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SUMO pathway, blood coagulation and oxidative stress in SARS-CoV-2 infection

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ABSTRACT

Severe Acute Respiratory Syndrome Corona Virus 2 (SARS CoV-2) is currently an international pandemic causing coronavirus disease 19 (COVID-19). Viral entry requires ACE2 and transmembrane protease serine 2 (TMPRSS2) for membrane fusion or through endosomal pathway. This Study aims to assess transcriptomic changes and differentially expressed genes (DFGs) in COVID-19.

Methods: Transcriptomic data of the publicly available dataset (GSE147507) was quantile normalized and analysed for DFGs, network analysis and pathway analysis.

Results: DFG sets showed that 8 genes (SAE1, AEBP2, ATP1A1, DKK3, MAFF, NUDC, TRAP1, and VAV1) were significantly dysregulated in all studied groups. Functional analysis revealed that negative regulation of glucocorticoid biosynthesis, protein SUMOylation (SAE1), blood coagulation (VAV1) and cellular response to stress were affected by SARS CoV-2 infection. Cell line transduction with ACE2 vector didn't show significant changes in the dysregulated pathways. Also, no significant change was observed in expression levels of ACE2 or TMPRSS2 in response to SARS CoV-2 infection. Further analysis showed dysregulation of several genes in the SUMOylation pathway and blood coagulation process in human and cell lines transcriptome. Also, several Cathepsins proteases were significantly dysregulated in case of SARS CoV-2 infection. Genes related to cellular response to stress such as TRAP-1 and NOX were dysregulated in cases of SARS CoV-2 infection.

Conclusion: Dysregulation in genes of protein SUMOylation, blood coagulation and response to oxidative stress pathways in SARS CoV-2 infection could be critical for disease progression. Drugs acting on SUMO pathway, VAV1, NOX genes could be studied for potential benefit to COVID-19 patients.

1. Introduction

Severe Acute Respiratory Syndrome Corona Virus 2 (SARS CoV-2) is currently an international pandemic causing coronavirus disease 19 (COVID-19). Genome of SARS CoV-2 codes for four main structural proteins: spike (S), nucleocapside (N), membrane (M) and envelope (E) proteins. The S protein is responsible for viral entry in host cells through interaction with angiotensin-converting enzyme 2 (ACE2). ACE2 is a zinc containing metallo-enzyme expressed in the cell membrane of several tissues including lung epithelium. Viral and cell membrane fusion requires host transmembrane protease serine 2 TMPRSS2 [1]. Both ACE and TMPRSS2 could be potential therapeutic targets for COVID-19. Serious clinical manifestations of COVID-19 include severe respiratory symptoms and irregular blood coagulation in COVID-19 that leads to a significantly increased risk of heart attacks and strokes [2]. These serious manifestations along with the absence of effective therapy dictate the need to suggest more potential target pathways.

This Study aims to assess transcriptomic changes in response to SARS CoV-2 infection in patients and several human cell lines. The differentially expressed genes (DFGs) could give an insight in molecular pathogenesis of COVID-19.

2. Materials and methods

Transcriptomic data of the publicly available dataset (GSE147507) [3] was analysed focusing on particular four subsets including cell lines of primary human lung epithelium (NHBE), transformed lung alveolar (A549), and A549 transduced with a vector expressing human ACE2 (A549/ACE2), with or without SARS-CoV-2 infection. In addition, transcriptomic data from uninfected human lung biopsies and lung samples from COVID-19 patient was analysed.

After determining DFGs either over expressed or under-expressed,

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the DEGs lists were analysed using several bioinformatics tools including network analysis of protein-protein interactions and functions by STRING [4]. Protein –protein interaction enrichment p-value < 0.05 was considered significant. Strength of the enrichment was calculated using the formula: ***Log10 (observed/expected)*** for proteins to be annotated with each term in the gene list compared to random network of the same size.

Also, PANTHER [5] and Reactome [6] were used for pathway analysis. PANTHER tool was used to analyse the DEGs list based on annotation classification for molecular function, biological process, and pathways. In Reactome pathway analysis, a statistical hypergeometric distribution test was performed to determine whether or not certain pathways are enriched in the submitted DEG list. The probability score calculated is corrected for false discovery rate (FDR) using the Benjamani-Hochberg method as described below. FDR <0.05 for pathway enrichment probability score was considered significant.

