Identifying potential novel insights for COVID-19 pathogenesis and therapeutics using an integrated bioinformatics analysis of host transcriptome

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Identifying potential novel insights for COVID-19 pathogenesis and therapeutics using an integrated bioinformatics analysis of host transcriptome

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Abstract

The molecular mechanisms underlying the pathogenesis of COVID-19 has not been fully discovered. This study aims to decipher potentially hidden parts of the pathogenesis of COVID-19, potential novel drug targets, and to identify potential drug candidates. Two gene expression profiles (GSE147507-GSE153970) were analyzed and overlapping differentially expressed genes (DEGs) were selected for which top enriched transcription factors and kinases were identified and pathway analysis was performed. Protein-protein interaction (PPI) of DEGs was constructed, hub genes were identified and module analysis was also performed. DGIdb database was used to identify drugs for the potential targets (hub genes and the most enriched transcription factors and kinases for DEGs). A drug-potential target network was constructed and drugs are ranked according to the degree. L1000FDW web-based utility was used to identify drugs that can reverse transcriptional profiles of COVID-19. We identified drugs currently in clinical trials and novel potential 8 drugs. Besides the well-known pathogenic pathways, It was found that axon guidance is a potential pathogenic pathway. Sema7A, which may exacerbate hypercytokinemia, is considered a potential novel drug target. Another potential novel pathway is related to TNF2 overexpression which may induce potential telomere dysfunction and hence DNA damage that may exacerbate lung fibrosis.

Keywords: COVID-19, Systems biology, Differentially expressed genes, Functional module analysis, Protein-protein interaction network, Enrichment analysis, host transcriptome and drug repurposing.

Background

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a member of the Coronaviridae family, isolated at the end of 2019. SARS-CoV-2 pandemic has about 3.06% mortality rate according to WHO. Nearly, the majority of COVID-19 patients, about 80%, have mild symptoms. Nearly 20% of cases are severe and develop severe respiratory distress syndrome [1].

Several studies reported that dysregulated immune response (hypercytokinemia) is associated with severe cases [2-6]. Uncontrolled host immune response may be responsible for the fatal outcome rather than unabated viral replication as it was observed that fatal cases had a low viral load [7] so that understanding the host response to SARS-CoV-2 is critical to find potential therapeutic targets for severe COVID-19 disease [7].

In this study, we tried to understand the underlying pathological pathways related to SARS-CoV-2 infection and identify potential drug targets and drugs that can be used for combating SARS-CoV-2-induced injuries. Two RNA-seq datasets (GSE147507-GSE153970) were analyzed and DEGs were identified based on the two datasets. Gene ontology and the Kyoto encyclopedia of genes and genomes pathway enrichment analysis for DEGs were performed. Also, top enriched transcription factors and kinases for DEGs were identified. Protein-protein interaction (PPI) of DEGs was constructed and hub genes were identified. DGIdb database was used to identify drugs for the potential targets (hub genes and the most enriched transcription factors and kinases for DEGs). A drug-potential target network was constructed and drugs were ranked according to the degree. L1000FDW was searched for drugs that can reverse transcriptional profiles of COVID-19. PPI interaction Network of DEGs was expanded and GO biological process enrichment analysis and KEGG pathway analysis of the top eight modules were performed. We identified drugs already in use in clinical trials against COVID-19 disease as well as new potential drugs. We also identified new potential drug targets and new

pathogenic pathways for COVID-19 disease. .

Methods

In Fig. 1, we present a Flow chart of our integrated novel bioinformatics approach to identify potential pathogenic pathways, drug targets, and drug candidates for COVID-19.

