RESEARCH ARTICLE

Transcriptome-based drug repositioning for coronavirus disease 2019 (COVID-19)

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One sentence summary: Transcriptome-based drug repositioning for COVID-19 recovered two antiviral drugs and identified several candidate drugs, including saquinavir, ribavirin, dinoprost and dexamethasone.

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ABSTRACT

The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) around the world has led to a pandemic with high morbidity and mortality. However, there are no effective drugs to prevent and treat the disease. Transcriptome-based drug repositioning, identifying new indications for old drugs, is a powerful tool for drug development. Using bronchoalveolar lavage fluid transcriptome data of COVID-19 patients, we found that the endocytosis and lysosome pathways are highly involved in the disease and that the regulation of genes involved in neutrophil degranulation was disrupted, suggesting an intense battle between SARS-CoV-2 and humans. Furthermore, we implemented a coexpression drug repositioning analysis, cogena, and identified two antiviral drugs (saquinavir and ribavirin) and several other candidate drugs (such as dinoprost, dipivefrine, dexamethasone and (-)-isoprenaline). Notably, the two antiviral drugs have also previously been identified using molecular docking methods, and ribavirin is a recommended drug in the diagnosis and treatment protocol for COVID pneumonia (trial version 5–7) published by the National Health Commission of the P.R. of China. Our study demonstrates the value of the cogena-based drug repositioning method for emerging infectious diseases, improves our understanding of SARS-CoV-2-induced disease, and provides potential drugs for the prevention and treatment of COVID-19 pneumonia.

Keywords: SARS-CoV-2; COVID-19; drug repositioning; neutrophil degranulation; saquinavir, ribavirin

INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread for several month since December 2019. The SARS-CoV-2 virus is a betacoronavirus, which includes Middle East respiratory syndrome CoV (MERS-CoV) and severe acute respiratory syndrome CoV (SARS-CoV). SARS-CoV-2 shares 95% sequence identity with a bat coronavirus, indicating that bats are likely the reservoir hosts for the virus (Zhou et al. 2020) and pangolins are possible intermediate hosts for SARS-CoV-2 (Lam et al. 2020). Both SARS-CoV-2 and SARS-CoV use angiotensin-converting enzyme II (ACE2) as the cell entry
The BALF of 8 COVID-19 patients (COVID) and healthy controls were used to extract RNA and prepare a library for RNA sequencing. Differentially expressed genes (DEGs), consisting of 872 genes with upregulated expression and 773 genes with downregulated expression, were identified using the filterByExpr function in the Bioconductor edgeR package. The enrichment score is the negative log2 of the adjusted p-value less than 0.05 to obtain differentially expressed genes (DEGs). Principal components analysis (PCA) was used to visualize the differences between the two groups. The raw read counts were filtered to identify low-expressed genes using the filterByExpr function in the Bioconductor edgeR package. The KEGG pathway analysis, Reactome pathway analysis and computational drug repositioning analysis were performed to identify drug candidates for COVID-19. The enrichment score is the negative log2 of the adjusted p-value less than 0.05 to obtain differentially expressed genes (DEGs). Principal components analysis (PCA) was used to visualize the differences between the two groups. The use of lower respiratory tract specimens, such as the BALF, is important to explore the mechanisms of disease development, as the disease mainly affects the lower respiratory tract, especially the lungs. To identify the key genes affected by COVID-19, we performed a transcriptomics differential expression analysis and identified a total of 1569 differentially expressed genes (DEGs), consisting of 872 genes with upregulated expression and 697 genes with downregulated expression. A heatmap of these DEGs with hierarchical clustering of both the samples and genes indicated that the two groups differed from each other, as the COVID samples closely clustered and the healthy controls were more dispersed.
Figure 1. General analysis of COVID-19-induced DEGs. (A) The first two dimensions of the principal components analysis for the DEGs of COVID-19. PC1 and PC2 are principal components 1 and 2, respectively. (B) Heatmap and hierarchical clustering of DEGs. C1-C8 represent the samples of COVID-19 patients, and H1-H20 represent healthy controls. The values are shown as the normalized gene expression. (C) Correlation between all the samples. The size of the circle represents the absolute Pearson correlation coefficient, and color indicates the direction of the correlation.

healthy samples clustered together (Fig. 1B). The Pearson correlation between samples also presented high consistency within each group, whereas there was a marked difference between the two groups (Fig. 1C). As a result, the identified DEGs are representative of COVID.

