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An *in Silico* Approach to Find the Molecular Targets and Potential Candidates for SARS-CoV-2

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Abstract

The rapid spread of SARS-CoV-2 has led researchers to seek novel drugs as well as re-purposing the existing drugs to prevent control or treat COVID-19. An interesting approach is to focus on the molecular pathways which could act as a drug target in this disease. Since the molecular pathways associated with SARS-CoV-2 are still unclear, the SARS-CoV infected patients and the convalescent individuals were selected as the model for SARS-CoV-2 infection and an *in silico* study was designed to identify the potential pathways which could act as the target for drug molecules. In the next step, the drugs with the ability to target these pathways were selected and introduced as potential compounds for further investigations to finding a drug for COVID-19. The results revealed that lycorine and GW-5074 are two small molecules with the ability to target the selected pathways. Interestingly, these compounds had shown antiviral activity against a broad range of viruses, including SARS-CoV. The results obtained in this *in silico* study could be considered as a primary study for further investigations.

Keywords: SARS-CoV-2, COVID-19, Molecular Pathways, Drug Candidate.

1. Introduction

There are several members of the family of *Coronaviridae*, usually spreading in the human population and cause common colds and respiratory tract infections. Also, they may be responsible for gastroenteritis in infants (1). Since these mild infections are usually self-limited by the human body, there is no need to develop a specific medication or vaccine for their treatment or prevention and international organizations such as World Health Organization (WHO) recommend social distancing as well as patient isolations. In recent decades, great attention has been directed to this virus family due to the emerging of two novel human coronaviruses from animal reservoirs (2). The severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV) caused severe respiratory disorders in humans (3). SARS and MERS resulted in the death of 774 and 858 patients, respectively (4). Although the total number of deaths for SARS and MERS was not too high, the emerging of the third pathogenic human coronavirus named 2019-nCoV/SARS-CoV-2 has led to a global pandemic (5). As of 31 March 2020, SARS-CoV-2 has had a total of 697,244 confirmed cases causing 33,257 deaths in 204 countries worldwide (6).

The time-consuming and costly process of drug or vaccine discovery have led researchers to use *in silico* approaches to find potential drug candidates as well as the molecular and signalling pathways associated with the diseases (7). It had been estimated that the development of a

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new drug costs around $2.6 billion in 2015 (8). The drug repurposing leads to the application of a therapeutic compound which already exists and its characteristics such as pharmacokinetic, pharmacodynamics, toxicity and distribution in human body is fully understood (9). Also, in the pandemic conditions such as COVID-19, a re-purposed drug could be tested in human clinical trials immediately since its safety has been proven for the primary application. On the other hand, two major strategies have been suggested for drug repurposing. First approach is based on virtual screening of the existing medications against the known molecular targets or pathways (10). Although bioinformatics methods including molecular docking provides a great opportunity for the researchers to find novel applications for the existing drug, the drawbacks of this method may limit its wide application. In this approach, the researchers need to know the molecular or cellular targets from the beginning of the study. Since SARS-CoV-2 is a novel virus and the three dimensional (3D) structure of interested proteins are not available, this approach may not lead to favorable results. Therefore, an alternative strategy has been proposed in which the interactions between the gene products involved in a previously-investigated disease with the highest similarity with the existing disease could be used as a model to find molecular pathways associated with the novel pathological condition (11). In order to find the genes associated with a disease, the genomic data and gene expression data could be used (e.g.; Genome-Wide Association Studies or RNA-seq differential expression analysis). The results of such studies identify the new targets for the interested disease. Using this approach, the possibility for the discovery of novel pathways and consequently molecular targets increase which provides the opportunity for the researchers to discover new drugs or repurpose existing drugs for the novel targets. Therefore, the process of drug repurposing could be carried out in a rationale manner (7).