1- Datasets

Two gene expression profiles (GSE147507- GSE15397) were obtained from the NCBI Gene Expression Omnibus (GEO) database (<http://www.ncbi.nlm.nih.gov/geo/>). The first dataset is stranded RNA-seq data from mock-treated and SARS-CoV-2 (USA-WA1/2020 at MOI of 2 for 24 h.)-infected primary human lung epithelium (NHBE). Each group included three independent samples. Each sample has four runs. The second dataset is unstranded RNA-seq data from mock-treated and SARS-

CoV-2 (USA-WA1/2020 at MOI of 0.5 for 48 h)-infected Normal Airway Epithelial cells (NAE). Each group included three independent samples. Each sample has two runs.

2- Identifying DEGs

We used Galaxy [<https://usegalaxy.org/>] as a platform for RNA-seq analysis. First, raw reads were subjected to quality control analysis using FastQC [11] before and after using trimmomatic tool [12] which was run with its default options. Trimmed reads were aligned against the reference genome (hg19) using HISAT2 [13]. All aligned runs for the same sample were merged using MergeSamFiles [14]. Aligned reads that overlap features in a GFF file (gencode.v19.annotation.gtf.gz) were count using htseq-count [15]. DESeq2 [16] was used to determine differentially expressed features count tables. Differentially expressed genes (DEGs) were considered using ($q. val < 0.05$).

3- Pathway and GO enrichment Analysis of DEGs

Gene Ontology (GO) term analysis and KEGG pathway enrichment analysis of DEGs were performed using STRING (<https://string-db.org/>). Transcription factors enrichment analysis and kinases enrichment analysis of DEGs were performed using ChEA3 [17] and KEA3 (<https://amp.pharm.mssm.edu/kea3/>) respectively using the integrated mean rank method.

4. Hub genes identification

PPI interaction network was obtained by submitting DEGs to the STRING and analysed by Cytoscape software (version 3.8.1) [18]. Hub genes were selected based on the intersection of three methods; CytoNCA [19] using two centrality methods (subgraph-degree) and Cytohubba [20].

5. Identifying potential drugs

5.1 Drug-potential target network

Using the Drug-Gene Interaction Database (DGIdb; http://www.dgiddb.org/search_interactions), drugs were selected based on the hub genes and the most enriched transcription factors and kinases for DEGs that served as promising drug targets [10]. Cytoscape software was used to draw the drug-

potential target network. Drugs are ranked according to the degree using Cyto-NCA. Only FDA approved drugs were selected. We neglected drugs that are not for internal use like topical cream and eye drop.

5.2 L1000FDW analysis

L1000FDW was used to identify potential drugs which can reverse the transcriptional profiles of COVID-19 [21]. DEGs were used as input. Only FDA approved drugs were selected.

6. Functional modules analysis

PPI interaction Network of DEGs was expanded using STRING and analysed by Cytoscape software (version 3.8.1) [18]. Functional modules were identified via the MCODE using ClusterViz [22-23] with the default parameters. GO biological process enrichment analysis and KEGG pathway analysis of genes in each functional modules were done using STRING.

Results

DEGs

We identified 422 DEGs between SARS-CoV-2 infected NHBE cells and mock-treated NHBE cells. and 1829 DEGs between SARS-CoV-2 infected NAE and mock-treated cells. 187 overlapping genes (48 downregulated and 139 upregulated genes) were selected using VENNY 2.1 (<https://bioinfogp.cnb.csic.es/tools/venny/index.html>) [24] (Fig. 2 and Table S1).

Fig. 2. Venn diagram for overlapping differentially expressed genes (DEGs) based on two datasets.

(A) the intersection of upregulated DEGs and (B) the intersection of downregulated DEGs identified from the two datasets, namely, GSE147507 and GSE15397.

Pathway and GO enrichment Analysis of DEGs

KEGG pathway analysis of DEGs was enriched to IL-17 signaling pathway, TNF signaling pathway, cytokine-cytokine receptor interaction, NF-kappa B signaling pathway, rheumatoid arthritis, and NOD-like receptor signaling pathway (Fig. 3A) whereas they were significantly involved in the immune process, inflammatory response, response to an external stimulus, response to biotic stimulus, defence response, and response to cytokine (Fig. 3B), highly enriched to cytokine activity, molecular function regulator, molecular function regulator, protein binding and cytokine receptor binding (Fig. 3C), and correlated to extracellular space, secretory granule, cytoplasmic vesicle, and vesicle (Fig. 3D).