Functional analysis of coexpressed genes

Coexpressed genes usually cooperate to implement similar functions, which is a specific way to connect key genes among DEGs with diseases and could provide an important clue to understand the pathogenesis of a disease. Three clusters of coexpressed genes were obtained via a divisive hierarchical clustering method, DIANA (Figure S3 and Table S2, Supporting Information). Compared with that of genes in the healthy group, the expression of coexpressed genes in cluster 1 in the COVID groups was increased, whereas the expression of others was decreased (Fig. 2A and Figure S3, Supporting Information). The KEGG pathway enrichment analysis for coexpressed genes is shown in Fig. 2B. Ribosome, chemokine signaling and endocytosis pathways were enriched in cluster 1. Generally, endocytosis or micropinocytosis plays a role in viral entrance into the early endosomes in cells (Zhang et al. 2020). Lysosome, renin-angiotensin system and asthma pathways were enriched in cluster 2, and calcium and MAPK signaling pathways were enriched in cluster 3. Lysosomes are involved in destroying invading viruses, and the downregulation of lysosome-associated gene expression suggests dysregulation of the innate immune system. This finding is consistent with the downregulation of the expression of lysosomal genes reported by Xiong et al. (2020).

In the Reactome analysis (Figure S4, Supporting Information), neutrophil degranulation and the innate immune system were enriched in both clusters 1 (upregulated genes) and 2 (downregulated genes). Interestingly, neutrophils, the proportion of which was higher in the COVID group than in the healthy control group, play a role in the response to viral infection (Liu et al. 2020). Neutrophil degranulation was not fully activated or was partly blocked by the virus, as the expression of approximately half of the genes in this pathway was downregulated (Fig. 2C). The coexpression pathway analysis revealed that a large gene expression change was induced by COVID to facilitate the viral life cycle.

Computational drug repositioning for COVID-19 pneumonia

We identified two FDA-approved antiviral drugs (saquinavir and ribavirin) in the coexpression-based drug enrichment analysis (Fig. 3A). Saquinavir, a protease inhibitor ranked 10th in cluster 2, is used to help control HIV infection. Saquinavir has recently been reported as a candidate drug for treating COVID-19 by several individual researchers via molecular docking with 3CLpro, PLpro and spike proteins (Ayman et al. 2020; Ruan et al. 2020) (Table S3, Supporting Information). By tightly binding to 3CLpro and S proteins, saquinavir can inhibit the replication of this
virus both extracellularly and intracellularly (Table S3, Supporting Information).

Ribavirin was ranked 19th in the drug enrichment analysis for cluster 2 (Fig. 3C). Ribavirin can be used in combination with other antiviral medications to treat chronic hepatitis C and for SARS-CoV and MERS-CoV infections (Chong et al. 2015; Yin and Wunderink 2018). Ribavirin was reported as a candidate drug by a team of researchers who used molecular docking or protein–protein interaction methods for PLpro and Nsp14 (Gordon et al. 2020; Li et al. 2020) (Table S3, Supporting Information). More importantly, it has been included as a possible therapeutic drug in the diagnosis and treatment protocol for COVID pneumonia (trial version 5–7) released by the National Health Commission & National Administration of Traditional Chinese Medicine of the P.R. of China on March 3, 2020 (Table S3, Supporting Information) (Commission, N.H., Medicine, N.A.o.T.C 2020), although ribavirin did not perform well at treating the virus in a prior in vitro drug evaluation experiment (Wang et al. 2020).

The recovery of the two approved antiviral drugs using our transcriptome-based drug repositioning method indicated the reliability of our method. Moreover, we have successfully applied our drug repositioning method for psoriasis and periodontal disease (Jia et al. 2016; Kang et al. 2019). Additionally, the consistency among the results produced by two totally different drug repositioning methods, our transcriptome-based and docking-based methods, further validated our results from based on data other than transcriptomics data. The former method was used to find drugs that could restore normal gene expression following viral infection, whereas the latter method aimed to find the drug that directly targets viral or human proteins and inhibits the replication or entry process of the virus. Additionally, one advantage of cогена-based drug repositioning is that it is less computationally intensive than docking methods. Consequently, the results of the cогена-based drug repositioning are robust.

