A protein interaction map identifies existing drugs targeting SARS-CoV-2

Claudia Cava (✉ claudia.cava@ibfm.cnr.it)

  Institute of Molecular Bioimaging and Physiology, National Research Council (IBFM-CNR), Via F.Cervi 93, 20090 Segrate-Milan, Milan, Italy   https://orcid.org/0000-0002-5540-4104

Gloria Bertoli

  Institute of Molecular Bioimaging and Physiology, National Research Council (IBFM-CNR), Via F.Cervi 93, 20090 Segrate-Milan, Milan, Italy   https://orcid.org/0000-0002-5901-4269

Isabella Castiglioni

  Department of Physics "Giuseppe Occhialini", University of Milan-Bicocca Piazza dell'Ateneo Nuovo, 1 - 20126, Milan, Italy   https://orcid.org/0000-0001-7191-5417

Research Article

Abstract

Background: Severe acute respiratory syndrome coronavirus (SARS-CoV-2), an emerging Betacoronavirus, is the causative agent of COVID-19. Angiotensin converting enzyme 2 (ACE2), being the main cell receptor of SARS-CoV-2, plays a role in the entry of the virus into the cell. Currently, there are no specific antiviral drugs for the treatment and neither preventive drugs such as vaccines.

Results: We proposed a bioinformatics analysis in order to test in silico existing drugs as a fast way to identify an efficient therapy. We found differentially expressed genes in COVID-19 patients correlated with ACE-2 and we explored their direct relations with a network approach.

Conclusions: In this network we integrated drug-gene interactions and we proposed several drugs with antiviral activity that, alone or in combination with other treatment options, could be considered as therapeutic approaches against COVID-19.

Introduction

Coronaviruses are a group of single-stranded RNA viruses with a wide range of vertebrate hosts. Infection with these viruses can cause acute respiratory syndrome, which may lead to damages in lung function, pneumonia, arrhythmia, or death [1].

Betacoronavirus (β-CoV) are the second of four genera of coronaviruses: alpha, beta, gamma and delta. Over the past two decades, three highly pathogenic human betacoronaviruses have spread from animals to humans: Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) in 2002–2003, MERS-CoV in 2012 and SARS-CoV–2 in 2019. Compared to SARS-CoV or MERS-CoV, SARS-CoV–2 spreads more rapidly making it difficult to control [1].

Recently, a first model of infection has been proposed [2] in which after an initial phase of viral exposure, the local innate immune response generates natural IgA and IgM antibodies. In this first stage (5–6 days after infection) patients show their first symptoms limited to upper airways (cough, sore throat) with fever, fatigue and muscle ache. If SARS-CoV–2 (the virus of COVID–19) blocks the innate immunity, it spreads initially from the upper airways to the alveoli, causing pneumonia and dyspnea, and releasing high amounts of viral particles. While many subjects remain asymptomatic or with mild symptoms, in other individuals the response of adaptive immunity, releasing IgM and IgG antibodies, causes severe symptoms such as acute respiratory distress syndrome (ARDS), due to spreading of inflammation, often leading to complications (dyspnea, respiratory failure, fulminant myocarditis) that require the use of intensive therapy. In the last phase, the COVID–19 could cause death due to ARDS, acute cardiac and kidney injury, sepsis, and secondary infections. High mortality has been observed in patients with older age or comorbidities (hypertension, diabetes, cardiovascular disease, chronic lung disease and cancer).

By studies on SARS-CoV, the first site of infection seems to be pneumocytes and enterocytes of small intestine as well as type II pneumocytes, enterocytes and macrophages [3]. The molecular mechanisms

of SARS-CoV–2 entry, as suggested also for SARS-CoV [4], is mediated by the binding of the viral spike glycoprotein to the angiotensin-converting-enzyme–2 (ACE2) receptor, in collaboration with a serine protease, TGRBSS2. This allows the viral capsid fusion with the cell membrane [4].

SARS-CoV–2 is a new virus for our immune system. The IgG antibodies produced by other β-CoV do not recognise this SARS-CoV–2, making this virus currently invincible. A possibility would be the use of immunoglobulin collected from immunized COVID–19 patients to be injected in patients suffering from COVID–19 [5]. Another possibility could come from the in silico test of already existing antiviral drugs, a method that could allow to test a large amount of possible therapies in silico very quickly with very low costs., Furthermore, immunotherapy approach combined with antiviral drugs detected by in silico analyses may be a stronger treatment until definitive options such as vaccines are available.