RELB, ARNTL2, GRHL3, PLSCR1, SNAI, CSRN1, ZNF750, OVOL1, FOSL1, and ZNF267 were the top-ranked transcription factors for DEGs whereas EGFR, RIPK1, CHUK, IKBKB, SRC, TBK1, PAK1, IRAK2, FYN,8, and LYN were the most enriched kinases (Table. 1). We considered the top enriched transcription factors and kinases for DEGs as potential drug targets [10].

Hub genes identification

The PPI network included 186 nodes, 654 edges with an average node degree of 7.03 and avg. local clustering coefficient of 0.49, and its PPI enrichment p-value was $< 1.0e-16$ (Fig. 4). We used three methods for identifying hub genes; CytoNCA using two centrality methods (subgraph-degree) and Cytohubba. The top 20 hub genes were identified from each method. 18 overlapping hub genes (IL6, CXCL8, CXCL1, CCL20, CXCL2, CXCL3, IL1B, IL1A, MMP9, CXCL5, CSF3, ICAM1, IL1RN, CSF2, PTGS2, SAA1, NFKB1, and TNFAIP3) were selected based on the intersection of the three methods (Table. 2). The PPI network of the hub genes was constructed using STRING (Fig. 5) and consisted of 18 nodes and 142 edges, while its average local clustering coefficient was 0.943, and its

PPI enrichment P-value was lower than $1.0e - 16$. This showed that these hub genes highly interact with each other.

Fig. 3. Enrichment analysis of DEGs. A. KEGG pathway enrichment analysis of DEGs. B. Biological function enrichment analysis of DEGs. C. Biological process enrichment analysis of DEGs. D. Biological component enrichment analysis of DE.

Table 1. Top enriched transcription factors and kinases for DEGs.

Rank	Transcription factors	Kinases
1	RELB	EGFR
2	ARNTL2	RIPK1
3	GRHL3	CHUK
4	PLSCR1	IKBKB
5	SNAI1	SRC
6	CSRNP1	TBK1
7	ZNF750	PAK1
8	OVOL1	IRAK2

9	FOSL1	FYN
10	ZNF267	LYN

Figure 4. Unexpanded PPI network of DEGs using STRING.

Drug-potential target network

Potential targets consisted of 18 hub genes, top 10 enriched kinases and transcription factors for DEGs were searched for promising drugs using DGIdb. We didn't find drugs for all potential transcription factors except FOSL1. However, all hub genes and potential kinases have drugs except CXCL1, CCL20, CXCL3, CXCL5 and IRAK2. We included only two FDA unapproved botanical derived drugs. 215 potential drugs were obtained (Supplementary data 2). We concentrated on drugs with degree ≥ 3 as Fostamatinib, Entrectinib, Ponatinib, Dasatinib, Bosutinib, Ibrutinib, and Acalabrutinib were the top interactive drugs that act on multi-drug targets (Table 3, Fig. 6).

Table 2. Identified hub genes by CytoNCA using two centrality methods (degree and subgraph) and Cytohubba.