Additionally, the Venn diagram between genes in cluster 2 and the upregulated genes produced by saquinavir and ribavirin is shown in Fig. 3B and Table S4 (Supporting Information). Inositol polyphosphate-5-phosphatase B (INPP5B) was shared between the 3 sets. AP2-associated protein kinase 1 (AAK1) and Arrestin Beta 1 (ARRB1), shared between saquinavir and COVID-19, are involved in endocytosis, and AAK1 is considered a target to inhibit the virus (Richardson et al. 2020). Leucyl and cystinyl aminopeptidase (LNPEP), shared between ribavirin and COVID-19, is involved in the renin-angiotensin system, and ACE2, the target of SARS-CoV-2, was also a member of this system. These genes provide insight into the mode of action of the two drugs for treating COVID-19.

Other drugs enriched in the cluster, especially those with a rank higher than that of the two antiviral drugs, could be further investigated to discover more effective drugs for COVID-19. Generally, drugs with similar indications in a cluster possess a higher confidence priority for consideration. Dinoprost, having two instances ranked 1st and 17th, is a smooth muscle activator (Fig. 3A). (-)-Isoprenaline, a bronchodilator useful in obstructive
Figure 3. Drug repositioning for COVID pneumonia. (A) Cogena-based drug repositioning using cluster 2. The top 20 enriched drugs are shown. The names of drugs followed by the cell line, concentration of drug used and instance ID in CMap data are shown on the y-axis. The color indicates the degree of statistical significance, and the enrichment score is shown as $-\log (q\text{-value})$. (C) Venn diagram of upregulated genes by saquinavir and ribavirin and genes in cluster 2. The overlapping numbers of genes and certain gene symbols are shown in some sets.

CONCLUSION

Based on the COVID group BALF transcriptome data, we showed that several key pathways, such as endocytosis, the lysosome and neutrophil degranulation, in the disease, represent the intense battle between SARS-CoV-2 and humans. We provided several candidate drugs for treating COVID pneumonia using the cogena-based drug repositioning method. A total of two antiviral drugs, saquinavir and ribavirin, were recovered in our results. Coincidentally, these two drugs were also identified by several research groups using molecular docking methods, a totally different but classical strategy for drug development. Furthermore, several candidate drugs for preventing and treating COVID-19 were also identified. Our study shows that cogena is a powerful and efficient drug repositioning tool for emerging infectious diseases, enhances the understanding of SARS-CoV-2-induced disease and importantly provides drug candidates for the prevention and treatment of COVID-19.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSPD online.

AUTHOR CONTRIBUTORS

Conceptualization: K.H. and W.W.; data analysis: Z.J., J.S., X.S. Writing & Editing: Z.J. All authors read and approved the final manuscript.

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DATA AVAILABILITY STATEMENT
The datasets analyzed for this study can be found in the Genome Warehouse in the National Genomics Data Center available at https://bigd.big.ac.cn/gsa with project number PRJCA002273.

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Conflicts of Interests. None declared.