In the present study, we focused on the data obtained from the global gene and protein expression profiles in SARS patients as the most relative family of coronaviruses to SARS-CoV-2. In the present investigation, we analyzed the data of patients versus healthy persons as well as healthy versus convalescent people. The aim of the study was to find out which signaling pathways in the cells of SARS patients have been up- or down-regulated in order to facilitate the process of drug discovery or repurposing for COVID-19.

2. Materials and methods

2.1. Gene Expression Dataset Selection

Due to the novelty of SARS-CoV-2 and lack of data on this virus, the microarray data of SARS-CoV as the most similar virus to SARS-CoV-2 was used for this research. “Severe acute respiratory syndrome” keyword searched on Gene Expression Omnibus (GEO) DataSets database (https://www.ncbi.nlm.nih.gov/gds) and the results filtered for *Homo sapiens*. GEO Dataset provides original and curated submitter-supplied records such as Series, Samples and Platforms for a wide range of organisms (12). GSE5972 dataset with the title of “Gene expression profiling of patients with the severe acute respiratory syndrome (SARS)” was chosen for further analysis (13). This dataset contains the microarray dataset of SARS-infected patients (pre and post pO2 nadir), convalescents and healthy controls. Data are normal and median-centred based on value distribution. *P*-value adjustment applied by Benjamini & Hochberg – false discovery rate method (14). In this method, the rate of type 1 errors in null hypothesis testing for a multiple comparison test model. Hence, the false rejection of the null hypothesis would be controlled by this model.

2.2. Gene expression analysis

Different groups of results (pre pO2 nadir, post pO2 nadir, convalescent and healthy control), combined into binary sub-groups to identify the fold change of expression between them. The first binary analyzing group is patients (pre and post pO2 nadir) over healthy controls. These two sub-groups compared by GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r/). Geo2R is a web tool to identify gene expression differences between two or more groups (12).

2.3. Gene set enrichment analysis

GSEA (gene set enrichment analysis) is a method to identify a group of genes or proteins

that are mostly responsible for a specific biological or pathological function in an organism (15). Genes with an adjusted p-value of <0.05 selected for GSEA. GSEA performed by Enrichr (https://amp.pharm.mssm.edu/Enrichr). Enrichr is an intuitive enrichment analysis web-tool to perform GSEA base on different gene-sets (16). Gene ontology (GO) enrichment analysis was performed on selected genes by Enrichr. GO is a collaborative language to descript genes and gene product attribution on molecular function, biological process and cellular components (17). Pathway enrichment was analyzed on Bioplanet (18), Reactome (19) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (20) databases by Enrichr. Related drugs for gene sets are investigated on DSigDB (21) by Enrichr. The same analyses were carried out on the other groups, including healthy versus convalescents as well as post versus pre pO2 nadir patients.

2.4. Network reconstruction

Based on GEO2R results, protein-protein interaction (PPI) network and protein-drug interaction (PDI) constructed by network analyst (www.networkanalyst.ca) for healthy versus patient datasets. Molecular interaction networks are a whole set of interactions between different molecules from related or different groups (22).

3. Results

GSE5972 dataset, as an investigated dataset for this study, contains expression profiling of SARS-infected persons (pre and post pO2 nadir and convalescents) and healthy controls by the array. In this study, the GLP4387 platform provides 19200 rows of genes to be investigated. The results for convalescent patients investigated versus healthy individuals and post pO2 nadir and pre pO2 nadir patients led to no significant changes between the groups. Therefore, the results limited to patients versus healthy individuals.

3.1. Fold-change analysis

The profile of gene expression for the patients (pre and post pO2 nadir) and healthy individuals were compared in this group in order to identify the potential differences between the profiles of gene expression in the SARS patients compared to healthy individuals. The results led to the selection of 229 genes for GSEA (adjusted p-value <0.05). Three genes consisting of *CCT8* (log FC=9.0), *MKI67IP* (logFC=7.5) and *SCG5* (logFC=-7.4) showed the highest rank genes in terms of fold change between patients and healthy individuals. LogFC has been defined as the binary logarithm of fold change for two experimental components. Positive values show the over-expression of a certain gene in the patients, while the negative values show down-regulation on a binary logarithmic scale (12).