In this work we propose an in silico study to identify genes deregulated in COVID–19 positive patients correlated with ACE2. Then, we performed a network analysis based on protein-protein interactions with the aim of identifying existing drugs targeting SARS-CoV–2 deregulated genes, to be proposed as candidate drugs for COVID–19, alone or in combination with other therapeutic strategies. The protein interactions that emerge from our study could help deciphering their mechanism of action with respect complex biological processes at the basis of COVID–19 infection and progression.

Material And Methods

Gene expression datasets

We collected the gene expression levels of lung tissue infected by SARS-CoV–2 from the Gene Expression Omnibus (GEO) database. Specifically, we selected from GSE147507 2 lung biopsies from postmortem COVID–19 positive male patients and 2 lung biopsies from COVID–19 negative male controls.

Furthermore, from the Genotype-Tissue Expression (GTEx) project we collected gene expression levels of lung tissue from healthy subjects (320 healthy volunteers).

Differential expression, pathway and network analysis

Differential expression analysis was performed on GSE147507 gene expression levels with the R-package TCGABiolinks [6]. We normalized and filtered RNA-seq raw counts using the reference of hg19, following the pipeline of the R/ Bioconductor package TCGAbiolinks [6]. We defined the differentially expressed genes if FDR<0.01 and |log.FC|>1.

Pathway enrichment analysis was performed with the R-package clusterProfiler [7]. In particular, we performed an enrichment test for KEGG pathways based on hypergeometric distribution [8].

We generated a protein-protein interaction network considering the direct connections among differentially expressed genes using SpidermiR [9] and we selected the network with the most connected

nodes. Drug-gene interactions were derived by the package rDGIdb [10].

Correlation

A correlation analysis was performed between ACE2 and the other genes in GTEx data (lung tissues from normal volunteers) to obtain the genes co-expressed with ACE2. The corresponding p-values of the Pearson's correlation were considered and only ACE2 and genes significantly correlated (p-value < 0.001) were used for the subsequent analysis.

Community detection

We implemented the fast greedy modularity optimization algorithm for finding community structure of our network [11]. Indeed, a network can be divided into communities and each community is characterized by many edges within communities and only a few between communities.

Molecular modelling: ligands and protein structures preparation

Docking study explored the binding mode of reported drugs on the 3D model of protease of SARS-CoV−2.

Based on the above analysis, the structural coordinates of potential drugs were downloaded from PubChem [12].

Ligand preparation was performed using DiscoveryStudio [13].

Crystal structure of SARS-CoV−2 main protease (PDB IDs: 5R7Y, 5R7Z, 5R80, 5R81 and 5R82) were obtained from Protein Data Bank. Polar hydrogen atoms were added, and water molecules were removed to the SARS-CoV−2 model.

Molecular modelling is performed using AUTODOCK 1.5.6 [14].

Results

Differential expression and pathway analysis reveal novel aspects of SARS-CoV−2 biology

Our study revealed 1269 differentially expressed genes between lung biopsies from postmortem COVID−19 positive patients and lung biopsies from negative controls (Supplementary 1). Volcano plot was designed to visualize the distribution of each gene related to the fold change and p-value (Figure 1A).

We found 22 pathways enriched with 1269 differentially expressed genes. The top differentially expressed mRNAs were associated with Alzheimer's disease, human T-cell leukemia virus 1 infection, Shigellosis, hepatocellular carcinoma, viral carcinogenesis, apoptosis, non-alcoholic fatty liver disease (NAFLD), mTOR signaling, cellular senescence, protein processing in endoplasmic reticulum and cell cycle.

Differentially expressed genes in COVID−19 are correlated with ACE2

From the correlation analysis in GTEx data we obtained 11011 genes that are correlated with ACE2. We selected differentially expressed genes in COVID−19 that are also correlated with ACE2 in GTEX data: 65% of differentially expressed genes (825 genes out of 1269) were found.

Protein-protein interaction reveals existing drugs targeting SARS-CoV−2

We studied the interactions among differentially expressed genes correlated with ACE2. From the analysis of protein-protein interaction network we obtained a network of 474 genes and 1130 interactions. We queried DGIdb and we obtained 714 drugs that interact with 119 of 474 genes involving 950 interactions.

We integrated the protein-protein interactions with the drug-gene interactions obtaining a network of 1188 nodes (including 474 genes and 714 drugs) and 2080 interactions. The overview of such network is shown in Figure 2A.

The drugs with the highest degree centrality (d.c.) are: CHEMBL1161866 (d.c. = 11), bortezomib (d.c. = 6), puromycin (d.c. = 6), metformin hydrochloride (d.c. = 6), NV−128 (d.c. = 6), ME−344 (d.c. = 6), methylene blue (d.c. = 5), anisomycin (d.c. = 5) and everolimus (d.c. = 5) (Figure 2B).

