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Pathway Analysis of Patients with Severe Acute Respiratory Syndrome

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ABSTRACT

Background   Coronaviruses are emerging threats for human health, as demonstrated by the ongoing coronavirus disease 2019 (COVID-19) pandemic that is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 is closely related to SARS-CoV-1, which was the cause of the 2002–2004 SARS outbreak, but SARS-CoV-1 has been the subject of a relatively limited number of studies. Understanding the potential pathways and molecular targets of SARS-CoV-1 will contribute to current drug repurposing strategies by helping to predict potential drug-disease associations.

Methods   A microarray dataset, GSE1739, of 10 SARS patients and 4 healthy controls was downloaded from NCBI's GEO repository, and differential expression was identified using NCBI's GEO2R software. Pathway and enrichment analysis of the differentially expressed genes was carried out using Ingenuity Pathway Analysis and Gene Set Enrichment Analysis, respectively.

Results   Our findings show that the drugs dexamethasone, filgrastim, interferon alfacon-1, and levodopa were among the most significant upstream regulators of differential gene expression in SARS patients, while neutrophil degranulation was the most significantly enriched pathway.

Conclusion   An enhanced understanding of the pathways and molecular targets of SARS-CoV-1 in humans will contribute to current and future drug repurposing strategies, which are an essential tool to combat rapidly emerging health threats.

Introduction

The coronaviruses (CoV) are a group of enveloped, single-stranded RNA viruses with the ability to infect birds and mammals. There are seven species of human coronaviruses, all of which cause respiratory tract infections that vary in severity [1]. Four human coronaviruses are responsible for 15 % to 30 % of common cold cases, while three – severe acute respiratory syndrome CoV 1 (SARS-CoV-1), Middle East respiratory syndrome CoV (MERS-CoV), and SARS-CoV-2 – cause more severe symptoms and have a significant mortality rate [2].

Drug repurposing, i. e., investigating approved drugs for alternative therapeutic purposes, has emerged as a shorter, less costly al-

ternative to traditional drug discovery and development, especially in the face of emerging infectious diseases with pandemic potential [3]. The coronavirus disease 2019 (COVID-19) pandemic, which is caused by SARS-CoV-2 infection, has illustrated the importance of drug repurposing strategies, which identified the therapeutic benefits of dexamethasone for COVID-19 patients who require mechanical ventilation or supplemental oxygen and remdesivir for those who require supplemental oxygen, among others [3, 4].

The main objective of the current study is to identify the canonical pathways and upstream regulators associated with SARS-CoV-1 infection in order to predict drug-disease associations based on pathway analysis.

Methods

Data acquisition

The microarray dataset GSE1739 was downloaded from the Gene Expression Omnibus (GEO) repository. GSE1739 included gene expression profiles of peripheral blood mononuclear cells (PBMCs) from adult SARS patients (n = 10) and healthy controls (n = 4). The Affymetrix GeneChip Human Genome Focus Array (HG-Focus) was used to produce the gene expression profiles [5].

Identification of differential expression

A list of 8,793 differentially expressed genes between SARS patients and healthy controls were identified using NCBI's GEO2R interactive web tool, which compares groups of samples from the GEO repository. Enhanced Volcano, a Bioconductor package, was used to create a labeled volcano plot of the 8,793 differentially expressed genes.

Pathway and enrichment analysis

QIAGEN's Ingenuity Pathway Analysis (IPA) was then utilized to scrutinize the differentially expressed genes between SARS patients and healthy controls. IPA revealed a total of 1,430 significantly differentially expressed genes (adjusted p-value < 0.05), with 928 downregulated genes and 502 upregulated genes. Through IPA Core Analysis, the canonical pathways and upstream regulators associated with the differentially expressed genes were inferred.