As the second group, convalescent patients investigated versus healthy individuals to determine the changes in the gene expression in convalescent after treatment in comparison with healthy persons. Convalescent and healthy persons were compared in terms of gene expression by Geo2R. The results led to the selection of 33 genes for GSEA (adjusted p-value <0.05).

3.2. Gene set enrichment analysis

All of the 229 genes symbols entered on Enrichr database. In the case of multiple names for a gene, all of the attributed names entered to prevent missing results in case of multiple gene names.

3.2.1. Gene Ontology

Gene ontology data demonstrated in three aspects, including biological process, molecular function and cellular components.

3.2.1.1. Biological Process

Gene ontology analysis of biological process was performed (adjusted p-value<0.05). According to the obtained results, three biological processes have been found to be significant (Table 1), including chaperone-mediated protein folding requiring cofactor (GO:0051085), positive regulation of peptidyl-threonine phosphorylation (GO:0010800) and 'de novo' posttranslational protein folding (GO:0051084)). The rank of competent biological processes is based on the combined score. The combined score is a variable of the p-value of the Fisher exact test and Z-Score calculated based on the deviation from the expect-

Table 1. Gene ontology analysis of biological process influenced by SARS-CoV in the patients compared with the healthy individuals.

Term	Adjusted *P*-value	Odds Ratio	Combined Score	Genes
chaperone mediated protein folding requiring cofactor (GO:0051085)	0.04042	17.6	206.5	*GAK;BAG1;HYOU1; HSPA14;HSPA1A*
positive regulation of peptidyl-threonine phosphorylation (GO:0010800)	0.025019	16.9	194.6	*AXIN1;PRKAG2;CHI3L1; CALM3;CALM1*
'de novo' posttranslational protein folding (GO:0051084)	0.042628	14.1	149.0	*GAK;BAG1;HYOU1; HSPA14;HSPA1A*

ed rank (16).

3.2.1.2. Molecular Function

According to the results obtained by gene ontology analysis on molecular functions, four molecular functions considered significant (adjusted p-value<0.05) (Table 2). These molecular functions include adenyl ribonucleotide binding (GO:0032559), purine ribonucleoside triphosphate binding (GO:0035639), ATP binding (GO:0005524) and protein kinase binding (GO:0019901).

3.2.1.3. Cellular Components

Gene ontology analysis of cellular components for patients versus healthy individuals did not show any significant changes in the cellular components of these two groups.

3.2.2. Pathway analysis

The pathway analysis was carried out to find the significant pathways in the cells affected by the infection. Adjusted p-value<0.05 was considered as the level of significancy for the selection of affected pathways by up- or down-regulated genes.

Table 2. Gene ontology analysis of Molecular function influenced by SARS-CoV in the patients compared with the healthy individuals.

Term	Adjusted *P*-value	Odds Ratio	Combined Score	Genes
adenyl ribonucleotide binding (GO:0032559)	0.011685	3.9	40.9	*SRPK2;ABCD4;PRKAG2; HSPA14;RUNX3;SYN1; CDK6;WNK1;AKT2; MKNK1;MYH9;HYOU1; HSPA1A*
purine ribonucleoside triphosphate binding (GO:0035639)	0.01254	3.4	36.6	*SRPK2;ABCD4;GNAZ; PRKAG2;HSPA14;RUNX3;SYN1;CDC42;CDK6; WNK1;AKT2;MKNK1; SCG5;MYH9;HYOU1; HSPA1A*
ATP binding (GO:0005524)	0.013603	4.3	48.8	*SRPK2;ABCD4;PRKAG2; HSPA14;RUNX3;SYN1; CDK6;WNK1;AKT2; MKNK1;MYH9;HYOU1; HSPA1A*
protein kinase binding (GO:0019901)	0.026467	2.9	26.9	*TCF7L2;PLEK;AXIN1; PRKAG2;ZBTB4;SYN1; CDC25A;CDC42;WNK1; KIT;CDK5RAP1;CALM3; RICTOR;CSK;PRKACA; CALM1;RAF1*

3.2.2.1. BioPlanet

Pathways analysis on Enrichr shows 28 significant pathways (adjusted p-value<0.05). The rank of these pathways is based on the combined score and the first 10 results are shown in Table 3.