The proteins with the highest degree centrality are: ERBB2 (d.c. = 146), APP (d.c. = 143), SUMO2 (d.c. = 106), CUL3 (d.c. = 89), CA2 (d.c. = 72), GSK3B (d.c. = 61), HSPA4 (d.c. = 60), NRAS (d.c. = 53) and MTOR (d.c. = 50) (Figure 2C).

Moreover, communities detection algorithm identified 19 communities (Figure 2D), the biggest communities consisting of 239, 175 and 174 nodes (see Figure 2D)

In the first community we found 3 genes with the highest degree centrality (d.c.): CUL3 (d.c. = 54), SUMO2 (d.c. = 53), and APP (d.c. = 41). The drugs with key roles in the network are 7: bortezomib, puromycin, ixazomib citrate, carfilzomib, oprozomib, methylene blue and anisomycin. The drugs with the major

number of drug targets in the network are: puromycin, methylene blue and anisomycin. They interact with 5 proteins: RPL11, RPL15, RPL26L1, RPL37 and RPL8 (Figure 3A).

In the second community we found 3 genes with the highest degree centrality (d.c.): ERBB2 (d.c. = 25), NRAS (d.c. = 22) and RB1 (d.c. = 10). The drugs involved in this community are 27: metformin, abemaciclib, ribociclib, palbociclib, pictilisib, fluorouracil, trametinib, vorinostat, sirolimus, entinostat, everolimus, cetuximab, crizotinib, sorafenib, afatinib, ethyl nitrosourea, gefitinib, osimertinib, mk−2206, buparlisib, gedatolisib, bgb−283, panitumumab, selumetinib, chembl1229592, vemurafenib and dabrafenib. The drug with the major number of drug targets in the network is palbociclib, and it interacts with 4 proteins: CCND3, ERBB2, NRAS and RB1 (Figure 3B).

In the third community we found 5 genes with the highest degree centrality (d.c.): PARP1 (d.c. = 16), PCNA (d.c. = 14) SMAD4 (d.c. = 14), CDK8 (d.c. = 14) and SMAD2 (d.c. = 14). The drugs involved in this community are 17: veliparib, olaparib, rucaparib, niraparib, talazoparib, talazoparib tosylate, rucaparib camsylate, camptothecin, temozolomide, azacitidine, lomustine, gemcitabine, roniciclib, irinotecan, PHA−793887, AT−7519 and AZD−5438. The drug with the major number of drug targets in the network is camptothecin. It interacts with 4 proteins: MAPK9, HUS1, MGMT and TOP1 (Figure 3C).

Molecular Docking

Docking study was performed on 9 potential drugs (CHEMBL1161866, bortezomib, puromycin, metformin hydrochloride, NV−128, ME−344, methylene blue, anisomycin, and everolimus) against SARS-CoV−2. 2 drugs (Puromycin and anisomycin) obtained a negative binding energy value as shown in the Table 1.

Puromycin showed promising results in 3 out of 5 protein structures of SARS-CoV−2 with a binding energy lower than −6.4. Anisomycin was found to interact with 2 out 5 protein structures of SARS-CoV−2 with a binding energy lower than −6.

Table 1. Binding energy of two drugs identified in the docking study

Ligand	Binding Energy kcal/mol				
	5R7Y	5R7Z	5R80	5R81	5R82
puromycin	-5.3	-6.59	-6.41	-5.62	-6.84
anisomycin	-5.72	-6.22	-5.77	-6.17	-5.47

Docking interactions of puromycin with PDB ID 5R82 and anisomycin with PDB ID 5R7Z are presented in Figure 4.

Discussion

Our in silico study highlighted protein interactions between genes differentially expressed in COVID−19 patients with respect to negative controls. Such proteins are involved in multiple complexes and biological processes including cell cycle, immune regulation, transcription and protein trafficking. Against these proteins, following in silico tests, we found several drugs, including FDA approved components as potential treatments for COVID−19.

Pathway analysis

We found 22 pathways enriched with 1269 differentially expressed genes between lung biopsies from postmortem COVID−19 patients and lung biopsies from negative controls. Intriguing, the top differentially expressed mRNAs were found associated with Alzheimer's disease.

