J Appl Biotechnol Rep. 2021 September;8(3):242-253

Journal of
Applied Biotechnology Reports

doi 10.30491/JABR.2021.275694.1360

Original Article

A Comprehensive Study on SARS-CoV-2 Through Gene Expression Meta-Analysis and Network Biology Approach

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Received March 1, 2021; **Accepted** June 13, 2021; **Online Published** September 25, 2021

Abstract

Introduction: Coronaviruses are significant pathogens of both human and animals and are globally distributed. Out of seven CoVs strains, the most lethal coronavirus strains being portrayed is SARS-CoV-2. It can cause bronchial asthma, and severe pneumonia and acute respiratory disease. Due to its contagion in infants, adults, and immunocompromised patients which further results in making this a deadly disease, thus there is an urgent need to develop effective and safe therapeutics against it.

Materials and Methods: Meta-analysis of publicly available gene expression datasets belonging to SARS-CoV-2, SARS-CoV, MERS-CoV and HCoV-229E were carried out to identify the potential differentially expressed genes exclusively associated with SARS-CoV-2 and then a network model was developed to decipher significant drug targets, associated pathways and drug candidates which can be repurposed for this infection.

Results: The COVID-19 infection mainly targets immune responses and regulatory processes. A novel role of Relaxin signaling pathway was identified in SARS-CoV-2 infection. Anti-inflammatory, anti-tumor, nutraceutical and anthelmintic agents were found to be good prospective candidates for repurposing against COVID-19.

Conclusions: This theoretical study resulted in the identification of approved drug targets that may have the potential to be repurposed for COVID 19 treatment.

Keywords: COVID-19, Microarray Data, RNA-Seq Data, Systems Biology, Drug Targets, Pathogenesis
Citation: Raj U, Rai S, Mathur SK, Saxena A, Kaushik AC. A Comprehensive Study on SARS-CoV-2 Through Gene Expression Meta-Analysis and Network Biology Approach. J Appl Biotechnol Rep. 2021;8(3):242-253. doi:10.30491/JABR.2021.275694.1360

Introduction

Coronavirus disease 2019 (COVID-19) has rapidly spread across China and numerous different nations[1-8] after its advent in Wuhan, a city of more than 11 million people and the capital of Hubei province in China in the month of December 2019.[9] Till date (4:34 PM CEST, 4 April 2021), COVID-19 has influenced more than 130,422,190 patients around 223 Countries, territories or areas and has turned into a significant worldwide health concern (https://covid19.who.int/). On March 11, 2020, the World Health Organization (WHO) declared COVID-19 a pandemic not because of the changes associated to the characteristics of a disease but due to the concerns over its geographic spread.

Coronaviruses (CoVs) are the largest known RNA viruses and are classified into four genera: (i) α- (ii) β- (iii) γ- (iv) δ-.[10,11] Out of which, α-CoVs and β-CoVs can infect mammals, whereas γ-CoVs- and δ-CoVs are found to infect birds and may also infect mammals.[12] Two α-CoVs including HCoV-229E and HCoV-NL63 and two β-CoVs comprising of HCoV-HKU1 and HCoV-OC43 have been recognized as human-susceptible virus and cause mild respiratory symptoms. Contrary to this, two pathogenic human CoVs (HCoVs): severe acute respiratory syndrome coronavirus SARS-CoV and Middle East respiratory syndrome coronavirus MERS-CoV (the other two forms of β-CoVs) lead to serious and conceivably deadly respiratory tract infections.[10] However, a highly pathogenic, non-segmented, enveloped and [+] ssRNA coronavirus that belong to class of β–coronaviruses is 2019-nCoV/SARS-CoV-2 which has greatly affected the worldwide population.

There are number of cascades by which SARS-CoV-2 interferes with the immune system (Figure 1).[13] As SARS-CoV-2 infects, the expression of TNF-α increases in response to increase in cytokines. Furthermore, the infection induces stress in endoplasmic reticulum triggering the unfolded

protein response. As a result of constant stress, the NF-κB pathway is activated leading to inflammation and symptoms like shortening of breath. Moreover, as the level of cytokines increases, interleukins such as IL6 and IL8 starts moving towards the liver leading to induction and accumulation of serum amyloid A. This further causes deposition of amyloid fibrils in the liver and in other organs leading to dysfunctionality.

SARS-CoV-2 shares genetic similarity with both SARS and the MERS coronaviruses, however similarity with SARS is greater as compared to MERS.[1] Therefore, in this study, we aim to perform meta-analysis of the gene-expression datasets of different β-CoVs strains. In addition to this, one gene-expression dataset from α-CoV i.e., HCoV-229E was also taken because they can also infect bats which is suspected to be the zoonotic originator of SARS-CoV-2.[14] Besides, HCoV-229E is also responsible for a mild disease, common cold which is characterized by coughing, sore throat, runny nose, sneezing, headache, and fever.