3.2.2.2. Reactome

Reactome pathway analysis of competent genes shows that Rap1 signalling Homo sapiens R-HSA-392517 is the only significant pathway (Table 4).

3.2.2.3. KEGG

Pathway analysis has performed on the KEGG database was employed for pathway analysis and 35 pathways were suggested which the first 10 pathways were shown in Table 5.

3.2.3. DSigDB Analysis

GSEA on DSigDB was performed to find potential drug compounds for the genes which have shown up-or down-regulation (adjusted p-value<0.05) (Table 6). According to these results GW-5074 (3-[(3,5-dibromo-4-hydroxyphenyl) methylene]-1,3-dihydro-5-iodo-2H-indol-2-one), valproic acid, lycorine (1,2,4,5,12b,12c-Hexahydro-7H-[1,3]dioxolo[4,5-j]pyrrolo[3,2,1-de] phenanthridine-1,2-diol) and aspirin (acetylsali-

Table 3. BioPlanet pathway analysis.

Term	Adjusted *P*-value	Odds Ratio	Combined Score	Genes
Rap1 signaling	0.047235	21.09705	218.8287	*RASGRP2;PRKACA;RAF1;SIPA1*
Melanocyte development and pigmentation pathway	0.030948	19.4742	150.9407	*KIT;RPS6KA1;RAF1*
Angiotensin II-mediated activation of JNK pathway via Pyk2-dependent signaling	0.046725	10.819	100.4514	*MAP2K4;MEF2C;CALM3; CALM1;RAF1*
Fc epsilon receptor I signalling in mast cells	0.035794	10.29124	93.03697	*MAP2K4;NFATC1; CALM3;CALM1;RAF1*
T cell receptor calcium pathway	0.030344	11.63975	92.24077	*NFATC1;CALM3;PRKACA;JUNB*
Skeletal myogenesis control by HDAC and calcium/calmodulin-dependent kinase (CaMK)	0.032129	11.63975	92.24077	*MEF2C;NFATC1;CALM3;CALM1*
Signaling pathway from G-protein families	0.03122	11.25176	87.66035	*NFATC1;CALM3;CALM1;RAF1*
Cell differentiation by G alpha (i/o) pathway inferred from mouse Neuro2A model	0.037584	9.81258	86.44187	*MAP2K4;AKT2;RASGRP2; PRKACA;RAF1*
PIK3C1/AKT pathway	0.042184	9.644364	69.3405	*AKT2;RICTOR;PRKACA; RAF1*
MEF2D role in T cell apoptosis	0.043806	9.644364	69.3405	*NFATC1;CALM3;CALM1;CD3D*

Table 4. Reactome Pathway Analysis.

Term	Adjusted *P*-value	Odds Ratio	Combined Score	Genes
Rap1 signalling Homo sapiens R-HSA-392517	0.047861	21.09705	218.8287	*RASGRP2;PRKACA; RAF1;SIPA1*

cylic acid) were suggested. GW-5074 is a material for research purposes and has not been approved for human or animal application yet. This chemical has been considered as a potent, selective, and cell-permeable inhibitor of Raf-1 (IC50=9 nM) (23). This molecule has shown more than 100-fold selectivity for Raf-1 compared with several related kinases. The other material suggested by DSigDB Analysis was lycorine, which is an alkaloid compound extracted from different Amaryllidaceae genera and widely tested for various effects, including anticancer or antiviral activities.