The risk of contracting COVID−19 in patients with Alzheimer disease (AD) is higher. This is due to their difficulty in following the recommendations from public health authorities and the difficulty of maintaining isolation during behavioural and psychological symptoms of dementia. In addition, patients with AD often have age and comorbid medical conditions associated with a poor prognosis and mortality from COVID−19 [15]. Few publications are currently available on a direct connection between the pathology of AD and COVID−19. From the little we know in the inflammatory response triggered by SARS-CoV−2, during this infection lymphopenia is observed, with loss of CD4 + and CD8 + T cells, hyperproduction of IL6, IL10, IL2R, TNFa and CCL2 [16]. The devastating effect of the cytokine storm on the respiratory system is already known. Less known is the effect of this inflammatory state on the nervous system. Chronic neuroinflammation associated with high levels of cytokine/chemokines has been associated with the pathophysiology of some neurodegenerative diseases (multiple sclerosis, Parkinson's disease, Alzheimer's disease, Alzheimer's disease, Huntington's disease or amyotrophic lateral sclerosis) [17]. In the case of AD, it has been described that microglia cells lose the ability of degrading Aβ protein in the presence of proinflammatory cytokines (mainly IL1 or IL6), leading to the pathogenic deposits of this protein in the brain [18]. It is thus possible that the cytokine storm produced by SARS-CoV−2 infection could worsen the status of AD patients.

Among the top differentially expressed mRNAs we also found mRNAs associated with human T-cell leukemia virus 1 infection, Shigellosis, hepatocellular carcinoma, viral carcinogenesis, apoptosis, non-alcoholic fatty liver disease (NAFLD), mTOR signaling, cellular senescence, protein processing in endoplasmic reticulum and cell cycle.

Chronic lymphocytic leukemia (CLL) is the most common adult leukemic disorder in the Euro-American population. It is caused by human retrovirus named human T lymphotropic viruses 1 (HTLV−1). HTLV−1 is very similar and relevant to human coronavirus (HCoVs) in animal-to-human transmission event. Although it is not yet fully known how SARS-CoV−2 interacts with host antiviral immunity, similar mechanisms are found from other HCoVs and human pathogenic viruses in other families that are very similar including HTLV−1 and the human immunodeficiency viruses (HIVs) [19]. Several therapeutic options are currently at hand for the first-line or relapsed/refractory CLL, including combination of drugs

(i.e., bendamustine, alemtuzumab, ofatumumab) with corticosteroids. Corticosteroids are frequently used to treatment persons with these coronavirus infections in order to counteract high interleukin−6 concentrations, although some studies are still uncertain about the real efficacy and benefit of the use of corticosteroids in COVID−19 patients [20].

Virus infection alters the gut microbiome in humans and makes the organism more susceptible to intestinal bacterial infections such as Shigellosis. Shigellosis is an infectious intestinal disease, also called bacillary dysentery, caused by the genus Shigella spp. The expression of ACE2 is abundant in the epithelia of the lungs as well as in intestinal epithelial cells. In line with this scenario, COVID−19 may be associated with gut microbiota [21]. The connection between gastrointestinal tract and the respiratory tract, takes place through shared mucosal immune system which could justify the main symptoms of COVID−19: pneumonia and digestive infections [22,23].

In addition to the lung and intestine, several evidences showed possible implications of hepatic involvement in SARS-Cov−2 infection. Patients with liver diseases, including hepatocellular carcinoma and Non-alcoholic fatty liver disease (NAFLD) (a metabolic disorder due to the accumulation of fat in the liver), can be more susceptible to the serious consequences of the COVID−19. This could be due to reduced innate immunity to the virus, as the liver plays crucial role in innate and adaptive immunity. As previously reported, the expression of ACE2 is abundant also in the epithelia of intestine, and as there is a rich circulation of blood from the intestine to liver, the spread of the virus in the liver is expected. Reduced hepatic innate immune status could contribute to progression of COVID−19 [24−26].

The mammalian target of rapamycin (mTOR) signaling pathway is a conserved serine/threonine kinase and promotes the cellular proliferation, protein synthesis and DNA replication. mTOR has already been associated with the development of influenza by promoting influenza virus replication [27]. The antiviral properties of mTORs inhibitors have been attributed to a variety of processes. Virus-infected cells initiate the stress response by promoting autophagy to destroy the infecting organism or by activating apoptosis to reduce virus spread. Since mTOR signaling pathway not only inhibits apoptosis but also reduces stress-activated autophagy, mTOR activation could have also a role in vaccine production to enhance virus replication [28,29].

Apoptosis, cellular senescence, protein processing in endoplasmic reticulum and cell cycle are all processes that are altered as consequence of the virus infection. The infected cell promotes an activation of apoptosis and cell senescence, and an inhibition of protein synthesis and cell cycle in order to remove the infecting organism and reduce the spread of the virus.