To perform in-depth analysis in order to find out the uncommon characteristics about 2019-nCoV, recently, an outstanding study has been carried out which helps in the rapid identification of repurposable drugs or its combination(s) against 2019-nCoV[15] by taking SARS-CoV and MERS-CoV affected patients into consideration. To the best of our knowledge, no study has been conducted on network-based drug repurposing for all the three categories of β-CoVs (SARS-CoV-2, SARS-CoV and MERS-CoV) in comparison with one α-CoV (HCoV-229E) through meta-analysis, differential gene expression and system biology approaches.

Furthermore, there is a need for the advancement of effective prevention and treatment strategies against sudden corona outburst. Identification of drug targets plays a vital role in designing new medications and fighting ailments. However, the process of screening drug targets in the lab is a costly and tedious affair. In the previous decade, the growth of different kinds of omics data made it conceivable to create *in-silico* approaches to identify putative drug targets. One of the best ways to identify possible drug targets is microarray data analysis.[16-18] Microarray technology can be used to investigate the functions of genes, or be used in the diagnosis of diseases.[19,20] Another way is to go for network-based studies that potentially serve as a useful tool for efficient screening of potentially new indications for approved drugs, as well as drug combinations.[15,21-24]

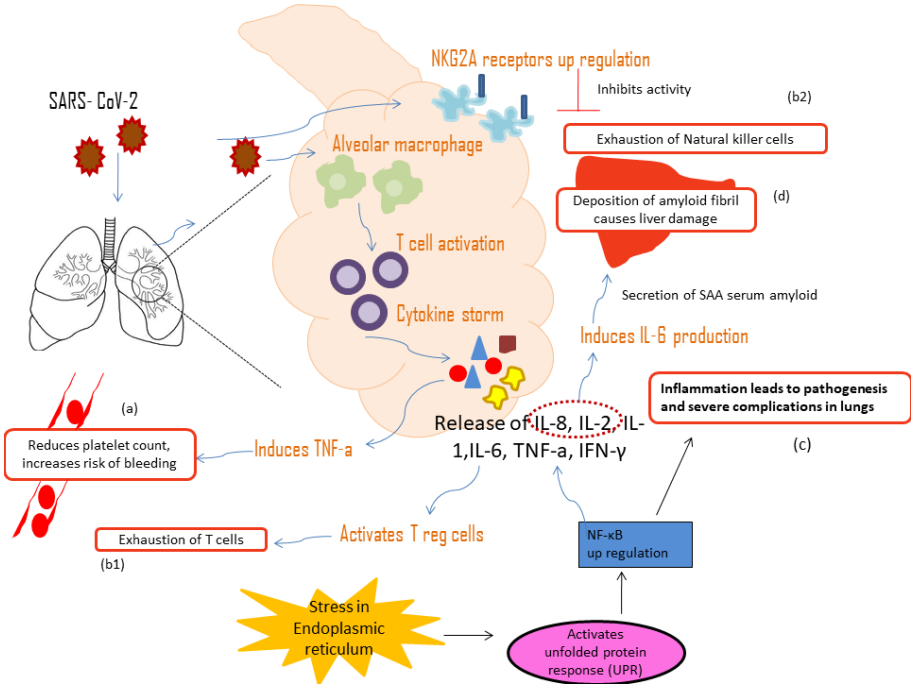

Figure 1. The Diagrammatic Representation Illustrates the Different Cascades by which SARS-CoV-2 Interferes with the Immune System. (a) Upon infection, the expression of TNF-α elevates due to sudden increase in the cytokines, which leads to reduction in platelet count and increases the chances of bleeding. (b1) The cytokine storm induces Treg cells, which exhausts the function of T cell. (b2) Similarly, the (natural killer) NK cells exhaust by the up regulation of NKG2A (natural killer cell receptors) due to SARS CoV-2 infection. (c) SARS-CoV-2 infection induces stress in endoplasmic reticulum, which triggers the unfolded protein response (UPR). As a consequence of persistent stress is the activation of NF-κB pathway, which results into inflammation and finally leads to th pathogenesis and other related issues such as shortening of breath. (d) Macrophages activates a cascade which induces cytokine stress in the cell by producing different inflammatory cytokines such as interleukin (IL-8, IL-2, IL-1, IL-6), tumor necrosis factor-alpha (TNF-a), interferon-gamma (IFN-γ). An elevated level of IL-6 due to the IL-2 and IL-8 moves towards liver through bloodstream, where it induces serum amyloid A (SAA). Accumulation of SAA causes the amyloid fibril deposition in the liver and in the other organs which eventually causes their dysfunction.