The 1,430 significantly differentially expressed genes in SARS patients were also scrutinized using the Gene Set Enrichment Analysis (GSEA) software [6, 7]. GSEA was applied to enrich the immune-related pathways from the list of genes.
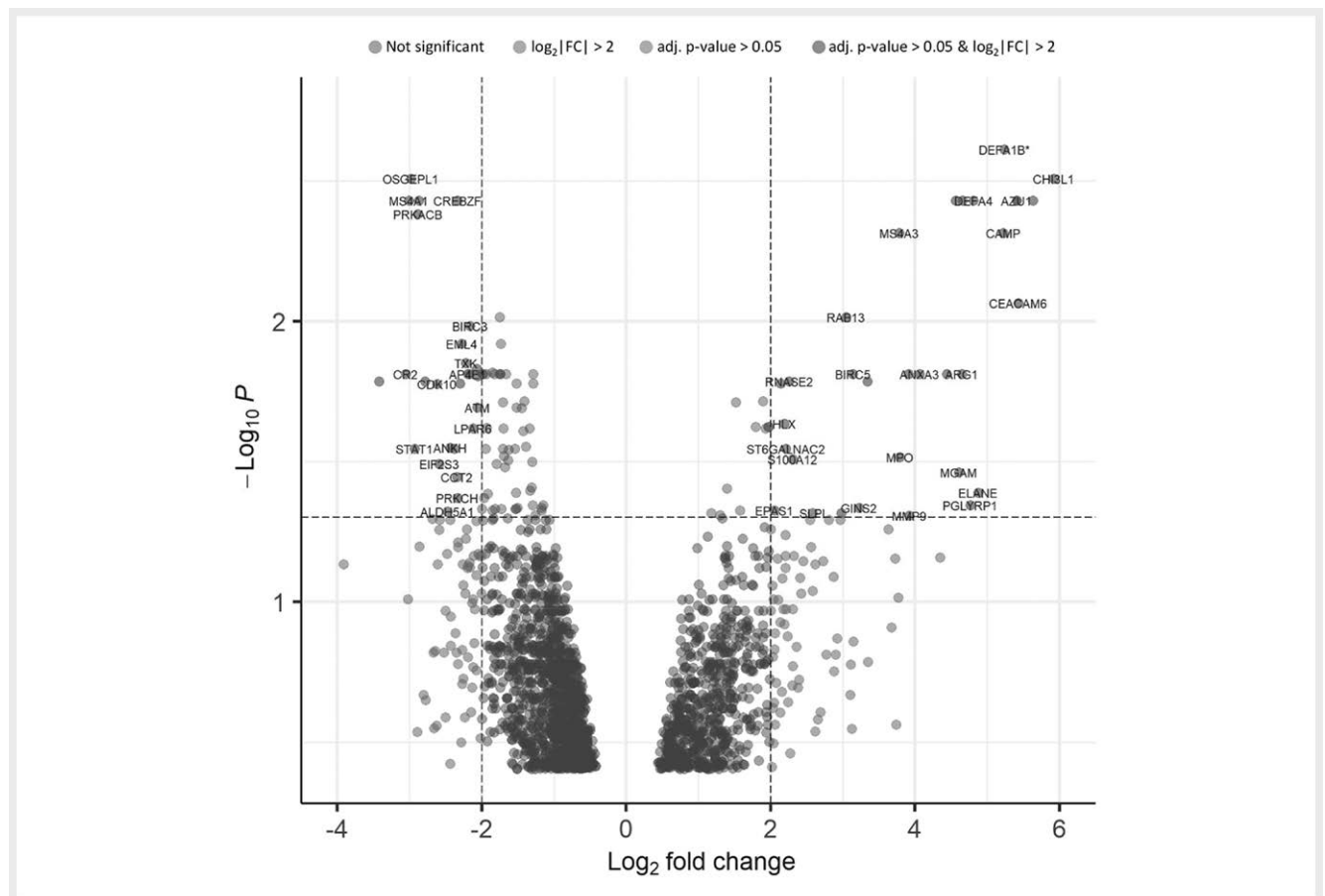
▶ **Fig. 1** Volcano plot showing the differentially expressed genes in SARS-CoV-1 patients compared to healthy controls as obtained from GEO2R. The full list of differentially expressed genes can be found in Supplementary Table 1.

Results and Discussion

Differentially expressed genes

The list of differentially expressed genes obtained from GEO2R are displayed in the form of a volcano plot (▶ **Fig. 1**), showing 26 significantly downregulated genes (adjusted p-value < 0.05, $\log_2$FC < -2) as well as 35 significantly upregulated genes (adjusted p-value < 0.05, $\log_2$FC > 2).

Canonical pathways

The integrin-linked kinase (ILK) signaling pathway was identified by IPA as the most significant canonical pathway in SARS patients compared to healthy controls (p-value = 1.9×10^{-11}, z-score = 2.5) (▶ **Fig. 2**).

ILK, a highly conserved and ubiquitously expressed intracellular protein, regulates signaling pathways for multiple cellular functions, and it has a major role in the contractility of cardiac and smooth muscles [8]. Dysfunction of the ILK signaling pathway has been associated with cardiomyopathies, glial scar formation, insu-

lin resistance, kidney disease, and tumorigenesis, with increased *ILK* expression connected to an unfavorable cancer prognosis and the multidrug resistance of tumor cells [8–13].

In the context of bacterial infection, the ILK signaling pathway modulates the production of tumor necrosing factor alpha (TNF-α), a proinflammatory cytokine, and the activation of nuclear factor kappa B (NF-κB) signaling, both of which are essential components of the innate immune response [14]. Similarly, ILK was shown to regulate the endothelium's inflammatory response to LPS exposure in a murine model [15].