Antiviral proprieties of existing drugs targeting SARS-CoV−2 genes

We investigated the role of existing drugs in the protein network finding the drugs with the highest degree centrality in the network:CHEMBL1161866 (d.c. = 11), bortezomib (d.c. = 6), puromycin (d.c. = 6),

metformin hydrochloride (d.c. = 6), NV−128 (d.c. = 6), ME−344 (d.c. = 6), methylene blue (d.c. = 5), anisomycin (d.c. = 5) and everolimus (d.c. = 5) (Figure 2B).

CHEMBL1161866 is an aminopurine drug with effects on liver alcohol dehydrogenase (ADH) and glutamate dehydrogenase, suggesting a possible beneficial application of this drug on liver infection by SARS-CoV−2. ADH enzymes, highly expressed in liver and colorectal tissues, catalyzed the conversion of alcohol to aldehydes during alcohol metabolism. In particular, ADH could have a role in hepatitis B virus (HBV) and hepatitis C virus infection, being the increased activity of ADH enzymes a clinical marker of liver injury [30]. Although a clear connection between COVID−19 and CHEMBL1161866 has been not proven yet, we could not exclude a possible beneficial effect of this drug on the COVID−19 patients.

Bortezomib (also known as velcade), a proteasome inhibitor approved against multiple myelomas, showed in several studies its antiviral proprieties. Proteasome inhibitors are encouraging antiviral agents that could inhibit viral entry. The ubiquitin-proteasome system (UPS) is involved in different steps of a broad range of viruses' replication cycle, including coronavirus. The UPS regulates many different processes, such as the cell cycle progression and apoptosis. In addition, ubiquitination is involved in the host immune response to viral infection regulating antigen presentation [31−33].

Recent studies suggested that a drug already approved for use against diabetes, metformin, may be effective against COVID−19. Metformin inhibits glucose production decreasing intestinal glucose absorption and enhancing glucose uptake by peripheral tissues. People with diabetes mellitus (DM) have poor prognosis and increased fatality in a wide range of viral infections, including SARS and MERS. The link between COVID−19 and DM could be explained by the use of angiotensin-converting enzyme inhibitors (ACEi) and angiotensin-receptor blockers (ARBs) in people with DM. Indeed, ACEi/ARBs act upregulating the receptor for entry of the virus into host pneumocytes. However, Metformin does not interfere with ACE2, and it is not clear how it could act on patients' outcome [34,35].

Methylene blue is a fluorescent dye used for nucleic acid stain. It has been approved by the Food and Drug Administration (FDA) as treatment of methemoglobinemia. Due to its antimicrobial, anti-inflammatory, and antioxidant effects, Methylene blue has been successfully utilized in vitro. Its antiviral proprieties have been attributed to its ability to intercalate into nucleic acids and inactivate RNA viruses. Virus inactivation with Methylene blue was applied in plasma to mitigate the risk of transmission by transfusion in human immunodeficiency virus (HIV), hepatitis B virus, hepatitis C virus and Zika virus [36,37].

Anisomycin was originally defined as an antibiotic against protozoa and fungi. The antiviral effect of anisomycin was demonstrated in cell cultures of Dengue and Zika viruses, mainly involving viral macromolecular synthesis [38]. In addition, anisomycin was reported to inhibit the animal picornavirus encephalomyocarditis virus [39] and to suppress in vitro replication of poliovirus [40] and flavivirus Japanese encephalitis virus [41].

Everolimus is an mTOR inhibitor with well-known antitumor activity in advanced cancer, including kidney and breast cancer. Everolimus showed also in vitro antiviral effects against influenza A virus: in a lethal mouse model of MERS, it delayed death and reduced MERS-CoV infection by ~ 60%. It has also been correlated with a reduced frequency of cytomegalovirus infection in transplant patients [42–44].

Puromycin is an antibiotic able to inhibit protein synthesis. Although protein inhibition could be obtained by puromycin treatment, in cornea C-M virus-infected cells as well as in HeLa cells infected with Newcastle disease virus the viral particle continues to be produced due to viral protein intracellular accumulation. In this way, viral particles could be assembled, also in the absence of continuous protein synthesis [45]. It seems that puromycin could be effective only in the initial phases of a viral infection, when the viral protein is necessary to produce the viral particles for the initial virus spreading in the body. Although a clear connection between COVID−19 and puromycin is not reported, we could not exclude a possible beneficial effect of this drug on the COVID−19 patients.

There is not also a clear connection between NV−128, ME−344 and COVID−19, but we cannot exclude a future possible implication of these drugs in COVID−19 patients.

The docking studies estimated that two drugs (puromycin and anisomycin) have potential characteristics of binding to SARS-CoV−2.