REFERENCES
Arakelyan A, Nersisyan L, Nikoghosyan M et al. Transcriptome-guided drug repositioning. Pharmaceutics. 2019;11:677.
Ashour HM, Elkhatabi WF, Rahman MM et al. Insights into the recent 2019 novel coronavirus (SARS-CoV-2) in light of past human coronavirus outbreaks, Pathogens (Basel, Switzerland). 2020;9:186.
Ayman F, Ping W, Mahmoud A et al. Identification of FDA Approved Drugs Targeting COVID-19 Virus by Structure-Based Drug Repositioning, 2020, DOI: 10.26434/chemrxiv.12003930.v2.
Chong YP, Song JY, Seo YB et al. A drug repositioning approach identifies tricyclic antidepressants as inhibitors of human coronavirus outbreaks, Chin Med J 2020, DOI: 10.1097/CMJ.000000000000744.
Li Y, Zhang J, Wang N et al. Therapeutic drugs targeting 2019-nCoV main protease by high-throughput screening, bioRxiv 2020, DOI: 10.1101/2020.01.28.922922.
Matsuyama Y, Kawase M, Nao N et al. The inhaled corticosteroid ciclesonide blocks coronavirus RNA replication by targeting viral NSP15, bioRxiv 2020, DOI: 10.1101/2020.03.11.987016.
Members, B.L.G.D.C. Database resources of the BIG Data Center in 2019. Nucleic Acids Res 2019;47:D8–D14.
National Health Commission and State Administration of Traditional Chinese Medicine. Diagnosis and treatment protocol for novel coronavirus pneumonia (Trial Version 7). Chin Med J 2020;133.
Richardson P, Griffin I, Tucker C et al. Baricitinib as potential treatment for 2019-nCoV acute respiratory disease. Lancet (London, England) 2020;395:e30–1.
Ritchie ME, Phipson B, Wu D et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 2015;43:e47, DOI: 10.1093/nar/gkv007.
Robinson MD, McCarthy DJ, Smyth GK. edgeR: a bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 2010;26:139–40.
Ruan Z, Liu C, Guo Y et al. Potential inhibitors targeting RNA-dependent RNA polymerase activity (NSP12) of SARS-CoV-2, Preprints.org 2020, DOI: 10.20944/preprints202003.0024.v1.
Walls AC, Park Y-J, Tortorici MA et al. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 2020, DOI: 10.1016/j.cell.2020.02.058.
Wang J, Zhao S, Liu M et al. ACE2 expression by colonic epithelial cells is associated with viral infection, immunity and energy metabolism, medRxiv 2020, DOI: 10.1101/2020.02.05.20020545.
Wang L, Wang Y, Hu Q et al. Systematic analysis of new drug indications by drug-gene-disease coherent subnetworks. CPT Pharmacometrics Syst Pharmacol 2014;3:e146, DOI: 10.1038/sps.2014.44.
Wang M, Cao R, Zhang L et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res 2020;30:269–71.
Wang Y, Chen S, Chen L et al. Associating lncRNAs with small molecules via bivelvel optimization reveals cancer-related lncRNAs. PLoS Comput Biol 2019;15:e1007540.
Wang Y, Chen S, Deng N et al. Drug repositioning by kernel-based integration of molecular structure, molecular activity, and phenotype data. PLoS One 2013;8:e78518.
Wu Z, Wang Y, Chen L. Network-based drug repositioning. Mol Biosyst 2013;9:1268–81.
Xiong Y, Liu Y, Cao L et al. Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood volume of tumor-infiltrating lymphocytes in patients with lung carcinoma, Cell Res 2020;30:269–71.
Liberzon A, Birger C, Thorvaldsdottir H et al. The Molecular Signatures Database (MSigDB) hallmark gene set collection. Cell Syst 2015;1:417–25.
Li G, De Clercq E. Therapeutic options for the 2019 novel coronavirus (2019-nCoV). Nat Rev Drug Discov 2020;19:149–50.
Li H, Wang YM, Xu JY et al. Potential antiviral therapeutics for 2019 Novel Coronavirus. Chin J Tuberc Respir Dis 2020;43:170–2.
Liu J, Li S, Liu J et al. Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients, medRxiv 2020, DOI: 10.1016/j.ebiom.2020.102763.
Liu K, Fang Y-Y, Deng Y et al. Clinical characteristics of novel coronavirus cases in tertiary hospitals in Hubei Province. Chin Med J 2020, DOI: 10.1097/CMA.000000000000744.
mononuclear cells in COVID-19 patients. SSRN 2020, DOI: 10.1080/22221751.2020.1747363.
Yin Y, Wunderink RG. MERS, SARS and other coronaviruses as causes of pneumonia. Respirology 2018;23:130–7.
Zhang J, Ma X, Yu F et al. Teicoplanin potently blocks the cell entry of 2019-nCoV, bioRxiv 2020, DOI: 10.1101/2020.02.05.935387.
Zhao S, Lin Q, Ran J et al. Preliminary estimation of the basic reproduction number of novel coronavirus (2019-nCoV) in China, from 2019 to 2020: a data-driven analysis in the early phase of the outbreak. Int J Infect Dis 2020;92: 214–7.
Zhou P, Yang XL, Wang XG et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020;579:270–3.
Zhou Z, Ren L, Zhang L et al. Heightened innate immune responses in the respiratory tract of COVID-19 patients. Cell Host Microbe 2020;27:883–90.e2.