Presently, *in-silico* methods are progressively drawing the attention of biological scientists who wish to recognize the effective drug target. Hence, this study was designed to bring together the understanding of the gene expression data and biological interaction networks to build a network-based pipeline for the discovery of potential drug targets and drug repurposing candidates.[25]

Materials and Methods

Collection of Gene Expression Datasets

Microarray data from the SARS-CoV, MERS-CoV, HCoV-229E related expression profiles were retrieved and downloaded from the National Center for Biotechnology Information (NCBI) GEO database.[26] Queries were performed using "*coronavirus*" as a keyword. The search was restricted to the following specific fields: study type, species—Homo sapiens and expression profiling by array. We used the dynamic gene expression datasets GSE1739[27] linked to the gene expression patterns of peripheral blood mononuclear cells (PBMC) from SARS-CoV patients, GSE100504 (NCBI Accession: PRJNA391962; Submitter: *Baric R, Sims A, Heller N, Waters KM, Eisfeld AJ, Kawaoka Y*, 2018) associated with the MERS-CoV infected human airway epithelial cells and GSE89159[28] related to the changes in host gene expression upon infection of A549 lung epithelial carcinoma cells with Human corona virus-229E (HCoV-229E). This is actually due to the fact that there is no microarray dataset available for 2019-nCoV/SARS-CoV-2. Therefore, we used the gene count-matrix files of the recently submitted RNA-Seq data: GSE147507,[29] which are only related to the transcriptional response to SARS-CoV-2 infection in the Normal human bronchial epithelial (NHBE) cells for the comparative analysis and identifying their similarities and more importantly the uncommon significant genes with their roles in the SARS-CoV-2 infection.

Inclusion Criteria for Meta-Genes across Microarray Datasets and Gene Expression Signatures (GES) across RNA-Seq Data

We first downloaded the series gene expression matrices for all the three microarray datasets viz. GSE1739, GSE100504 and GSE89159 from NCBI-GEO using GEOquery package from Bioconductor.[30,31] A statistical method k/a Principal Component Analysis (PCA) was used for exploratory analysis of the datasets.[32] To carry out gene-level meta-analysis, the Network Analyst 3.0 - a web application[33-35] was used which enables adjustment of batch effects across multiple studies by Combat function and then integrate them using either of the following approaches: P-value, effect size, vote counting, and direct merging. This tool does data normalization with transformation along with a procedure that decreases false discovery rate (Benjamini and Hochberg method)[36] and considers the annotation information. Significant meta-signatures (genes) across all three microarray datasets were screened using a combined p-value of less than 0.01 as threshold. According to the recent benchmark study, this tool is found to offer robust gene ranking methods such as moderated T-test, signal-to-noise ratio, fold change etc.[37] Similarly, RNA-Seq data (GSE147507) analysis of the six samples (GSM4432378, GSM4432379, GSM4432380, GSM4432381, GSM4432382, GSM4432383) of SARS-CoV-2 infection was carried out by using RNA-Seq pipeline based on Jupyter platform[38] and was further validated by using the BioJupies web server.[39]

Modeling of Corona Protein–Protein Interaction Network (CPPIN)

The identified GES in NHBE cells in response to SARC-CoV-2 infection were used as seed genes for construction of CPPIN. Search Tool for Retrieval of Interacting Genes/ Proteins (STRING) server[40] was used for modeling the CPPIN from the seed nodes. The parameters used for STRING were all prediction sources enabled, medium confidence score of ≥ 0.400 and atleast 50 interactors in first and second shell. The final CPPIN was analyzed in Cytoscape 3.7.1[41,42] and disconnected components were removed.

The topological characteristics such as degree, betweenness centrality and bridging centrality of the CPPIN were calculated using CentiScape plugin[43] fully implemented in Cytoscape 3.7.1. For each centrality, mean was calculated and nodes having centrality value greater than mean were considered significant. The number of connections determine the degree of a node. Highly connected nodes participate in crucial regulatory functions of a cell and are referred to as hubs. The number of shortest paths passing through a node determine its betweenness centrality (BC). High BC nodes play an important role in information flow and are referred to as bottlenecks. High BC nodes that coincide with hubs are referred to as hub bottlenecks (HBN). Bridging centrality (BrC) on the other hand, identifies nodes that are located between highly connected modules. It is a product of BC and bridging coefficient which is the measure of how well a node is situated between highly connected modules. Bridging nodes are regulated independently and are less lethal,[44] therefore may serve as potential drug targets especially in human systems.

Pathway Enrichment Analysis of CPPIN

The enrichment analysis was done using KOBAS 3.0 web server[45,46] to identify signaling pathways and processes that are affected by COVID-19. The KOBAS 3.0 web server integrates information of 4000 species from databases including BioCyc,[47] Gene Ontology,[48] KEGG DISEASE,[49] KEGG PATHWAY,[50] OMIM,[51] NHGRI GWAS Catalog,[52] PANTHER,[53] and Reactome.[54] For the functional annotation, the entire list of CPPIN protein was given as an input and the used parameters were: annotate and identify; Homo sapiens as the species background and KEGG PATHWAY and

KEGG DISEASE database enabled. Criteria used to identify statistically significant hits were hypergeometric test, Benjamini-Hochberg FDR correction, and p- value ≤ 0.01.