3.3. Interaction Networks

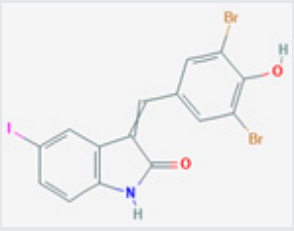
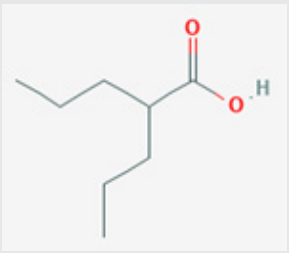
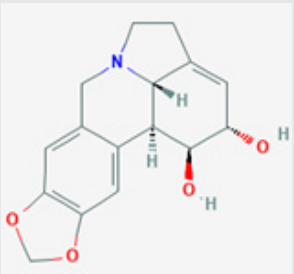
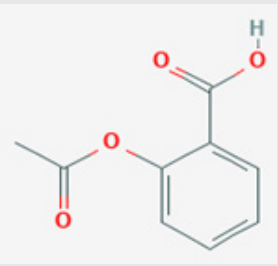
3.3.1. PPI Network

Network analysis of attributed genes from gene expression analysis shows hub proteins in the case of PPI in SARS-infected patients versus

Table 5. KEGG Pathway Analysis.

Term	Adjusted *P*-value	Odds Ratio	Combined Score	Genes
Apelin signaling pathway	0.001713	6.159722	74.52903	*GABARAPL2;MEF2C; MEF2B;AKT2;GNB1; PRKAG2;CALM3;PRKACA; RAF1;CALM1*
Terpenoid backbone biosynthesis	0.02999	11.50748	70.7245	*IDI1;ICMT;PCYOX1*
Influenza A	0.005931	4.934982	50.1619	*MAP2K4;CXCL10;NXF1; NXF3;AKT2;MX1;RAF1; HLA-DPA1;JAK1;HSPA1A*
Phototransduction	0.045934	9.041591	49.21665	*GNB1;CALM3;CALM1*
Kaposi sarcoma-associated herpesvirus infection	0.006023	4.536999	42.90156	*MAP2K4;GABARAPL2; CDK6;AKT2;GNB1;NFATC1;CAL M3;RAF1;CALM1;JAK1*
Long-term potentiation	0.028197	6.297626	42.40617	*RPS6KA1;CALM3;PRKACA; RAF1;CALM1*
Estrogen signaling pathway	0.011935	4.927777	41.22881	*NCOA1;AKT2;CALM3; PRKACA;RAF1;CALM1; CREB5;HSPA1A*
GnRH signaling pathway	0.021539	5.444399	38.56303	*CDC42;MAP2K4;CALM3; PRKACA;RAF1;CALM1*
MAPK signaling pathway	0.005551	3.718801	36.53793	*MAP2K4;MEF2C;NFATC1; RASGRP2;EREG;CDC42; AKT2;MKNK1;RPS6KA1; KIT;PRKACA;RAF1;HSPA1A*
Pancreatic cancer	0.030345	5.625879	35.04633	*CDC42;CDK6;AKT2;RAF1; JAK1*

Table 6. DSigDB Analysis.

Term	Structure	Adjusted *P*-value	Odds Ratio	Combined Score	Genes
GW-5074		0.041993	9.376465	94.5569	*GAK;MAP2K4;RPS6KA1;KIT;RAF1;JAK1*
Valproic acid		0.029399	3.796008	38.09505	*SRPK2;GABARAPL2;SEC24B;NRGN;HERC5;NXF1;MKNK1;CALM3;CALM1;WASF1;MYBL1;PCYOX1;HSPA1A*
Lycorine		0.017063	2.643249	32.70069	*ICAM3;PRKX;RASGRP2;SLC2A5;NRGN;HERC5;SLC9A3R1;LASP1;RPS6KA1;IDS;CSK;FAM65A;MYBL1;S100PBP;ARHGEF10;SERPINB1;ICMT;SEMA4D;TREX1;HPCAL1;NFATC1;ARHGEF9;XPOT;CHI3L1;PTPRCAP;PCYOX1;HSPA1A*
Aspirin		0.034434	2.858067	28.75161	*CD74;SERPINB1;G6PD;B4GALT1;MX1;ICAM3;CHD2;CDC25A;CDC42;POLR2A;AKT2;C9ORF16;CALM3;TIMP1;PTPRCAP;CALM1;RAF1;IL7R;HSPA1A*