With regard to viral infection, ILK was found to be enriched in an alveolar mucosa model following exposure to recombinant SARS-CoV-2 spike glycoprotein S1 [16]. ILK inhibition was associated with improved viability in mouse cardiomyocytes infected with coxsackievirus B3, the latter of which is the most common agent in viral myocarditis [17]. ILK was also shown to promote herpes simplex virus 1 (HSV-1) replication via the phosphorylation of Akt [18].
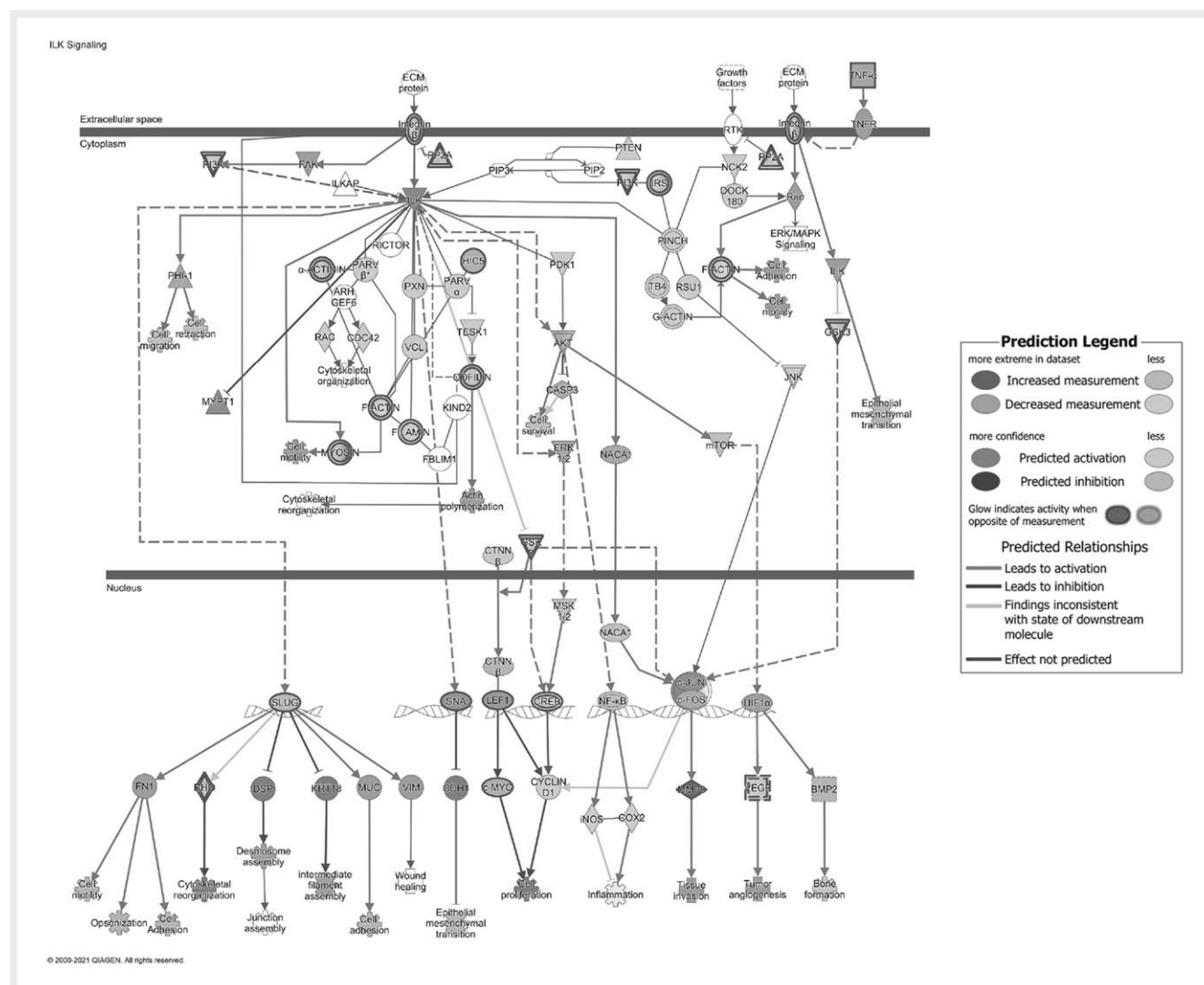
▶ **Fig. 2** Illustration of the integrin-linked kinase (ILK) pathway as differentially regulated between SARS patients and healthy controls. Illustration was generated using QIAGEN's Ingenuity Pathway Analysis (IPA).