Identification of Functional Clusters

Molecular complex detection (MCODE) plugin[55] was employed to identify clusters in the CPPIN. The MCODE is a clustering algorithm that finds highly connected regions in the network. The parameters used to run MCODE were: find clusters in whole network; degree cutoff 2; haircut included; node score cutoff 0.2; K-core 2 and maximum depth 100. Each cluster was further analyzed for over-represented Gene Ontology terms and KEGG Pathways.

Repurposing of Approved Drugs against COVID-19

The bridging nodes are less lethal and are regulated independently, hence may serve as good drug targets. We firstly identified bridging nodes in the CPPIN and further through DrugBank 5.1.5.[56] These bridging nodes were examined if they correspond with approved drug targets. The approved bridging nodes were searched in DrugBank 5.1.5 to identify the approved drugs that could be good candidates for repurposing.[57] These approved drugs were further screened for side effects from SIDER 4.1 database[58] and drugs with severe side effects were discarded.

Results and Discussion

Screening of Meta-Genes and GES with their Mapping to Detect Uncommon Significant Genes in SARS-CoV-2

To check the overall structure of the analyzed microarray datasets, Principal Component Analysis (PCA) was carried out for individual dataset as well as combined datasets (Figure 2).

Initially, significant gene signatures (meta-genes) were selected from the three microarray datasets GSE1739, GSE100504 and GSE89159 on the basis combined p-value (≤ 0.01) as defined in the cut-off criterion. Various other statistics such as combined t-statistic (t), and annotation features like Entrez ID and Gene Symbol were also obtained with the help of the Network Analyst 3.0 tool. These 1597 significant genes obtained after the meta-analysis of the above mentioned three microarray datasets, with their calculated statistics and annotated features are mentioned in the Supplementary Table S1. Thereafter, PCA plot was also calculated for the chosen RNA-Seq data (Figure 3A) because it provides fully unsupervised information on the dominant directions of highest variability in the data and may be utilized to investigate similarities between individual samples, or formation of clusters. In addition, the Volcano plot and Microarray (MA) plot were created to identify genes whose expression is significantly altered in a perturbation, and to assess the global similarity of gene expression in the two biological sample groups (Figure 3B and 3C). The RNA-Seq data analysis of GSE147507 results in identifying 140 GES across the SARS-CoV-2 infected samples based on adjusted p-value (≤ 0.01) as threshold criterion. Other statistical parameters viz. log fold-change, average gene expression, t-statistic and B-statistic were also calculated for these 140 GES and have been reported in Supplementary Table S2. Out of these two sets of 1597 meta-genes and 140 GES, 51 genes which are common in both the datasets were not so important for deciphering peculiar signatures about SARS-CoV-2 (Figure 3D).

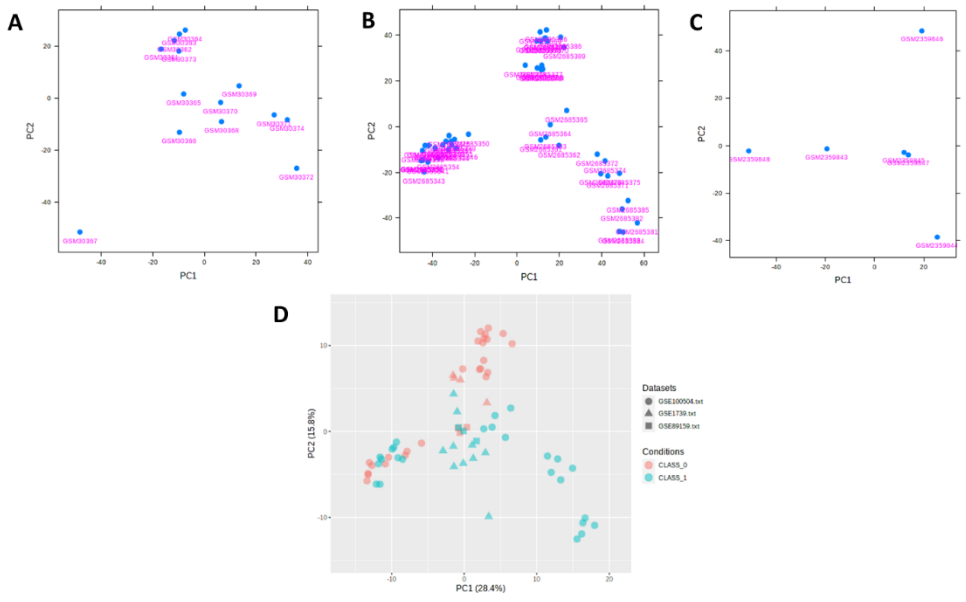

Figure 2. PCA Plots (PC1 vs PC2) of Individual Dataset GSE1739 Containing 14 Samples - (A), GSE100504 Comprising 50 Samples – (B), GSE89159 Covering 6 Samples – (C) and PCA Plot of Combined Datasets (Meta-Data) is Depicted in (D).