healthy individuals. Based on PPI results, UBC protein (Polyubiquitin-C) could be considered as the major hub protein in this network. Nevertheless, according to the results obtained by gene expression analysis, UBC has not shown any significant change among infected versus persons, while RAF1 (proto-oncogene serine/threonine-protein kinase) has shown the highest degree among genes or proteins with the LogFC=-1.38. PPI network of genes (with the first 12 hub proteins) has been shown in Figure 1.

3.3.2. PDI Network

PDI network (Figure 2) was constructed based on genes-drug molecules interaction and significant interactions have shown that three genes could be considered as candidates for further investigations to find suitable drugs. These genes include NCOA1, PRKACA and AKT2.

4. Discussion

The outbreak of COVID-19 as the result

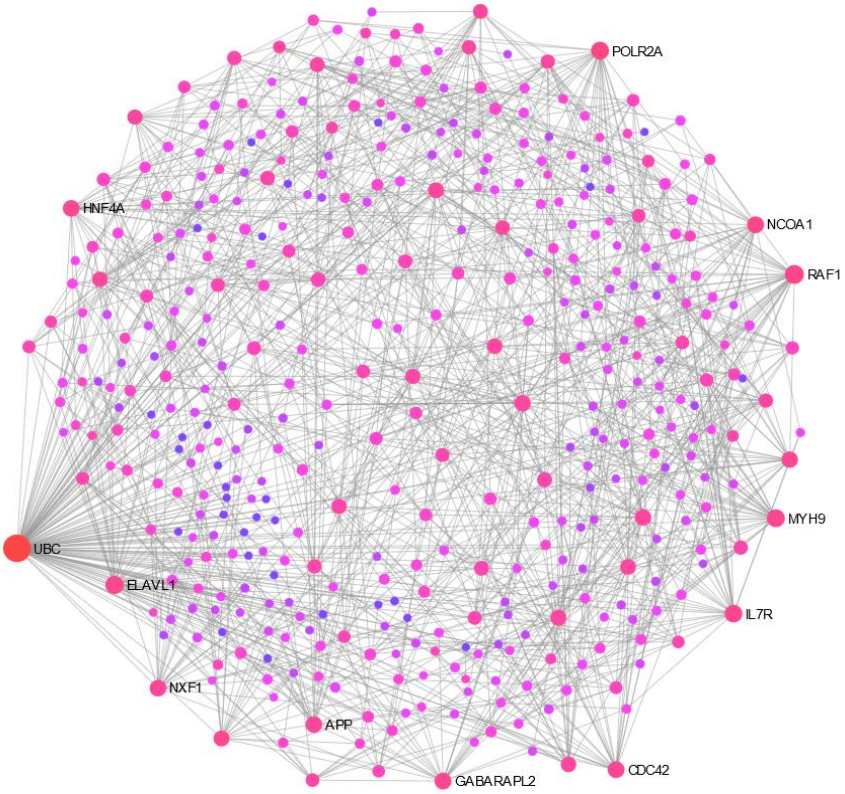


Figure 1. PPI Network of gene expression analysis.

of the rapid spread of the SARS-CoV-2 virus has led researchers to seek novel drug molecules as well as the repurposing of existing drugs for the virus. In order to suggest existing drugs for the control, treatment or prevention of COVID-19, the genomics or proteomics data of SARS-CoV-2 is not available yet. Therefore, we decided to use the microarray data obtained from the patients and convalescents following the infection with SARS-Cov. The up-regulated and down-regulated genes in these individuals were selected and associated with the metabolic pathways inside the cells. At