Conclusions

This study identified, in silico, a group of proteins, drug targets of conventional treatments, within multiple complexes and biological processes that could represent effective mechanism of therapeutic actions also for SARS-CoV−2 infection. The protein interactions that emerge from our analysis highlight such drugs as actionable proteins of SARS-CoV−2-specific deregulated gene network, they could be proposed with a rational against the virus, alone or in combination with other therapies.

List Of Abbreviations

acute respiratory distress syndrome (ARDS)

angiotensin-converting-enzyme−2 (ACE2)

Gene Expression Omnibus (GEO)

Genotype-Tissue Expression (GTEx)

Degree centrality (d.c.)

Alzheimer disease (AD)

human coronavirus (HCoVs)

Chronic lymphocytic leukemia (CLL)

human T lymphotropic viruses 1 (HTLV−1)

mammalian target of rapamycin (mTOR)

diabetes mellitus (DM)

angiotensin-converting enzyme inhibitors (ACEi)

angiotensin-receptor blockers (ARBs)

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets supporting the conclusions of this article are available in the Gene Expression Omnibus repository and Genotype-Tissue Expression (GTEx) project, [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE147507, https://gtexportal.org/home/]

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

CC has made substantial contribution to the acquisition, analysis and interpretation of data. GB has made substantial contribution to the interpretation of data. IC has revised the manuscript.

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Figures

A)