► **Table 1** Upstream regulators in SARS patients (sorted by p-value) as shown by QIAGEN's Ingenuity Pathway Analysis (IPA).

Upstream regulator	Regulator type	Predicted activation	Activation z-score	p-value
Dexamethasone	Chemical drug	Activated	2.939	1.21×10^{-41}
TGFB1	Growth factor	Activated	2.35	4.51×10^{-35}
Beta-estradiol	Chemical – endogenous	–	−1.796	3.26×10^{-32}
HNF4A	Transcription regulator	–	0.709	1.94×10^{-29}
TNF	Cytokine	–	1.715	2.01×10^{-28}
Lipopolysaccharide	Chemical drug	Activated	2.285	2.96×10^{-28}
ESR1	Ligand-dependent nuclear receptor	–	−1.546	4.15×10^{-27}
Tretinoin	Chemical – endogenous	–	1.327	2.77×10^{-26}
CD3	Complex	Inhibited	−2.104	2.74×10^{-25}
OSM	Cytokine	–	1.792	6.52×10^{-25}
TP53	Transcription regulator	–	−0.514	1.44×10^{-24}
Immunoglobulin	Complex	Inhibited	−3.814	5.97×10^{-24}
Filgrastim	Biologic drug	Activated	4.097	7.67×10^{-23}
IL1B	Cytokine	Activated	2.182	1.94×10^{-22}
IFNG	Cytokine	–	−0.655	6.44×10^{-22}
Camptothecin	Chemical drug	–	0.325	4.50×10^{-20}
IL2	Cytokine	–	−1.733	4.70×10^{-20}
Trichostatin A	Chemical drug	–	0.011	2.31×10^{-19}
GATA1	Transcription regulator	–	1.379	4.69×10^{-19}
CEBPA	Transcription regulator	Activated	4.295	1.90×10^{-18}
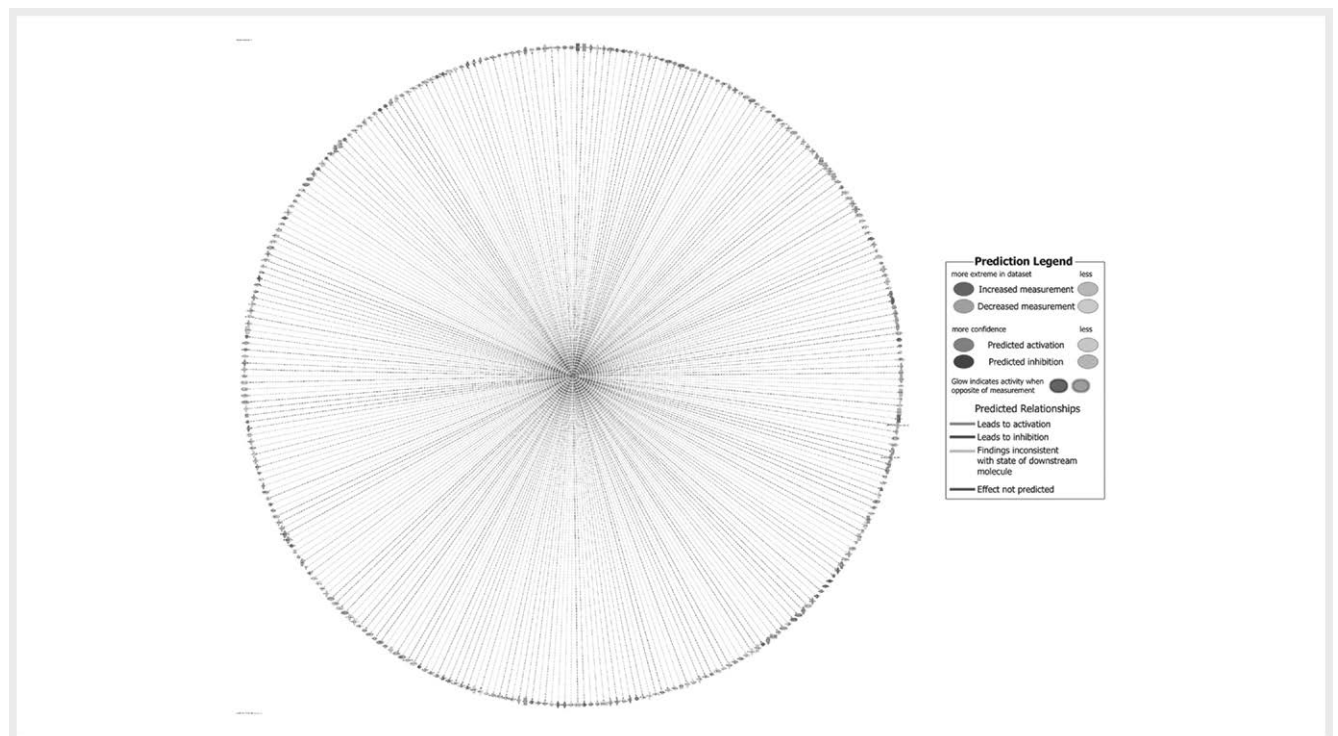
► **Fig. 3** Dexamethasone and its targeted genes in SARS patients. All targeted genes were significantly differentially expressed based on QIAGEN's Ingenuity Pathway Analysis (IPA). The full list of targeted genes can be found in Supplementary Table 2.