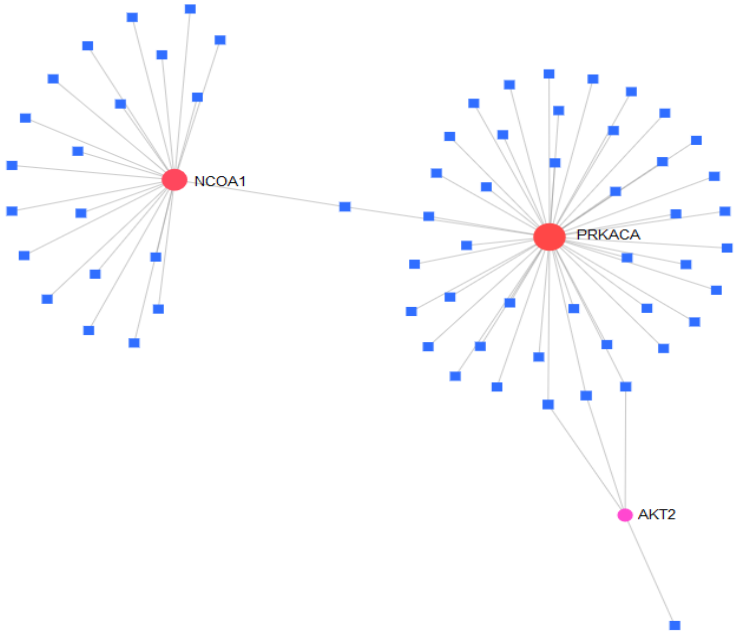


Figure2. PDI Network.

the next step, the potential drug candidates were suggested based on DSigDB Analysis. The results revealed that four compounds could be considered as potential material to target the pathways with the highest importance in the patients infected with SARS-CoV. The first compound was named GW-5074 since this is not an approved drug neither for animal nor human applications. This compound has been synthesized as a potent inhibitor of Raf-1 with high selectivity versus the other similar kinases (23). Raf-1 is a proto-oncogene serine/threonine protein kinase that transfers the signals from Ras to the MAPK/ERK signalling pathway. This pathway has shown basic functions in the cells, including differentiation, proliferation and cell survival. The selective Raf inhibition for cancer therapy has lead researchers to find Raf inhibitors as potent anticancer drugs such as sorafenib. Although the other Raf- inhibitors did not suggested by DSigDB Analysis, the potential of these drugs might not be neglected. Interestingly, there are some reports indicating the anti-viral activity of GW-5074. For example, Chen Hwang and the colleagues showed the antiviral activity of this compound against poliovirus (24). The anti poliovirus activity of this material has also been demonstrated in other studies (25). In another investigation by Yan et al., the potent antiviral activity of GW-5074 was demonstrated. This antiviral activity was not only against the dengue virus (DENV) but also against the zika virus and West Nile virus (26). Recently, the broad antiviral activity of GW-5074 against various different viruses has been demonstrated. Therefore, GW-5074 could be considered as a hit compound for additional investigations.

In this study, lycorine was also suggested as a potential compound that may influence the pathways associated with SARS disease. The previous investigations also have shown the potent antiviral activity of lycorine. In an investigation conducted by Li and colleagues, 200 Chinese medicinal herb extracts were evaluated for their potential as antiviral agents against SARS. The study led to the identification of lycorine as a potent anti-SARS-Cov with an EC50 value of 15.7 ± 1.2 nM, CC50 value of 14980.0 ± 912.0 nM and a selective index (SI) greater than 900 (27). Also, The replication of human enterovirus 71-which causes hand, foot and mouth disease in children could be reduced by lycorine (28). The anti-hepatitis C virus (HCV) activity of lycorine-derived phenanthridine has been shown in some investigations (29).

Altogether, it seems that lycorine and GW-5074 might be considered as hit compounds for further investigations towards finding potent drugs against COVID-19.

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Conflict of Interest

None declared.

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