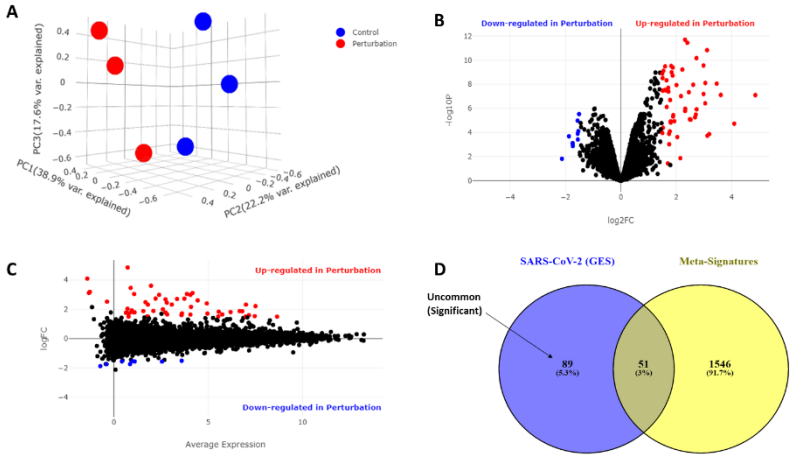

Figure 3. (A) PCA plot by calculating three principal components PC1, PC2 and PC3 for the RNA-Seq dataset GSE147507 comprising only six samples (GSM4432378, GSM4432379, GSM4432380, GSM4432381, GSM4432382, GSM4432383) used in this analysis. Each point represents an RNA-seq sample; (B) Volcano Plot which displays the log2-fold changes and statistical significance of each gene calculated by performing a differential gene expression analysis. Every point in the plot represents a gene, Genes that pass a threshold of padj < 0.01 and |log2foldChange| > 1 in differential expression analysis are colored in blue when they are down regulated and red when they are upregulated; (C) MA plot: a type of scatter plot that displays the average expression and statistical significance of each gene calculated by performing differential gene expression analysis; (D) Venn diagram representing the uncommon 89 significant gene signatures in SARS-CoV-2.

What is more significant here is the uncommon 89 significant gene signatures which are specific to only SARS-CoV-2 dataset and these have been used for further network-based analysis.

Furthermore, the Gene Ontology (GO) enrichment analysis of these 89 significant genes was carried out by ShinyGO v0.61.[59] Enrichment analysis based on hypergeometric distribution followed by FDR (False Discovery Rate) correction with a cut-off value of 0.01 was carried out. The top 20 significant functional categories i.e. significant pathways based on Gene Ontology (Biological Process) with their enrichment FDR and related participating genes from the list of 89 gene expression signatures have been depicted in Supplementary Table S3. To summarize the correlation among these significant pathways, a hierarchical clustering tree and network of these pathways were also constructed as depicted in Figure 4A and 4B.

CPPIN Structure and its Characteristics

The CPPIN network consisted of 141 nodes and 961 edges (Figure 5). Topological analysis revealed fifty-three hubs and thirty-two bottleneck proteins in CPPIN. twenty-one proteins were both hubs and bottleneck, therefore they were considered as HBNs. These HBNs correspond to the central elements of a biological network.[60] It is often found that viruses and parasites target central proteins to make a hold on host cells.[61,62] Furthermore, these central proteins may also serve in the identification of drug targets, biomarkers and therapeutic agents.[63-66] However in human diseases, targeting these central proteins may turn out to be lethal.[67-69] The central proteins are shown in Supplementary Table S4.

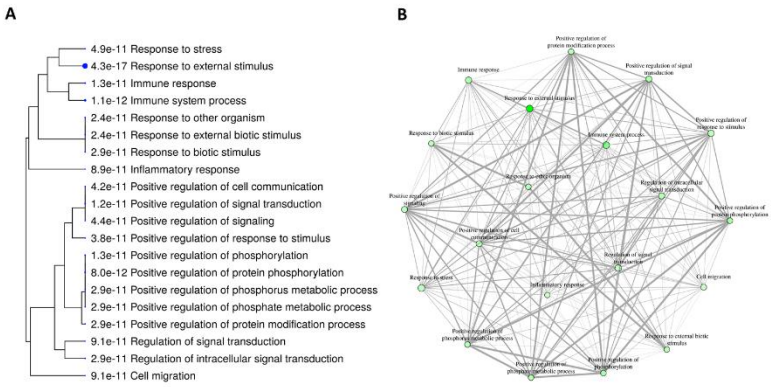

Figure 4. (A) Hierarchical clustering tree summarizing the correlation among the top 20 significant pathways based on the Enrichment FDR. Pathways with many shared genes are clustered together. Bigger dots (*blue in color*) indicate more significant P-values; (B) This interactive plot also shows the relationship between enriched pathways. Two pathways (nodes) are connected if they share 20% (default) or more genes. Darker nodes are more significantly enriched gene sets. Bigger nodes represent larger gene sets. Thicker edges represent more overlapped genes.