Rank	Cytohubba	CytoNCA-Degree	CytoNCA-Subgraph	Common hub genes	Description
1	IL6	IL6	IL6	IL6	Interleukin 6 (IL6)
2	CXCL8	CXCL8	CXCL8	CXCL8	C-X-C motif chemokine ligand 8 (CXCL8)
3	CXCL1	IL1B	IL1B	CXCL1	C-X-C motif chemokine ligand 1 (CXCL1)
4	CCL20	MMP9	MMP9	CCL20	C-C motif chemokine ligand 20 (CCL20)
5	CXCL2	CXCL1	CXCL1	CXCL2	C-X-C motif chemokine ligand 2 (CXCL2)
6	CXCL3	PTGS2	PTGS2	CXCL3	C-X-C motif chemokine ligand 3 (CXCL3)
7	IL1B	ICAM1	ICAM1	IL1B	Interleukin 1 Beta (IL1B)
8	IL1A	CSF2	CCL20	IL1A	Interleukin 1 Alpha (IL1A)
9	MMP9	CCL20	CSF2	MMP9	Matrix Metalloproteinase 9 (MMP9)
10	CXCL5	IL1A	IL1A	CXCL5	C-X-C motif chemokine ligand 5 (CXCL5)
11	CSF3	NFKB1	CXCL2	CSF3	Colony Stimulating Factor 3 (CSF3)
12	ICAM1	CXCL2	CXCL3	ICAM1	Intercellular Adhesion Molecule 1(ICAM1)
13	IL1RN	IL1RN	IL1RN	IL1RN	Interleukin 1 Receptor Antagonist (IL1RN)
14	CSF2	CSF3	CSF3	CSF2	Colony Stimulating Factor 2 (CSF2)
15	PTGS2	SAA1	SAA1	PTGS2	Prostaglandin-Endoperoxide Synthase 2 (PTGS2)
16	SAA1	CXCL3	CXCL5	SAA1	Serum Amyloid A1 (SAA1)
17	CXCL6	TNFAIP3	NFKB1	NFKB1	Nuclear Factor Kappa B Subunit 1(NFKB1)
18	NFKB1	PI3	TNFAIP3	TNFAIP3	TNF Alpha Induced Protein 3 (TNFAIP3)
19	TNFAIP3	NFKB2	CXCL6		
20	CXCL16	CXCL5	EDN1

Figure 5. The PPI network of the hub genes using STRING.

Table 3. Potential drugs with high degree of connectivity identified by CytoNCA from Drug-Potential targets network.

Rank	Drugs	Degree	Potential targets (DGIdb)
1	Fostamatinib	8	EGFR-RIPK1-IKBKB-SRC-TBK1-PAK1-FYN-LYN
2	Entrectinib	4	SRC-TBK1-FYN-LYN
3	Ponatinib	4	EGFR-RIPK1-SRC-LYN
4	Dasatinib	4	EGFR-SRC-FYN-LYN
5	Bosutinib	4	EGFR-SRC-FYN-LYN
6	Ibrutinib	4	EGFR-SRC-FYN-LYN
7	Acalabrutinib	4	EGFR-SRC-FYN-LYN
8	Resveratrol	4	IL1B-PTGS2-NFKB1-RIPK1
9	Nintedanib	3	SRC-FYN-LYN
10	Vandetanib	3	EGFR-SRC-FYN
11	Gefitinib	3	EGFR-IKBKB-LYN
12	Sulfasalazine	3	PTGS2-CHUK-IKBKB
13	Mesalamine	3	PTGS2-CHUK-IKBKB
14	Curcumin	3	MMP9-PTGS2-EGFR
15	Rilonacept	3	IL1B-IL1A-IL1RN
16	Alteplase	3	CXCL2-IL1B-FOSL1
17	Aspirin	3	CXCL8-IL1B-PTGS2
18	Bevacizumab	3	CXCL8-MMP9-EGFR
19	Paclitaxel	3	CXCL8-EGFR-SRC
20	Omeprazole	3	CXCL8-IL1B-CSF2
21	Naproxen	3	CXCL8-PTGS2-SAA1
22	Cisplatin	3	IL6-EGFR-SRC
23	Ribavirin	3	IL6-EGFR-CXCL8

L1000FDW analysis

Drugs with low similarity scores may potentially reverse the transcriptional profile of COVID-19 and are potential drugs for COVID-19. Sorafenib, Sirolimus, Selumetinib, Fostamatinib, Dasatinib were the top drugs (Table 4).

Figure 6. Drug-potential target network using Cytoscape.