3. Statistical analysis

Raw read counts were of the dataset GSE147507 were quantile normalized prior to analysis. Student's T test was performed to determine the DFGs. P values were corrected for multiple testing (and represented as Q value) using Benjamini Hochberg method. Q value < 0.05 was considered statistically significant. Benjamini Hochberg method was performed using the following formula: ***(i/20)*0.2 where i = rank of p-value.***

4. Results

Analysis of transcriptomic data of the four studied subsets revealed DEGs that were further analysed using Reactome to determine the most significant dysregulated pathways (p-value < 0.05) after SARS COV-2 infection or transfection (Table 1).

For both types of A549 cell line (A549 and A549/ACE2) the DEGs (p-value < 0.05) mostly belong to similar pathways.

Regarding human cells and NHBE cell line, the most significant dysregulated pathways were related to immunity and viral replication.

Lists of DEGs from the four studied group were compared to determine dysregulated genes in all the four groups. Only 8 significantly dysregulated genes (namely SAE1, AEBP2, ATP1A1, DKK3, MAFF, NUDC, TRAP1, and VAV1) were dysregulated in all the four groups (Fig. 1).

Predicted protein functional analysis performed with STRING for the 8 genes showed relation to negative regulation of glucocorticoid biosynthetic process GO:0031947 (ATP1A1 and DKK3, FDR = 0.0016). Further analysis showed relation to protein SUMOylation GO: 0016925 (SAE1, FDR = 0.0017), and blood coagulation process GO: 0007596 (VAV1, and MAFF, FDR = 0.023).

Gene lists of the three GO processes were compared to DEGs in the four studied groups (Table 2). Several genes of the three pathways were dysregulated. While the DEGs profiles were alike in negative regulation of glucocorticoid biosynthetic process, the DEGs related to protein SUMOylation and blood coagulation were quite different across the four studied groups.

5. Genes related to viral entry and replication

For all studied groups, sets of DEGs were analysed for the expression of genes involved in viral entry and processing (ACE2, TMPRSS2, ANGPT, AT1, and Cathepsins). Several members of the Cathepsins family were significantly dysregulated in case of SARS CoV-2 infection (Q-value< 0.05). On the other hand, no significant change in expression levels of ACE2, TMPRSS2, ANGPT, AT1 was observed. Genes related to virus replication (such as IMPDH1, IMPDH2, ADSSL1) were dysregulated in cases of SARS CoV-2 infection (Q-value < 0.05).

Table 1
The most significant pathways of DEGs (p-value < 0.05) after SARS COV-2 infection.

Human	NHBE	A549 cell line	A549/ACE
Nonsense Mediated Decay (NMD) independent of the Exon Junction Complex (EJC)	Interleukin-10 signaling	Class I MHC mediated antigen processing & presentation	Antigen Presentation: Folding, assembly and peptide loading of class I MHC
Eukaryotic Translation Initiation	Cytokine Signaling in Immune system	Antigen processing: Ubiquitination & Proteasome degradation	Endosomal/ Vacuolar pathway
Cap-dependent Translation Initiation	Signaling by Interleukins	Membrane Trafficking	ER-Phagosome pathway
Peptide chain elongation	Interleukin-4 and Interleukin-13 signaling	Antigen Presentation: Folding, assembly and peptide loading of class I MHC	Class I MHC mediated antigen processing & presentation
Formation of a pool of free 40S subunits	Interferon alpha/beta signaling	Neddylation	Antigen processing-Cross presentation
Eukaryotic Translation Elongation	TRAIL signaling	Endosomal/Vacuolar pathway	Cellular responses to stress
SRP-dependent cotranslational protein targeting to membrane		COPI-dependent Golgi-to-ER retrograde traffic	Cellular responses to external stimuli
GTP hydrolysis and joining of the 60S ribosomal subunit		Intra-Golgi and retrograde Golgi-to ER traffic	Neddylation
L13a-mediated translational silencing of Ceruloplasmin expression		ER-Phagosome pathway	Regulation of mRNA stability by proteins that bind AU-rich elements
Selenocysteine synthesis		Ub-specific processing proteases	Antigen processing: Ubiquitination & Proteasome degradation
Eukaryotic Translation Termination		M Phase	NIK–>noncanonical NF-kB signaling
Response of EIF2AK4 (GCN2) to amino acid deficiency		Golgi-to-ER retrograde transport	NGF-stimulated transcription
Major pathway of rRNA processing in the nucleolus and cytosol		Separation of Sister Chromatids	Response of EIF2AK1 (HRI) to heme deficiency
Interleukin-10 signaling		Mitotic Anaphase	FBXL7 down-regulates AURKA during mitotic entry and in early mitosis
		Mitotic Metaphase and Anaphase	Asymmetric localization of PCP proteins
		Deubiquitination	ESR-mediated signaling
		Mitotic G2-G2/M phases	Ubiquitin-dependent degradation of Cyclin D
			Translesion Synthesis by POLH