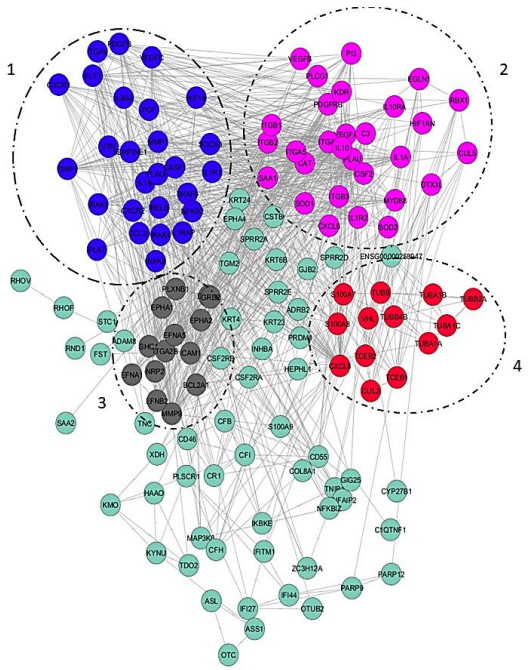

Figure 5. CPPIN Structure. The network consisted of 141 nodes and 961 edges. The blue nodes constitute cluster-1 (27 nodes); magenta nodes constitute cluster-2 (29 nodes); black nodes constitute cluster-3 (13 nodes) and red nodes constitute cluster-4 (13 nodes) as calculated through MCODE. Green nodes represent rest of the network nodes.

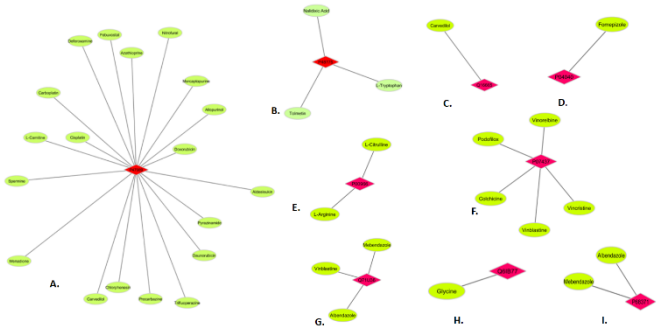

Figure 6. Drug- Target Interactions. The approved bridging nodes are represented as red diamonds while the approved drugs associated with each approved bridging nodes are represented as light-green ovals. A. Xanthine Dehydrogenase (XDH) having 19 interactions B. Tryptophan-2,3-dehydrogenase (TDO2) having three interactions C. Hypoxia-Inducible-Factor 1A having one interaction D. Catalase (CAT) having one interaction E. Argininosuccinate synthase (ASS1) having two interactions F. Tubulin beta chain (TUBB) having five interactions G. Tubulin alpha-1A chain (TUBA1A) having three interactions H. Glycine N-acyltransferase (GLYAT) having one interaction I. Tubulin beta-4B chain (TUBB4B) having two interactions.

Role of Central Proteins in Viral Infection

Viruses adopt various strategies to disrupt the cellular environment of the host for replication and survival. Gene Ontology analysis of the central proteins of the CPPIN revealed that they were involved in immune responses and regulatory processes such as regulation of apoptosis, angiogenesis, cell division and transcription. As the virus invades the body, the human immune system responses to the virus. The complement system is a constituent of innate immunity and links adaptive and innate immunity.[70] The three-complement activation pathway i.e., classical, alternative and lectin converges in C3[71] has a major role in neutralizing viral proteins.[72] Similarly, Interleukin 1α (IL-1α) and 1β (IL-1β) are pro-inflammatory cytokines that are produced by a variety of cells and act on all the organs of the body.[73] The viral infection induces the transcription of various genes that encode various cytokines and chemokines.[74] Induction of IL-1β increases the inflammation of lungs and blockade of IL-1β signaling is considered potential treatment for influenza induced inflammation.[74] Furthermore IL-8/CXCL8 is also released as a result of infection due to Influenza A[75] and Hepatitis C virus.[76] IL-8 is produced by many cells such as monocytes, fibroblast, epithelial cells and hepatocytes.[77] IL-8 also elicits chemotaxis of neutrophils, basophils and T-lymphocytes and oxidative burst, degranulation and release of lysosomal enzymes.[77] Similarly, IL-10 is considered as a critical player in protecting the host tissue damage during the acute phase of immune responses.[78] IL-10 is central to immune responses and viruses hijack this pathway to evade immune responses and establish infection.[78] MyD88 functions as an important adaptor molecule which bridges IL-1 receptor, type 1 to IL-1 receptor-associated kinase.[79] It has been reported that bacterial and viral proteins target MyD88 signaling to evade immune responses.[80]