Upstream regulators

IPA revealed that dexamethasone, lipopolysaccharide, and filgrastim are the most significant upstream drug regulators in SARS patients compared to healthy controls (► **Table 1**).

Dexamethasone, a glucosteroid medication with anti-inflammatory and immunosuppressive effects, was shown by IPA to be the most significant upstream regulator (p-value = 1.21×10^{-41}, z-score = 2.939), indirectly interacting with 313 of the significantly

differentially expressed genes in SARS patients (► **Fig. 3**). It was the first drug shown to reduce deaths from severe SARS-CoV-2 infection, and it is currently recommended for patients suffering from COVID-19 pneumonia who need mechanical ventilation or oxygen therapy [19, 20]. Dexamethasone decreases inflammation by suppressing neutrophil migration and, in the context of COVID-19, by modulating interferon signaling to downregulate IFN-stimulated genes and alter IFN-active neutrophils [20, 21].

Our findings revealed that lipopolysaccharide (LPS) was the second most significant upstream regulator (p-value = 2.96×10^{-28}, z-score = 2.285) in SARS patients. They are major components of Gram-negative bacterial membranes and strong immunostimulants, entering the bloodstream from gut microbiota or sites of in-

► **Table 2** Upstream regulators in SARS patients (sorted by z-score) as revealed by QIAGEN's Ingenuity Pathway Analysis (IPA).

Upstream regulator	Regulator type	Predicted activation	Activation z-score	p-value
Most activated regulators				
GABA	Chemical - endogenous	Activated	4.438	2.09×10^{-06}
CEBPA	Transcription regulator	Activated	4.295	1.90×10^{-18}
E. coli B4 lipopolysaccharide	Chemical toxicant	Activated	4.159	2.28×10^{-03}
Filgrastim	Biologic drug	Activated	4.097	7.67×10^{-23}
CSF3	Cytokine	Activated	3.764	1.50×10^{-14}
STAT3	Transcription regulator	Activated	3.585	2.68×10^{-10}
CST5	Other	Activated	3.573	3.45×10^{-05}
Interferon alfacon-1	Biologic drug	Activated	3.5	8.09×10^{-11}
Trinitrobenzenesulfone	Chemical reagent	Activated	3.497	2.35×10^{-03}
IL6	Cytokine	Activated	3.409	3.31×10^{-13}
mir-17	microRNA	Activated	3.399	5.96×10^{-03}
mir-16-5p (and others)	Mature microRNA	Activated	3.371	2.02×10^{-04}
PTTG1	Transcription regulator	Activated	3.364	9.91×10^{-05}
IL1A	Cytokine	Activated	3.33	1.91×10^{-07}
miR-1-3p (and others)	Mature microRNA	Activated	3.321	3.22×10^{-05}
Alefacept	Biologic drug	Activated	3.243	2.68×10^{-08}
YAP1	Transcription regulator	Activated	3.192	4.48×10^{-04}
CAMP	Other	Activated	3.169	3.03×10^{-05}
SP110	Transcription regulator	Activated	3.157	1.49×10^{-08}
IL17A	Cytokine	Activated	3.152	1.11×10^{-05}
Most inhibited regulators				
TGFBR2	Kinase	Inhibited	−3.981	6.01×10^{-11}
Immunoglobulin	Complex	Inhibited	−3.814	5.97×10^{-24}
GW3965	Chemical reagent	Inhibited	−3.1	8.16×10^{-05}
Z-LLL-CHO	Chemical - protease	Inhibited	−2.91	1.50×10^{-14}
Levodopa	Chemical - endogenous	Inhibited	−2.854	2.18×10^{-03}
ATP7B	Transporter	Inhibited	−2.828	3.19×10^{-03}
MYCN	Transcription regulator	Inhibited	−2.816	7.38×10^{-04}
IFNB1	Cytokine	Inhibited	−2.79	1.52×10^{-02}
EBI3	Cytokine	Inhibited	−2.749	2.92×10^{-02}
JAK3	Kinase	Inhibited	−2.747	3.82×10^{-03}
Phytohemagglutinin	Chemical drug	Inhibited	−2.744	2.89×10^{-15}
Alpha catenin	Group	Inhibited	−2.732	1.32×10^{-07}
NUP98-DDX10	Fusion gene/product	Inhibited	−2.714	1.64×10^{-04}
l-asparaginase	Biologic drug	Inhibited	−2.673	1.51×10^{-02}
NS-398	Chemical reagent	Inhibited	−2.661	7.50×10^{-04}
SENP3	Peptidase	Inhibited	−2.646	2.48×10^{-02}
PTEN	Phosphatase	Inhibited	−2.598	6.09×10^{-09}
ITGB2	Transmembrane regulator	Inhibited	−2.538	2.90×10^{-04}
MYCL	Transcription regulator	Inhibited	−2.538	4.20×10^{-02}
Medroxyprogesterone	Chemical drug	Inhibited	−2.524	2.70×10^{-15}