(*continued on next page*)

Table 1 (*continued*)

Human	NHBE	A549 cell line	A549/ACE
		ER to Golgi Anterograde Transport	
		APC/C:Cdh1 mediated degradation of Cdc20 and other APC/C:Cdh1 targeted proteins in late mitosis/early G1	EGR2 and SOX10-mediated initiation of Schwann cell myelination
		Processing of Capped Intron-Containing Pre-mRNA	Host Interactions of HIV factors
		G2/M Transition	Packaging Of Telomere Ends
			Dectin-1 mediated noncanonical NF-kB signaling
			Regulation of activated PAK-2p34 by proteasome mediated degradation
			Hedgehog 'off' state
			Estrogen-dependent gene expression

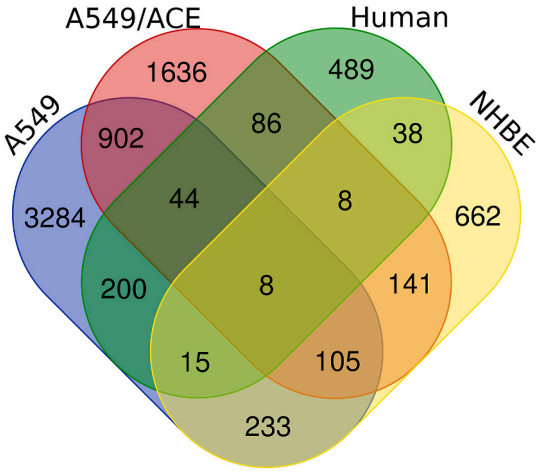

Fig. 1. Venn diagram of number of DEGs in all studied groups.

6. Cellular response to stress genes

Genes related to cellular response to oxidative stress (such as the NOX genes (NADPH oxidase complex) and C5aR2) were dysregulated in cases of SARS CoV-2 infection (Q-value < 0.05).

7. Discussion

Although ACE2 plays an important role in SARS CoV-2 molecular pathogenesis, the results of the current study showed that the expression of ACE2 didn't have much impact on neither the cell line transcriptome nor the pathways of DEGs. Pathway analysis of the transcriptome for the four studied subsets showed that the most significant dysregulated pathways in both A549 and A549/ACE2 after SARS CoV-2 transduction were quite similar. This could suggest that ACE2 expression although related to viral entry doesn't play major role in the disease molecular pathogenesis. This came in contradiction with previous reports that suggested an important role for ACE2 expression in acute lung injury (reported in 3%–20% of COVID-19 patients) [7]. Loss of ACE2 was shown to increase neutrophil accumulation, vascular permeability, and pulmonary edema in acute lung injury. Recombinant ACE2 was previously shown to decrease inflammatory reaction and improve ventilation in laboratory models [8]. This conflict opens questions about the uniqueness of the acute lung injury caused by SARS CoV-2 infection. Yet the special nature of A549 as an immortal cell line makes it insufficient to evaluate the effect of exogenous ACE2 on COVID-19 pathogenesis.

Lists of DEGs of the four studied group showed only 8 genes dysregulated in all groups due to SARA CoV-2 infection. Functional analysis of the proteins encoded by these genes suggests the mostly belong to three processes, glucocorticoid biosynthetic process, protein SUMOylation, and blood coagulation.

The process of negative regulation of glucocorticoid biosynthesis was dysregulated in relation to SARA CoV-2 infection in all studied groups via dysregulation of DKK3 and ATP1A1 in human cells and cell lines. In situ production of active glucocorticoids was reported in developing lung epithelium [9] and as a part of extra-adrenal glucocorticoid production in adults [10]. Down -regulation of DKK3 was observed in relation to SARS CoV-2 in the current study. This could raise a possibility that loss of negative regulation of glucocorticoid biosynthesis in lung cells may lead to increased glucocorticoids levels. DKK3 was previously reported to inhibit aldosterone biosynthesis in cultured human adrenocortical cells [11]. Also, it was suggested that DKK3 could act as a modifier of the hyper-aldosteronemea. DKK3 may be implicated in the pathogenesis of low-renin hyperaldosteronism in humans [12].