B)
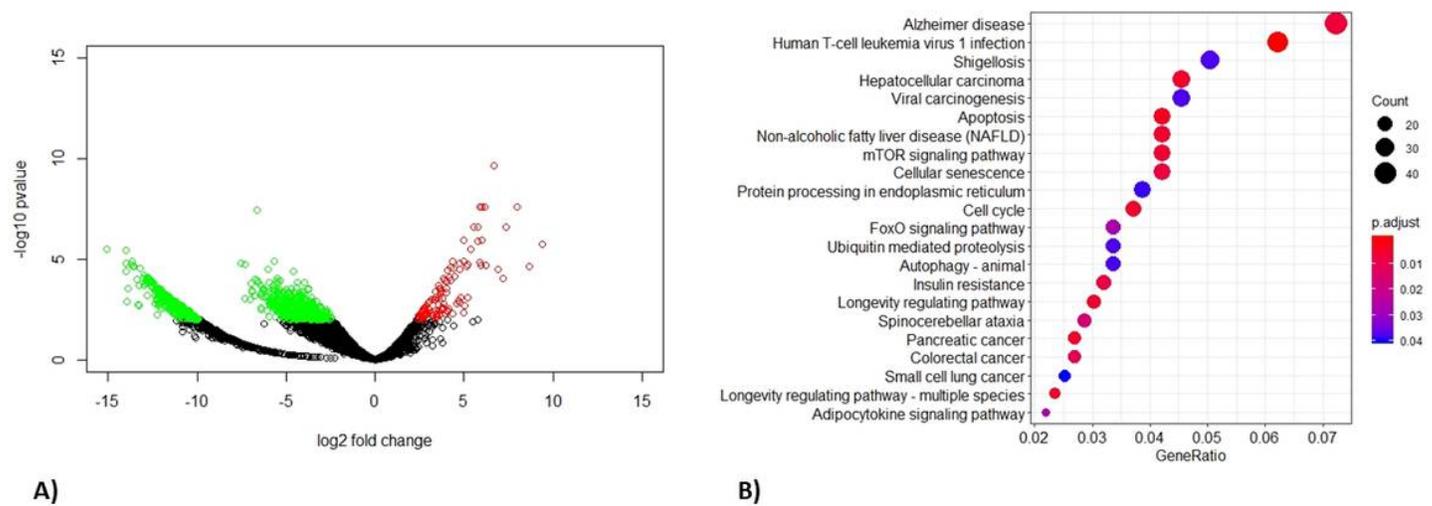
Figure 1

A) Volcano plot for differential gene expression. The dots represent the genes that are related to p-value versus fold change. Red dots are genes that are significantly up-regulated in Covid-19, and green dots are genes significantly down-regulated in Covid-19. B) Top enriched KEGG pathways. The size of the circles represents the number of differentially expressed gene in the pathway. The color intensity of the circle represents the p-value.
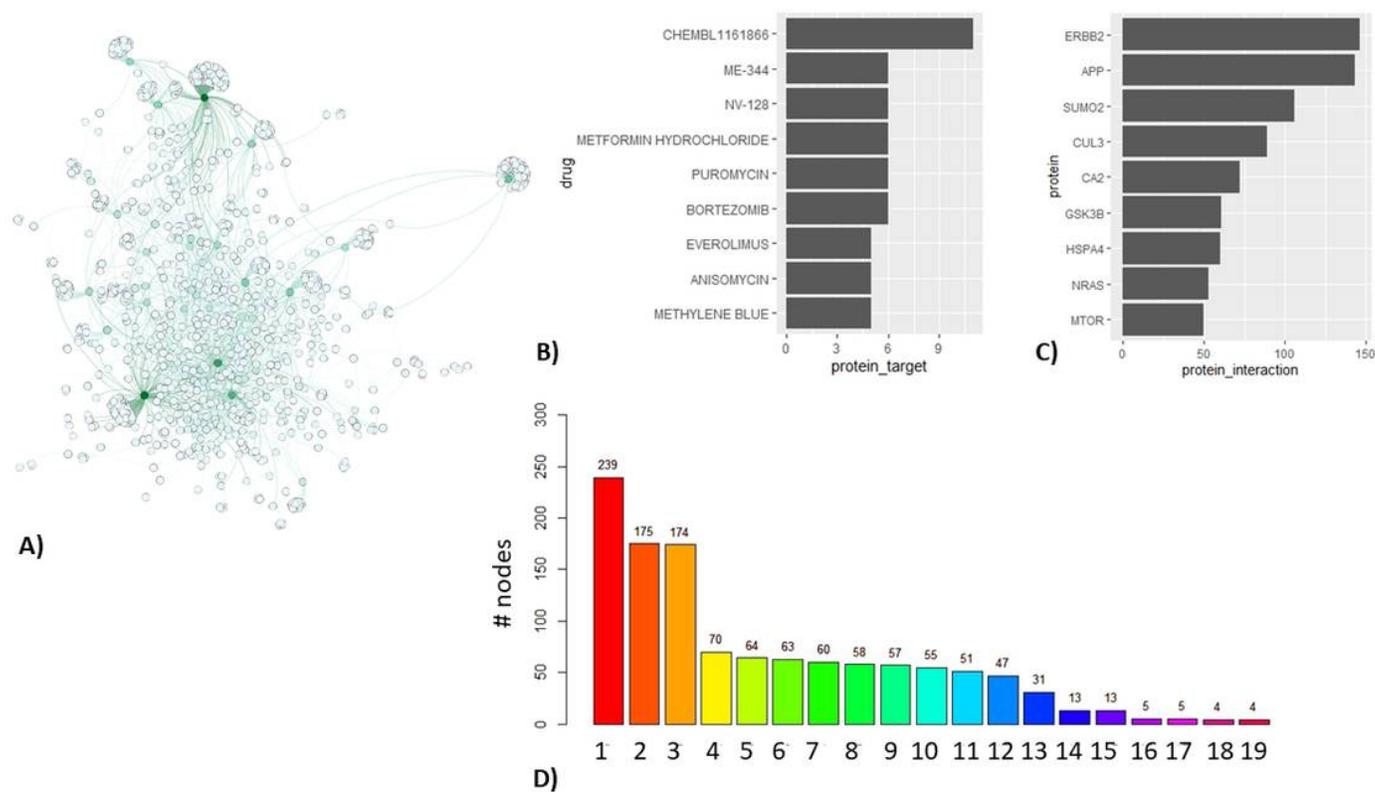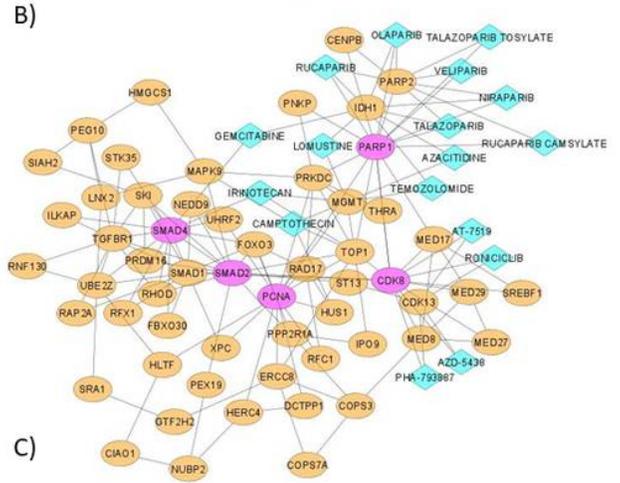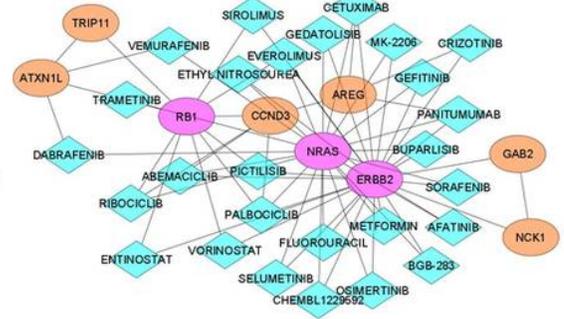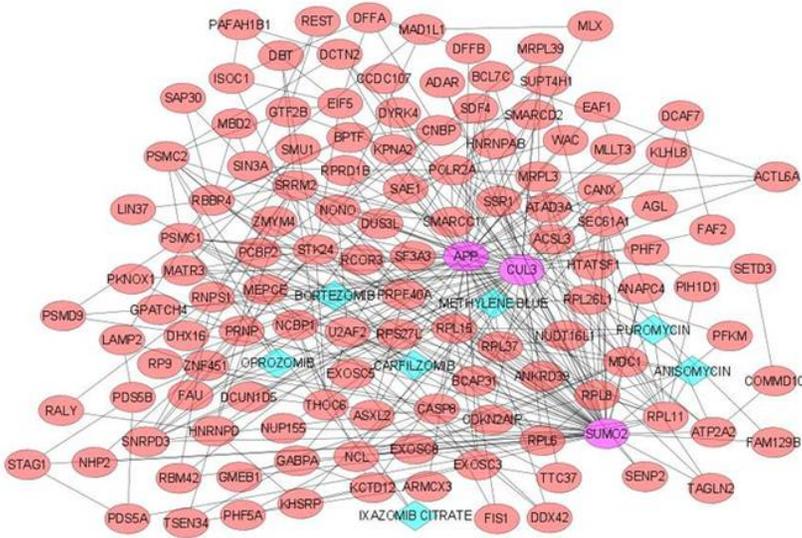
Figure 2