Table 4. L1000FDW analysis

Rank	Drugs	Similarity scores
1	Sorafenib	-0.19496855345912
2	sirolimus	-0.19496855345912
3	Selumetinib	-0.182389937106918
4	fostamatinib	-0.176100628930818
5	dasatinib	-0.169811320754717
6	vemurafenib	-0.157232704402516
7	Neratinib	-0.150943396226415
8	Exemestane	-0.144654088050314
9	Bosutinib	-0.138364779874214

Functional Modules Analysis

To understand the underlying pathologic pathways, we expanded the PPI network of DEGs to include 386 nodes, 5280 edges with an average node degree of 27.4 and avg. the local clustering coefficient of 0.587, and its PPI enrichment p-value was $< 1.0e-16$ (Fig. S1). Then, the significant modules were identified via the MCODE using ClusterViz. The top eight functional clusters of modules were selected. KEGG pathway analysis and biological process enrichment analysis of each module was performed by STRING.

Module 1, with MCODE score = 32.789, consisted of 77 nodes and 1246 edges (Fig. S2).

The KEGG pathway analysis of this module indicated that IL-17 signaling pathway, axon guidance, TNF signaling pathway, cytokine-cytokine receptor interaction, NF-kappa B signaling pathway, and NOD-like receptor signaling pathway, are the most related pathways (Table S3). Biological process enrichment analysis identified that cell surface receptor signaling pathway, signal transduction, response to cytokine, ephrin receptor signaling pathway, and cytokine-mediated signaling pathway are the top enriched process for the first top module (Table S3).

Module 2, with MCODE score = 16.049, consisted of 42 nodes and 329 edges (Fig. S3). The KEGG pathway analysis of this module indicated that NOD-like receptor signaling pathway, RIG-I-like receptor signaling pathway, NF-kappa B signaling pathway, TNF signaling pathway, influenza A, and hepatitis C are the most related pathways (Table S3). Biological process enrichment analysis identified that cytokine-mediated signaling pathway, response to cytokine, and cellular response to organic substance are the top enriched process for module 2 (Table S3).

Module 3, with MCODE score = 10.2, consisted of 11 nodes and 51 edges (Fig. S4). The KEGG pathway analysis of this module indicated that homologous recombination, non-homologous end-joining, and cellular senescence are the most related pathways (Table S3). Biological Process enrichment analysis identified that telomere capping, regulation of telomere maintenance, and negative

regulation of telomere maintenance are the top enriched process for module 3 (Table S3).

Module 4, with MCODE score = 10, consisted of 10 nodes and 45 edges (Fig. S5). The KEGG pathway analysis of this module indicated that focal adhesion, regulation of actin cytoskeleton, leukocyte transendothelial migration, tight junction, vascular smooth muscle contraction, and platelet activation are the most related pathways (Table S3). Biological Process enrichment analysis identified that muscle contraction, and muscle organ development is the top enriched process for module 4 (Table S3).

Module 5, with MCODE score = 9.33, consisted of 10 nodes and 42 edges (Fig. S6). The KEGG pathway analysis of this module indicated that hepatitis C, NOD-like receptor signaling pathway, influenza A, herpes simplex infection, and measles are the most related pathways (Table S3).

Biological Process enrichment analysis identified that type I interferon signaling pathway, innate immune response, defense response to virus, and cellular response to cytokine stimulus are the top enriched process for module 5 (Table S3).

Module 6, with MCODE score = 9, consisted of 6 nodes and 36 edges (Fig. S7). For KEGG pathway enrichment analysis, this module wasn't enriched (Table S3). Biological Process enrichment analysis identified that peptide cross-linking and cornification were the top enriched process for module 6 (Table S3).

Module 7, with MCODE score = 7.778, consisted of 10 nodes and 35 edges (Fig. S8). The KEGG pathway analysis of this module indicated that pathogenic *Escherichia coli* infection, gap junction, phagosome, apoptosis, and tight junction are the most related pathways (Table S3). Biological process enrichment analysis identified that microtubule-based process and cytoskeleton organization are the top enriched process for module 7 (Table S3).