▶ **Table 3** Enriched pathways in SARS patients as shown by Gene Set Enrichment Analysis (GSEA).

Pathway	Systematic name	No. of overlapping genes in pathway	p-value
Neutrophil degranulation	M27384	28	0.003
Innate Immune System	M1060	36	0.007
Genes over-expressed in CD34 cell types bone marrow derived of leukemic patients, compared to normal subjects	M1077	39	0.029
Genes overexpressed in CD34 hematopoietic cell type through expressing NUP98-HOXA9 melting	M27385	17	0.044
Genes down-regulated in CD133 cell type associated to the CD133 cell type	M27565	16	0.049

fection [22]. Interestingly, the spike proteins of SARS-CoV-2 were found to interact with and bind to LPS in the blood, boosting proinflammatory activity both in vitro and in vivo [23]. Moreover, circulatory LPS levels were connected to the severity of patient outcome in several viral infections, including SARS-CoV-2, HIV, and dengue virus [24].

Filgrastim was the third most significant upstream regulator (p-value = 7.67×10^{-23}, z-score = 4.097) in SARS patients. Used to treat neutropenia, filgrastim is a recombinant form of human granulocyte colony-stimulating factor that boosts neutrophil counts by acting on neutrophil progenitors [25]. One study has shown that the most activated biological processes in SARS patients are neutrophil activation and degranulation [26]. Similarly, another study comparing differentially expressed genes between SARS and H1N1 patients identified enriched hub genes involved in the antimicrobial humoral response as well as neutrophil activation and degranulation [27].

With regard to neutrophils, it has been hypothesized that lowering the neutrophil burden in patients with severe SARS-CoV infection by inhibiting the neutrophil elastase (*ELANE*) and lactotransfferin (*LTF*) genes directly results in lung protection [28]. Correspondingly, in neutropenic cancer patients with COVID-19, filgrastim administration was shown to increase the number of hospitalizations among outpatients as well as the number of deaths among inpatients [29].

To gain further insight from Reghunathan et al.'s (2005) data [5], IPA was used to identify the most activated and most inhibited upstream regulators in SARS patients compared to healthy controls (▶ **Table 2**).

Interferon alfacon-1 is a non-naturally occurring and synthetic type-1 interferon which is primarily used in the treatment of chronic hepatitis C infection [30]. Our findings show that it was the most activated drug upstream regulator (p-value = 4.097, z-score = 7.67×10^{-23}) in SARS patients, and an exploratory study has shown that interferon alfacon-1 demonstrates significant anti-viral activity in cell lines infected with SARS-CoV-1 [31]. Moreover, administration of interferon alfacon-1

alongside corticosteroids was associated with improved clinical parameters in SARS [32].

In contrast, levodopa, a dopamine precursor used for Parkinson's disease management, was shown by IPA to be the third most inhibited upstream regulator (p-value = −2.854, z-score = 2.18×10^{-03}) in SARS patients. Emerging reports point towards a potential association between SARS-CoV-2 infection and subsequent parkinsonism development, and Parkinson's disease patients infected with SARS-CoV-2 were observed to have a higher case fatality than the general population [33–35].

Enrichment analysis

GSEA revealed that the neutrophil degranulation and innate immune system pathways were the most significantly enriched pathways in SARS patients (▶ **Table 3**). Neutrophils play a key role in innate immunity , and the lungs are a major neutrophil reservoir in humans [36]. SARS-CoV-2 infection has been shown to alter the abundance, functionality, and phenotype of neutrophils in the nasopharyngeal epithelium, lungs, and blood [37].

Conclusions

The present findings illustrate the utility of pathway and enrichment analysis in drug repurposing research. The drugs dexamethasone, filgrastim, interferon alfacon-1, and levodopa were among the most significant upstream regulators of differential gene expression in SARS patients.