A) Protein-protein and drug-protein interactions consisting of 474 proteins and 714 drugs. Intensity colour of nodes represents the degree centrality. B) Drugs with the highest degree centrality in the network. C) Proteins with the highest degree centrality in the network. D) Barplot indicating the number of nodes for each community.

Figure 3

Community detection. The figure shows the biggest communities identified A) first community, B) second community, C) third community. Proteins with higher degree centrality are represented with the purple circles. Drugs are represented with light-blue triangle (we removed the nodes that have only one connection).
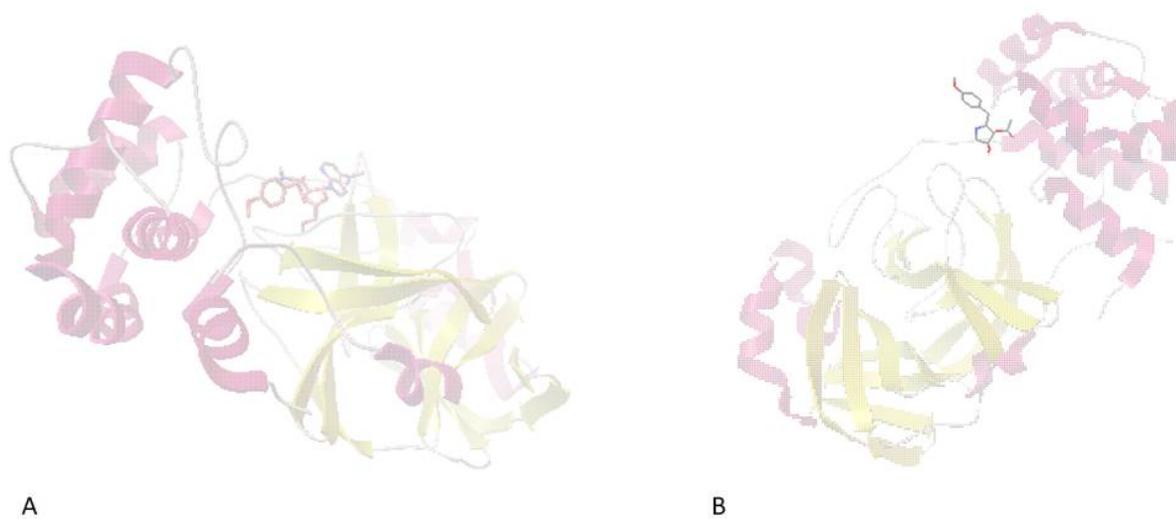
Figure 4

Docking interactions of A) puromycin with PDB ID 5R82 and B) anisomycin with PDB ID 5R7Z.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- tableDEAGSE147507.txt