Module 8, with MCODE score = 6.061, consisted of 10 nodes and 35 edges (Fig. S9). The KEGG pathway analysis of this module indicated that apoptosis, cytokine-cytokine receptor interaction,

complement and coagulation cascades, natural killer cell-mediated cytotoxicity, and Jak-STAT signaling pathway are the most related pathways (Table S3). Biological process enrichment analysis identified that cell surface receptor signaling pathway, signal transduction and regulation of response to stress are the top enriched process for module 8 (Table S3).

Discussion

In this study, we used an integrated bioinformatics approach to analyse publicly available two gene expression profile of SARS-CoV-2 to discover potential novel insights regarding COVID-19 pathogenesis and therapeutics. We identified 187 overlapping DEGs for which KEGG pathway analysis and GO term analysis were performed and significant transcription factors and kinases were identified. From functional module analysis of the expanded PPI network of DEGs, many known pathogenic pathways and biological processes were identified as well as potential novel ones. Most of the DEGs in module 1 (NFKB2, CSF3, NFKB1, CSF2, TNFAIP3, CXCL1, CXCL2, MMP9, IL1RN, PTGS2, IL6, CCL20, SEMA7A, CXCL5, CXCL3, IL1A, ICAM1, IL1B) and module 2 (IL7R, EDN1, MAP3K8, PI3, BIRC3, IKBKE, CXCL16, BCL2A1, LIF, and CXCL6) were upregulated except two genes (IL6R and EPHA4) were down-regulated. Pathway analysis of DEGs, module 1 and module 2 revealed the significance of the following pathways, IL-17 signaling pathway, TNF signaling pathway, cytokine-cytokine receptor interaction, NF-kappa B signaling pathway, Rheumatoid arthritis, NOD-like receptor signaling pathway, Toll-like receptor signaling pathway [2, 4, 25- 36]. In addition to these pathways, axon guidance was highly enriched for module 1. We found only one study which mentioned that axon guidance was top enriched for postmortem lung samples from patients who died of COVID-19 (7). In our finding, there were two DEGs involved in axon guidance (SEMA7A and EPHA4). SEMA7A was upregulated, however, EPHA4 was down-regulated. SEMA7A, a semorphin member, has been involved in both axon guidance [37] and regulation of immune cell activation [38-39]. Sema7A and its receptor, PlexinC1 has been implicated in lung injury and fibrosis [40-42]. Roth

JM et al (2016) found that proinflammatory cytokines and Sema7A can induce each other and explore the critical role of Sema7A in pulmonary inflammation [43]. Sema7A–plexinC1 signaling is considered a promising drug target for the treatment of acute lung injury [44]. With regards to EPHA4, there is a lack of studies that define the role of down-regulated EPHA4 in lung fibrosis and inflammation. Here, we considered that axon guidance, specially Sema7A and its receptor, plexinC1, is an attractive area for management COVID-19. From pathway analysis, it was found that COVID-19 share significant pathways with rheumatoid arthritis [45], Kaposi's sarcoma-associated herpesvirus infection, influenza A, hepatitis C, pathways in cancer [46-47], legionellosis, salmonella infection, herpes simplex infection, and measles. Apoptosis and RIG-I-like receptor signaling pathway were significantly enriched for module 2. This indicates that COVID 19 induces these important pathogenic pathways [48-52]. Apoptosis is a promising target for combating SARS-CoV-2 virus infection [48-51]. It was found that Telomere capping was the top enriched biological process enriched for module 3 (Table 5). TINF2 (TIN2) was up-regulated in our final DEGs. TIN2 is induced by NF-kB transcription factor [53] and is a core part of the Shelterin complex which protects telomeres and chromosomes. High TIN2 expression can produce telomere dysfunction-induced DNA damage and aberrant chromosomal alterations [54]. Telomere dysfunction limits the proliferative capacity of human cells by activation of DNA damage responses, inducing senescence or apoptosis [55]. Short telomere is manifested as pulmonary fibrosis-emphysema in adults [56]. Telomere dysfunction may provide a novel pathway for the pathogenesis of COVID-19. So, it's was found that homologous recombination, non-homologous end-joining, and cellular senescence are the only KEGG pathway enriched for module 3. Module 4 displays the interplay between platelets activation and leukocyte transendothelial migration which mediates thrombus formation a major contribution for the severity of COVID-19 [7, 57-58]. Focal adhesion, regulation of actin cytoskeleton, leukocyte transendothelial migration, tight junction, vascular smooth muscle contraction, and platelets activation are potential targets for COVID-