It has also been reported that VEGF play an important role in many viral infections.[81] Many viruses upregulate the expression of VEGF while others carry VEGF homologs with them to the infected cells.[81] Similarly, the Matrix Metalloproteinases (MMPs) are a family of proteases that are involved in degradation of extracellular matrix. During inflammation, MMPs are involved in degradation of basement membrane and parenchymal extracellular matrix, thus enabling infiltration of leukocytes.[82] It has been reported that the corona virus infection increases the expression of MMP2 and MMP9 in human microglial and astrocytic cell lines.[82] The viral infection increases the expression of IL-6, tumor necrosis factor – α and monocyte chemoattractant protein-1 which are inducers of MMPs.[83] Other central proteins such as C-C Motif Chemokine Ligand 20,[84-86] Intercellular Adhesion Molecule 1,[87,88] Suppressor Of Cytokine Signaling 3,[89,90] Hypoxia-Inducible-Factor 1A,[91,92] catalase,[93,94] Tumor necrosis factor receptor associated factor 6,[95,96] Kinase Insert Domain Receptor,[97,98] Serum Amyloid A1,[99,100] also have a profound role in viral infection.

Enrichment Analysis Revealed that COVID-19 Ameliorates an Array of Signaling Pathway

Twenty-nine signaling pathways (Supplementary Table S5) were overrepresented in CPPIN. Most of these pathways

Table 1. Description of Approved Bridging Nodes, Approved Drugs, and Their Present Application

Approved drug target	Drugs	Present use of Drugs
GLYAT	Glycine	Antispastic, antipsychotic, antioxidant, and anti-inflammatory
ASS1	L-Citrulline	Promote energy level, stimulate immune system, and detoxify Ammonia
	L-Arginine	Improves immune responses to bacteria, viruses, and tumor cells. It also possesses anti-atherogenic properties
TDO2	Tolmetin	Non-steroidal anti-inflammatory agent
	L-Tryptophan	Increases serotonin production, improves sleep, and helps in managing depression
	Nalidixic Acid	Antimicrobial agent
XDH	*Azathioprine*	Treatment of inflammatory conditions such as rheumatoid arthritis and is also used as immunosuppressant
	Carvedilol	Heart failure, left ventricular dysfunction, and hypertension
	Carboplatin	ovarian carcinoma
	Daunorubicin	leukemia and other neoplasms
	Chlorphenesin	muscle relaxant
	L-Carnitine	Stimulates gastric and pancreatic secretions and in the treatment of hyper lipoproteinemia
	Mercaptopurine	Leukemia.
	Deferoxamine	Treatment of acute iron or aluminum toxicity
	Aldesleukin	renal cell carcinoma
	Febuxostat	Hyperuricemia
	Nitrofural	topical anti-infective agent
	Trifluoperazine	antipsychotic and antiemetic
	Allopurinol	Gout
	Procarbazine	Hodgkin's disease
	Cisplatin	Cancers
	Doxorubicin	Antibiotic
	Pyrazinamide	Antitubercular agent
	Menadione	Assist in normal blood clotting
	Spermine	Treating dietary shortage or imbalance
CAT	Fomepizole	Used as an antidote in confirmed or suspected methanol or ethylene glycol poisoning
HIF1A	Carvedilol	Heart failure, left ventricular dysfunction, and hypertension
TUBB	Vincristine	Antitumor
	Vinblastine	Antitumor
	Podofilox	Treatment of warts
	Vinorelbine	Cancer
	Colchicine	Gout
TUBA1A	Mebendazole	Broad-spectrum anthelmintic
	Vinblastine	Antitumor
	Albendazole	Broad-spectrum anthelmintic
TUBB4B	Mebendazole	Broad-spectrum anthelmintic
	Albendazole	Broad-spectrum anthelmintic

have well established a role in viral infection. However, we found a novel role of Relaxin signaling pathway in viral infection. Relaxin is a peptide hormone that mediates a variety of functions including vasodilation angiogenesis, anti-apoptosis and anti-inflammatory.[101] It also shares crosstalk with Notch 1, angiotensin II type 2 receptor, and peroxisome proliferator activated receptor gamma (PPARγ).[101] Relaxin signaling activates PPARγ,[102] whose activation have anti-viral and anti-inflammatory effects.[103-105] Moreover, Relaxin has demonstrated potential therapeutic benefit in cardiovascular and fibrotic diseases.[101] Thus, it could be concluded that Relaxin may have a potential role in viral infection. Furthermore, the enrichment analysis also predicted that COVID-19 may lead to cancer, immune system diseases, allergies, cardiovascular diseases, skin diseases and congenital malformations. The comparison with Figure 4, which shows the pathways overrepresented by the GES revealed that the first interactors of viral antigens only elicit responses to stress, immune responses and regulation of signal transduction and protein phosphorylation. However, GES along with the second interactors of viral antigens evoke a number of signaling pathways, which further contributes to severity of disease.