ATP1A1 expression was dysregulated in relation to SARS CoV-2 infection in all studied groups. In addition to its role in negative regulation of glucocorticoid biosynthesis, ATP1A1 is known to interact with M-protein in SARS CoV-2, thus plays an important role in viral entry. ATP1A1 was previously reported to supports CoV infection. Targeting ATP1A1 by small dose of cardiotonic steroids such as ouabain and bufalin that bind to ATP1A1 resulted in inhibition of MERS-CoVs infection at an early entry stage. Also knockdown of ATP1A1 inhibited CoV infection during the virus entry stage [13]. These results along with the dysregulation of ATP1A1 expression found in the current study in SARS CoV-2 infection might raise a question about potential role of cardiotonic steroids in SARS CoV-2 cell entry similar to their role in early entry stage of MERS-CoVs. This point remains open for further studies.

In the current study, SUMOylation activating enzyme −1 (SAE1) was downregulated in relation to SARS CoV-2 infection in all studied groups except NHBE, in which it is upregulated. It was suggested recently that down regulation of SAE1 could favor viral replication [14]. Previously, only adenovirus was reported to target SAE1, hence inhibiting SUMOylation processes [15].

In the current study, human cells transcriptome showed dysregulation of other genes in the SUMOylation pathway including NUP85, BCL11A, CDKN2A, PIAS2, SUMO1P1 in case of SARS CoV-2 infection. Downregulation of the PIAS2 was reported to reduce SUMOylation of IFIH1 decreasing interferon production, as IFIH1 SUMOylation is known to stimulate type I IFN production. Viruses were reported to be able to evade the immune response by suppressing interferon production via decreasing IFIH1 SUMOylation in an IFIH1 expression independent manner [16]. Decreased SUMOylation of specific antiviral proteins has been suggested to be important in regulating anti-viral immune responses [15].

On the other hand, some protein SUMOylation was reported to play an important role in formation of SARS CoV ribonucleoprotein complexes and nucleocapsid assembly through modulation of cellular SUMOylation [17]. Based on this, several studies tested SUMOylation inhibitors for potential effect on viral replication. The SUMO inhibitor 2-D08 was shown to reduce replication and pathogenicity of some viruses significantly [18].

Taken together, these observations and previous reports about virus –host interaction regarding protein SUMOylation suggest that viruses

Table 2
Predicted protein functional analysis for the 8 genes that are significantly dysregulated in all the four groups and related networks.

		Human	NHBE	A549 cell line	A549/ACE2
Negative regulation of GC biosynthesis	DKK3	Q-value< 0.05	Q-value< 0.05	Q-value< 0.05	Q-value< 0.05
	ATP1A1	Q-value< 0.05	Q-value< 0.05	Q-value< 0.05	Q-value< 0.05
	BMP2		Q-value< 0.05		
	REST			Q-value< 0.05	Q-value< 0.05
Protein SUMOylation	SAE1	Q-value< 0.05, Downregulated	Q-value< 0.05, Upregulated	Q-value< 0.05, Downregulated	Q-value< 0.05 Downregulated
	NUP85	Q-value< 0.05			Q-value< 0.05
	BCL11A	Q-value< 0.05, Upregulated			
	CDKN2A	Q-value< 0.05, Upregulated			
	PIAS2	Q-value< 0.05, Downregulated			
	SUMO1P1	Q-value< 0.05, Upregulated			
	IFIH1		Q-value< 0.05, Upregulated	Q-value< 0.05, Upregulated	
	HDAC4		Q-value< 0.05 Upregulated	Q-value< 0.05, Downregulated	
	EGR2		Q-value< 0.05, Downregulated		Q-value< 0.05, Upregulated
	UBA2			Q-value< 0.005, Upregulated	
	SUMO3			Q-value< 0.05	Q-value< 0.05
	SENP		SENP8 down	Up SENP6, SENP8	Up SENP1. SENP7 Down SENP2
Blood coagulation	VAV1	Q-value< 0.05	Q-value< 0.05	Q-value< 0.05	Q-value< 0.05
	MAFF	Q-value< 0.05	Q-value< 0.05	Q-value< 0.05	Q-value< 0.05
	FGG	Q-value< 0.05			Q-value< 0.05
	MAPK1	Q-value< 0.05			Q-value< 0.05
	HPS6	Q-value< 0.05		Q-value< 0.05	
	IL6		Q-value< 0.05	Q-value< 0.05	
	THBS1		Q-value< 0.05	Q-value< 0.05	Q-value< 0.05
	SAA1		Q-value< 0.05	Q-value< 0.05	
	THBD			Q-value< 0.05	Q-value< 0.05
	LCP2	Q-value< 0.05			
	RAC2	Q-value< 0.05			
	CDC42	Q-value< 0.05			
Cell response to stess	Nox genes	Q-value< 0.05 Nox2 (CYBB) (Up) DUOX1 (up)		Q-value< 0.05 NOX1(Down) DUOX2(Up)	Q-value< 0.05 DUOX2(Up)
	C5aR2	Q-value< 0.05			
	Cathepsins	Q-value< 0.05 (Up)CTSS, CTSW	Q-value< 0.05 (Up) CTSC (Down)CTSH, CTSF	Q-value< 0.05 (Up)CTSC, CTSS (Down) CTSA, CTSF CTSD,	Q-value< 0.05 (Up)CTSB, (Down)CTSD