19. MYLK was found to be downregulated and its role in the pathogenesis of COVID-2 is unclear. Besides, Genes of module 5 (MX2, IFI27, and GBP5) were upregulated and highlighted the role of type I interferon signaling pathway and innate immune response in COVID-19 pathogenesis [2, 59-61]. SARS-CoV-2-infection up-regulated genes (TGM1, SPRR1B, LOR, SPRR2E, SPRR2D, SPRR1A, IVL, FLG, and SPRR2A) involved in cornification and peptide cross-linking which were enriched for module 6, this may lead to cell death and involved in lung fibrosis respectively [62-64]. Module 7 genes (TUBA1B, TUBB4B, TUBA1C, and TUBA1A) were up-regulated and involved in the microtubule-based process and cytoskeleton organization which may explore the potential significance of gap junction, phagosome, and tight junction in COVID-19 pathogenesis. Colchicine, which acts on tubulin, was repurposed for COVID-19 [65]. Genes of module 8 (SAA1, DDX58, SERPINB2, F3, TNFRSF10A, BID, PLAUR, HBEGF, ANXA1, and PLAUR) were up-regulated, four of them (F3, PLAUR, PLAUR, and SERPINB2) were involved in complement and coagulation cascades and considered of potential importance in coagulation disorders associated with COVID-19 infection. Many papers explored the significance of Jak-STAT signaling pathway, and natural killer cell-mediated cytotoxicity [66-67].

From the unexpanded PPI network of DEGs, 18 hub genes (IL6, CXCL8, CXCL1, CCL20, CXCL2, CXCL3, IL1B, IL1A, MMP9, CXCL5, CSF3, ICAM1, IL1RN, CSF2, PTGS2, SAA1, NFKB1, and TNFAIP3) were identified by the overlap of three methods and it was found that these hub genes were highly interactive with each other. Hub genes along with the top enriched transcription factors and kinases for DEGs were used for constructing drug-potential target network. Drugs are ranked according to the degree. Drugs with a high degree (≥ 3) were prioritized. The top potential drugs as Fostamatinib (Degree = 8), Acalabrutinib (Degree = 4), and Ibrutinib (Degree = 4) were already suggested to be repurposed for COVID-19 in recent papers [68-70]. Phase II clinical trials were conducted to study the safety and efficacy of Fostamatinib, Acalabrutinib, Ibrutinib, and