Functional Cluster Analysis

Four functional clusters were identified in the CPPIN as shown in Figure 5. Cluster-1 (blue colored nodes) consisted of 27 nodes and 111 edges; Cluster-2 (magenta colored nodes) consisted of 29 nodes and 99 edges; Cluster-3 (black colored nodes) consisted of 13 nodes and 41 edges and Cluster-4 (red colored nodes) consisted of 13 nodes and 27 edges. The gene ontology analysis of these clusters revealed that proteins of Cluster-1 were involved in IL binding, regulation of cell division and proliferation. Cluster-2 proteins were involved in chemokine, chemoattractant activities, regulation of transcription by RNA polymerase II and regulation of apoptosis. On the other hand, the proteins of Cluster-3 were involved in ephrin and neurotrophin receptor binding whereas Cluster-4 proteins were involved in microtubule-based process, cytoskeleton organization, GTP binding and GTPase activity.

Identification of Potential Repurposing Candidates for COVID-19

Thirty-one bridging nodes were identified in the CPPIN. Out of these 31 bridging nodes, nine were identified as

approved drug targets from DrugBank and were denoted as approved bridging nodes. Based on a study of drug-target interactions, 33 approved drugs were identified which could be good candidates for repurposing against COVID-19 infection. The approved bridging nodes along with their drug interactions are shown in Figure 6 and the details of the approved bridging nodes, approved drugs as well as their present use is given in Table 1.

It is evident from Table 1, that anti-inflammatory agents (including drugs for rheumatoid arthritis and Gout); nutraceuticals (such as Glycine, L-Citrulline, L-Arginine, L-Tryptophan and spermine); anthelmintics and antitumor provide good starting points for repurposing. These categories of drugs are either used or are under study for COVID-19 treatment. A well-known anthelmintics is Ivermectin which has been used for COVID-19 treatment.[106,107] Similarly, anti-inflammatory drugs,[108] anti-cancer drugs[109,110] and nutraceuticals[111] have also been reported to treat COVID-19 patients.

Through our study, we have identified some of the drug candidates that could be studied for their potential to get repurposed against COVID-19. The search was refined through side effect analysis and the role of targets in the COVID-19 infection. The side effect analysis revealed that Doxorubicin, Carvedilol, Vincristine as well as Vinorelbine have a large number of side effects and Nitrofural induces cancer.[112] Therefore, these drugs must not be considered as repurposing candidates. The literature search revealed that out of the nine proposed drug targets, XDH,[113] ASS1,[114] HIF1A,[115,116] TUBB[117] and TUBA1A[118] were found to be the promising targets because of its involvement in the COVID-19 infection. The drug-target interactions (Figure 6A) identified 19 drugs associated with XDH, 1 drug with HIF1A (Figure 6C), 2 drugs with ASS1 (Figure 6E), 5 drugs with TUBB (Figure 6F) and 3 drugs with TUBA1A (Figure 6G) which again provides promising repurposing candidates for the COVID-19 infection. However, additional confirmatory studies are required to analyze the potency of these drugs against the COVID-19 infection.

Conclusion

In last 20 years, various viral diseases such as SARS-CoV, HINI influenza, MERS-CoV have emerged and posed major public health problems. Presently, the world is facing global pandemic due to new CoV (i.e., SARS-CoV2). The CoV are major pathogens that cause respiratory diseases. In this context, we developed a network model using GES associated COVID-19 and found that this virus mainly targets the proteins involved in immune responses and regulatory processes. A novel role of Relaxin signaling pathway in viral infection was also revealed. The cluster analysis was performed to identify gene ontology categories that are affected by SARS-CoV-2. Clustering on functional basis revealed four main groups of proteins that have an important role in COVID-19 infection. These belong to proteins involved in cell division and proliferation; chemokine and chemoattractant activities; ephrin and neurotrophin binding activities and those involved in microtubule-based processes. The network analysis revealed 33 approved drugs that were associated with approved bridging nodes. However, five of these approved drugs had severe side effects. Therefore, the remaining 28 drugs that belong to nutraceutical, anti-inflammatory, anti-tumor, and anthelmintic categories could be good candidates for repurposing. These proposed drugs may be more effective against COVID-19 because they are already approved drugs, and hence could be also tested against COVID-19 cell lines. However, more experimental studies are required to establish the confirmatory role of these medications for repurposing against COVID-19 infection.

Authors' Contributions

UR Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing-original draft, Writing-review and editing. SR Conceptualization, Investigation, Methodology, Validation, Visualization, Writing-original draft. SKM Writing-review and editing. AS Data curation, Supervision, Validation, Visualization, Writing-review and editing. ACK Supervision, Validation, Writing-review and editing.

Conflict of Interest Disclosures

The authors declare that they have no conflict interests.

Supplementary Materials

Supplementary Tables S1, S2, S3, S4 and S5 are attached with this manuscript.

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