benefit from dysregulation (whether upregulation or down regulation) of protein SUMOylation. Viruses have several mechanisms by which they could evade immune response or help viral replication through inhibition or induction of protein SUMOylation, [15].

A previous study reported that most mortalities of COVID-19 had disseminated intravascular coagulation (more than 70% of studied mortalities) [19]. Abnormal coagulation was observed in late stages of the disease. Concentrations of D-dimer and other fibrin degradation products were significantly increased in patients with poor prognosis [20]. Members of VAV family are phosphorylation-dependent guanosine nucleotide exchange factors. VAV1 is highly expressed in hematopoietic cells. Also, VAV1 plays an important role in several signaling pathways including T cell receptor (TCR)-induced Ca2+ entry [21]. The encoded protein has been identified as the specific binding partner of Nef proteins from HIV-1 [22].

In the current study, VAV1 was overexpressed in association with SARS CoV-2 infection. Azathioprine, which is a potent anti-inflammatory and immune suppressant, was reported to inhibit VAV1 functions in human patients [23]. However, whether or not this inhibition would improve COVID-19 patients needs further studies.

For all studied groups, several members of the Cathepsins family were significantly dysregulated in case of SARS CoV-2 infection. Cathepsins are proteases activated mostly at low lysosome pH. Cathepsins B, C, F, H, K, L, V, O, S, W, and X are cysteine proteases, Cathepsins D and E are aspartyl proteases, and Cathepsins A and G are serine proteases. In the current study, most dysregulated Cathepsins were cysteine proteases. Transcriptome of SARS CoV-2 infected cells revealed that Cathepsins C, S, W were upregulated, while Cathepsins D and F were downregulated. Due to the important role of Cathepsins C and S in the inflammatory process [24], their upregulation with SARS CoV-2 infection could contribute to the severity of lung injury.

Cathepsin C was reported to play an important role in human natural killer cells and in neutrophils. Cathepsin S is mostly present in antigen presenting cells such as dendritic cells and B lymphocytes. Cathepsin W is present in cytotoxic cells, mostly in natural killer cells and, also in $CD8^+$ T lymphocytes [25]. It was previously reported that Cathepsin S inhibition is prophylactic to ameliorate airway inflammation [26]. Hence, upregulation of these Cathepsins could suggest that Cathepsin S inhibitors could improve the lung condition in SARS CoV-2 cases.

On the other hand, the aspartic protease Cathepsin D was reported to trigger and apoptosis in human fibroblasts through permeabilization of the mitochondrial outer membrane [27]. Other mechanisms by which Cathepsin D contribute to apoptosis include the release of cytochrome C and caspase activation [28]. Down regulation of Cathepsin D observed in relation to SARS CoV-2 needs more investigations especially because there is no Known endogenous inhibitor for Cathepsin D [29].

Cathepsin F, which participates in intracellular degradation and turnover of proteins, was found in the current study to be downregulated with SARS CoV-2 infection. Cathepsin F was previously reported to be upregulated by angiotensin II which also increase the secretion of Cathepsin F in a mechanism involving angiotensin II receptors [30].

It should be noted that SARS CoV-2 virus may enter the cells through endosomal uptake. In fact, direct entry from the cell surface or early endosomes was reported to be the preferred way of viral entry in vivo [31]. Endosomal vesicles within the cells form contain several classes of proteolytic enzymes, including cathepsins [32]. Collectively, these studies along with the current study results add more evidence to the importance of cathepsines in viral entry through ACE2 or endosomes.