Bevacizumab with (degree = 3) for hospitalized patients with COVID-19 (ClinicalTrials.gov). Another top potential drug, Nintedanib with (degree = 3), is under phase III for treatment of SARS-Cov-2 Induced Pulmonary Fibrosis (ClinicalTrials.gov). Sulfasalazine was considered a potential drug [71]. Other top potential drugs as Dasatinib, Bosutinib, Entrectinib, Ponatinib, Vandetanib, Gefitinib, Mesalamine, Rilonacept, Alteplase, Omeprazole, Naproxen, Cisplatin, and Ribavirin are considered novel potential drugs for COVID-19. There is a controversy about using Ribavirin in COVID-19 (72-75). Some papers addressed the beneficial effects of the two botanical-derived drugs, Resveratrol and Curcumin, in COVID-19 [76-79]. Along with the degree, we added another dimension for prioritization. We selected those novel potential drugs which act on the same targets as those under clinical investigation. It was found that Dasatinib and Bosutinib shared the same targets (EGFR-SRC-FYN-LYN) as with Acalabrutinib and Ibrutinib (EGFR-SRC-FYN-LYN) whereas targets of Entrectinib (SRC-TBK1-FYN-LYN), Ponatinib (EGFR-RIPK1-SRC-LYN), and Vandetanib (EGFR-SRC-FYN) included three of them (Table 3). L1000FDW analysis added new potential drugs as in table 5. Fostamatinib, Dasatinib, and Bosutinib were common between the two methods (drug-potential target network and L1000FDW analysis). Other potential drugs revealed by L1000FDW analysis were Sorafenib, Sirolimus [80], Selumetinib [80], Neratinib [81], Vemurafenib, Exemestane, and Bosutinib.

Conclusion

In this study, we tried to decipher potentially hidden parts of the pathogenesis of COVID-19 as well as repurposing FDA-approved drugs using an integrative bioinformatics approach. Besides the well-known pathogenic pathways like IL-17 signaling pathway, TNF signaling pathway, Cytokine-cytokine receptor interaction, NF-kappa B signaling pathway, NOD-like receptor signaling pathway, Toll-like receptor signaling pathway, platelets activation and leukocyte transendothelial migration, axon guidance is a potential pathogenic pathway. Sema7A and its receptor, plexinC1, is a potential novel

drug target. Sema7A may exacerbate cytokines production. Another potential novel pathway is related to TNF2 overexpression which may induce potential telomere dysfunction and hence DNA damage that may exacerbate lung fibrosis. We identified repurposed drugs that are under investigation for hospitalized patients with COVID-19 and new potential drugs such as Dasatinib, Bosutinib, Entrectinib, Ponatinib, and Vandetanib were prioritized based on two factors (The degree and the similarity of targets with those drugs which were under clinical investigation). Others like Sorafenib, Vemurafenib, and Exemestane were identified as potential novel drugs using L1000FDW analysis. Our findings provide new insight into the mechanisms of COVID-19 complications as well as potential new drugs. Further validation of these findings is needed. Although we didn't do an experimental validation, finding known pathogenic pathways and drugs currently in clinical trials for COVID-19 in our analysis is one way of validation for our method.

Supplementary Materials

Fig. S1: Expanded PPI network of DEGs, Fig. S2: Module 1, Fig. S3: Module 2, Fig. S4: Module 3, Fig. S5: Module 4, Fig. S6: Module 5, Fig. S7: Module 6, Fig. S8: Module 7, Fig. S9: Module 8, Table S1. List of differentially expressed genes (DEGs), Table S2. List of Drugs ranked by degree, Table S3. KEGG pathway, GO Biological Process of functional modules, and DEGs involved in each module.

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Author Contributions

Conceptualization, A.M., S.E., and M.E.; methodology, S.E. .; formal analysis, S.E.; writing—original draft preparation, S.E. .; writing—review and editing, A.M., S.E., and M.E ; visualization, S.E., and M.E. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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Figures

Figure 1

we present a Flow chart of our integrated novel bioinformatics approach to identify potential pathogenic pathways, drug targets, and drug candidates for COVID-19.

Figure 2

Venn diagram for overlapping differentially expressed genes (DEGs) based on two datasets. (A) the intersection of upregulated DEGs and (B) the intersection of downregulated DEGs identified from the two datasets, namely, GSE147507 and GSE15397.

Figure 3

Enrichment analysis of DEGs. A. KEGG pathway enrichment analysis of DEGs. B. Biological function enrichment analysis of DEGs. C. Biological process enrichment analysis of DEGs. D. Biological component enrichment analysis of DE.

Figure 4
Unexpanded PPI network of DEGs using STRING.

Figure 5

The PPI network of the hub genes using STRING.

Figure 6

Drug-potential target network using Cytoscape.

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