Acknowledgements

Conflict of Interest

The authors declare that there are no conflicts of interest associated with this manuscript.

References

[1] Liu DX, Liang JQ, Fung TS. Human Coronavirus-229E, -OC43, -NL63, and -HKU1 (Coronaviridae). In: Bamford DH, Zuckerman M, Hrsg. Encyclopedia of Virology (Fourth Edition). Oxford: Academic Press; 2021: 428–440

[2] Kesheh MM, Hosseini P, Soltani S et al. An overview on the seven pathogenic human coronaviruses. Reviews in Medical Virology 2022; 32: e2282. doi:10.1002/rmv.2282

[3] Ng YL, Salim CK, Chu JJH. Drug repurposing for COVID-19: Approaches, challenges and promising candidates. Pharmacol Ther 2021; 228: 107930. doi:10.1016/j.pharmthera.2021.107930

[4] Venkatesan P. Repurposing drugs for treatment of COVID-19. The Lancet Respiratory Medicine 2021; 9: e63. doi:10.1016/S2213-2600(21)00270-8

[5] Reghunathan R, Jayapal M, Hsu L-Y et al. Expression profile of immune response genes in patients with Severe Acute Respiratory Syndrome. BMC Immunol 2005; 6: 2. doi:10.1186/1471-2172-6-2

[6] Subramanian A, Tamayo P, Mootha VK et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. Proceedings of the National Academy of Sciences 2005; 102: 15545–15550. doi:10.1073/pnas.0506580102

[7] Mootha VK, Lindgren CM, Eriksson K-F et al. PGC-1α-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet 2003; 34: 267–273. doi:10.1038/ng1180

[8] Hannigan GE, McDonald PC, Walsh MP et al. Integrin-linked kinase: Not so 'pseudo' after all. Oncogene 2011; 30: 4375–4385. doi:10.1038/onc.2011.177

[9] Brodehl A, Rezazadeh S, Williams T et al. Mutations in ILK, encoding integrin-linked kinase, are associated with arrhythmogenic cardiomyopathy. Transl Res 2019; 208: 15–29. doi:10.1016/j.trsl.2019.02.004

[10] Zheng C-C, Hu H-F, Hong P et al. Significance of integrin-linked kinase (ILK) in tumorigenesis and its potential implication as a biomarker and therapeutic target for human cancer. Am J Cancer Res 2019; 9: 186–197

[11] Bugler-Lamb AR, Hasib A, Weng X et al. Adipocyte integrin-linked kinase plays a key role in the development of diet-induced adipose insulin resistance in male mice. Molecular Metabolism 2021; 49: 101197. doi:10.1016/j.molmet.2021.101197

[12] Raman A, Reif GA, Dai Y et al. Integrin-Linked Kinase Signaling Promotes Cyst Growth and Fibrosis in Polycystic Kidney Disease. JASN 2017; 28: 2708–2719. doi:10.1681/ASN.2016111235

[13] Pan L, North HA, Sahni V et al. β1-Integrin and Integrin Linked Kinase Regulate Astrocytic Differentiation of Neural Stem Cells. PLOS ONE 2014; 9: e104335. doi:10.1371/journal.pone.0104335

[14] Ahmed AU, Sarvestani ST, Gantier MP et al. Integrin-linked Kinase Modulates Lipopolysaccharide- and Helicobacter pylori-induced Nuclear Factor κB-activated Tumor Necrosis Factor-α Production via Regulation of p65 Serine 536 Phosphorylation*. Journal of Biological Chemistry 2014; 289: 27776–27793. doi:10.1074/jbc.M114.574541

[15] Hortelano S, López-Fontal R, Través PG et al. ILK mediates LPS-induced vascular adhesion receptor expression and subsequent leucocyte trans-endothelial migration†. Cardiovascular Research 2010; 86: 283–292. doi:10.1093/cvr/cvq050

[16] Rahman M, Irmler M, Keshavan S et al. Differential Effect of SARS-CoV-2 Spike Glycoprotein 1 on Human Bronchial and Alveolar Lung Mucosa Models: Implications for Pathogenicity. Viruses 2021; 13: 2537. doi:10.3390/v13122537

[17] Esfandiarei M, Suarez A, Amaral A et al. Novel role for integrin-linked kinase in modulation of coxsackievirus B3 replication and virus-induced cardiomyocyte injury. Circ Res 2006; 99: 354–361. doi:10.1161/01.RES.0000237022.72726.01

[18] Tsai M-S, Chen S-H, Chang C-P et al. Integrin-Linked Kinase Reduces H3K9 Trimethylation to Enhance Herpes Simplex Virus 1 Replication. Front Cell Infect Microbiol 2022; 12: 814307. doi:10.3389/fcimb.2022.814307