Nuclear distribution gene C (NudC) was shown in the current study to be significantly dysregulated in SARS CoV-2 infection in all studied groups. Several studies had pointed out the importance of the of intracellular transport motors dynein and kinesin in viral life cycle. These motors are critical for replication and spread of many viruses via transport on microtubules. Different binding and regulatory proteins including Nud family interact with a specific subunits in the dynein motor complex [33]. NudC also plays important roles in cell migration, ciliogenesis, thrombopoiesis, and the inflammatory response [34]. Effect of NudC dysregulation on microtubule transport of the virus needs further study.

No significant change in expression levels of ACE2, TMPRSS2, ANGPT, AT1 was observed in relation to SARS CoV-2 infection in any studied group. Human ACE2 is known to be the receptor responsible of SARS-CoV-2 entry. The entry in cells was reported to require the action of a protease such as TMPRSS2 [35]. TMPRSS2 inhibitors such as camostat mesylate were reported to block SARS-CoV-2 entry in lung cells in vitro [36]. The absence of differential expression of ACE2 and TMPRSS2 in SARS CoV-2 groups suggests that their concentrations don't correlate with viral growth and disease progression.

In the current study, several genes related to cellular response to stress (such as the NOX genes and C5aR2) were dysregulated in cases of SARS CoV-2 infection. A previous study showed that some viruses trigger NOX-derived Reactive oxygen species secretion by inducing NOX signaling in the very early stages of the viral infection. NOX signaling inhibitors such as diphenyliodonium chloride improve the exaggerated inflammatory responses induced by viral infection [37]. Whether or not these drugs could help SARS CoV-2 patients needs more studies. It worth noting that NOX inhibitors affect the GTP-binding proteins: RAC1/RAC2. RAC2 was also found in the current study to be dysregulated in SARS CoV-2 infection among other genes related to blood coagulation process GO:0007596.

Tumor Necrosis Factor Receptor-Associated Protein 1 (TRAP-1), which was found in the current study to be dysregulated in SARS CoV-2 infection, is also related to cellular response to stress. It plays an important role in mitochondrial integrity and oxidative stress response [38].

Overproduction of reactive oxygen species after infection and inflammation induces DNA damage and affects epigenetic regulators that are important in normal cell development and differentiation. Adipocyte Enhancer Binding Protein 2 (AEBP2) is a part of Polycomb repressive complex 2 (PRC2) which is a key epigenetic regulators that maintain transcriptional repression of many genes and plays an important role of some viruses pathogenesis [39]. AEBP2 was found in the current study to be dysregulated in SARS CoV2 infection. Collectively, these data suggests that oxidative stress could be related to COVID-19 pathogenesis.

The small numbers in each group of the studied dataset dictates the necessity of further studies on COVID-19 transcriptome. Several researchers had reported transcriptomic changes in COVID-19 patients in agreement with the results of the current study. COVID-19 patients were reported to have over expression of pro-inflammatory cytokines such as IL-2, IL-6, IL-7, IL-10, granulocyte-colony stimulating factor, interferon-γ inducible protein 10, and TNF-α. This was observed to increases the vascular permeability and accumulation of fluid and blood cells into the alveoli, leading to dyspnea and respiratory distress [40,41]. Another transcriptome profiling study suggested that SARS-CoV-2 RNA could be acting as an RNAi. This might explain many of the down-regulatory expression effects including its effect on mitochondrial functions [42]. This could support the results of the current study that mitochondrial integrity and oxidative stress response could be affected by SARS CoV-2 infection (through TRAP-1 dysregulation in the current study).

The processes found in the current study to be dysregulated –particularly protein SUMOylation, negative regulation of glucocorticoid biosynthesis and blood coagulation-require attention especially they could be targeted by already known drugs such as SUMO inhibitor 2-D08, cardiotonic steroids, and Azathioprine, respectively. Also, the role of Cathepsins in viral entry and the role of NOX signaling could be of therapeutic value, perhaps using the NOX signaling inhibitor, diphenyliodonium chloride.

Author statement

I am submitting a revision to the manuscript BBREP-D-20-00122R1 for consideration of publication in "**Biochemistry and Biophysics Reports**". The manuscript is entitled "***SUMO Pathway, Blood Coagulation and Oxidative Stress in SARS-Cov-2 Infection***". It is currently neither submitted nor published elsewhere.

I declare that this study is an analysis of publically available dataset with no human subjects enrolled in this study. However, ethical considerations and privacy rights of human subjects of the original study were observed and no personal data were collected.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bbrep.2021.100938.

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