge of the molecular mechanism of COVID-19 and drug repurposing through its gene regulatory network. Since SARS-CoV-2 is a new virus, in vivo omics data accessible for it are limited. This study uses the network analysis of in vitro data to fill the knowledge gap in vivo.

Comparing the motif-related subnetworks of DEGs between respiratory cells treated with SARS-CoV-2 and SARS-CoV revealed the shared molecular mechanisms and signaling pathways underlying both viral infections. NLRs signaling pathway was the first pivotal pathway between SARS and COVID-19 up-regulated DEGs networks. NLR signaling plays an essential role in pro-inflammatory mediators of host antiviral immunity. NLRs are sensors (intracellular) for PAMPs and mediate in several immune-related pathways, including NF- κ B, MAPK, IFN type I response, and ROS generation. Some are vital components of the host's immune response to viral infections. The "metabolic pathways signaling" term was also the most significant pathway term for the down-regulated DEGs network. The metabolism is an essential factor that regulates immune cell function and differentiation and influences the immune response process. We suggest that studies related to metabolic pathways signaling will likely need more attention in the route to discover the molecular aspects of the host response to SARS-CoV and SARS-CoV-2.

The motif-related subnetworks helped candidate six transcription factors as probably shared critical regulators of SARS-CoV-2 and SARS-CoV pathogenesis, including *NFKB1*, *JUN*, *STAT1*, *FOS*, *KLF4*, and *EGRI*. Besides, the top three non-shared biochemical pathways enriched specifically for COVID-19 included Complement-coagulation cascade, Staphylococcus aureus infection, and Phagosome. Some top crucial nodes in the network specific to SARS were *WEE1*, *PMAIP1*, and *TSC22D2*. Besides, *MYPN*, *SPRY4*, and *APOL6* are the crucial nodes specific to COVID-19 in vitro. Our findings suggest that they probably play roles in the molecular regulatory mechanisms mediating the host response to SARS-CoV-2.

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Data availability The authors confirm that the data supporting the findings of this study are available within the article or its supplementary materials.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethical approval and consent to participate Not applicable.

Consent for publication Not applicable.

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