[19] Ledford H. Coronavirus breakthrough: dexamethasone is first drug shown to save lives. Nature 2020; 582: 469–469. doi:10.1038/d41586-020-01824-5

[20] Maláska J, Stašek J, Duška F et al. Effect of dexamethasone in patients with ARDS and COVID-19 (REMED trial) – study protocol for a prospective, multi-centre, open-label, parallel-group, randomized controlled trial. Trials 2022; 23: 35. doi:10.1186/s13063-021-05963-6

[21] Sinha S, Rosin NL, Arora R et al. Dexamethasone modulates immature neutrophils and interferon programming in severe COVID-19. Nat Med 2022; 28: 201–211. doi:10.1038/s41591-021-01576-3

[22] Ghosh SS, Wang J, Yannie PJ et al. Intestinal Barrier Dysfunction, LPS Translocation, and Disease Development. J Endocr Soc 2020; 4: bvz039. doi:10.1210/jendso/bvz039

[23] Petruk G, Puthia M, Petrlova J et al. SARS-CoV-2 spike protein binds to bacterial lipopolysaccharide and boosts proinflammatory activity. J Mol Cell Biol 2020; 12: 916–932. doi:10.1093/jmcb/mjaa067

[24] Kruglikov IL, Scherer PE. Preexisting and inducible endotoxemia as crucial contributors to the severity of COVID-19 outcomes. PLOS Pathogens 2021; 17: e1009306. doi:10.1371/journal.ppat.1009306

[25] Price TH, Chatta GS, Dale DC. Effect of Recombinant Granulocyte Colony-Stimulating Factor on Neutrophil Kinetics in Normal Young and Elderly Humans. Blood 1996; 88: 335–340

[26] Hemmat N, Derakhshani A, Bannazadeh Baghi H et al. Neutrophils, Crucial, or Harmful Immune Cells Involved in Coronavirus Infection: A Bioinformatics Study. Frontiers in Genetics 2020; 641. doi: 10.3389/fgene.2020.00641

[27] Mousavi SR, Lotfi H, Salmanizadeh S et al. An experimental in silico study on COVID-19: Response of neutrophil-related genes to antibiotics. Health. Sci Rep 2022; 5: e548. doi:10.1002/hsr2.548

[28] Ramesh P, Veerappapillai S, Karuppasamy R. Gene expression profiling of corona virus microarray datasets to identify crucial targets in COVID-19 patients. Gene Rep 2021; 22: 100980. doi:10.1016/j.genrep.2020.100980

[29] Zhang AW, Morjaria S, Kaltsas A et al. The Effect of Neutropenia and Filgrastim (G-CSF) on Cancer Patients With Coronavirus Disease 2019 (COVID-19) Infection. Clinical Infectious Diseases 2022; 74: 567–574. doi:10.1093/cid/ciab534

[30] Melian EB, Plosker GL. Interferon Alfacon-1. Drugs 2001; 61: 1661–1691. doi:10.2165/00003495-200161110-00009

[31] Kumaki Y, Day CW, Wandersee MK et al. Interferon alfacon 1 inhibits SARS-CoV infection in human bronchial epithelial Calu-3 cells. Biochemical and Biophysical Research Communications 2008; 371: 110–113. doi:10.1016/j.bbrc.2008.04.006

[32] Loutfy MR, Blatt LM, Siminovitch KA et al. Interferon Alfacon-1 Plus Corticosteroids in Severe Acute Respiratory SyndromeA Preliminary Study. JAMA 2003; 290: 3222–3228. doi:10.1001/jama.290.24.3222

[33] Rao AR, Hidayathullah SM, Hegde K et al. Parkinsonism: An emerging post COVID sequelae. IDCases 2022; 27: e01388. doi:10.1016/j.idcr.2022.e01388

[34] Li W-S, Chan L-L, Chao Y-X et al. Parkinson's disease following COVID-19: causal link or chance occurrence? Journal of Translational Medicine 2020; 18: 493. doi:10.1186/s12967-020-02670-9

[35] Artusi CA, Romagnolo A, Ledda C et al. COVID-19 and Parkinson's Disease: What Do We Know So Far? J Parkinsons Dis 2021; 11: 445–454. doi:10.3233/JPD-202463

[36] Giacalone VD, Margaroli C, Mall MA et al. Neutrophil Adaptations upon Recruitment to the Lung: New Concepts and Implications for Homeostasis and Disease. Int J Mol Sci 2020; 21: 851. doi:10.3390/ijms21030851

[37] Reusch N, De Domenico E, Bonaguro L et al. Neutrophils in COVID-19. Frontiers in Immunology 2021; 652470. doi: 10.3389/fimmu.2021.652470