

# In vitro analysis of the renin–angiotensin system and inflammatory gene transcripts in human bronchial epithelial cells after infection with severe acute respiratory syndrome coronavirus

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## Abstract

**Introduction:** Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a recently identified coronavirus family member that triggers a respiratory disease similar to severe acute respiratory syndrome coronavirus (SARS-CoV). SARS-CoV and SARS-CoV-2 are very similar to each other in many respects, such as structure, genetics, and pathobiology. We hypothesized that coronaviruses could affect pulmonary tissues via integration with the critical immune genes after their interaction with renin–angiotensin system (RAS) elements. The aim of the present bioinformatics study was to assess expression changes of the RAS and non-RAS genes, particularly immune response genes, in the lung epithelial cells after infection with SARS-CoV.

**Methods:** Linear regression, hierarchical clustering, pathway analysis, and network analysis were performed using the E-GEOD-17400 data set.

**Results:** The whole-genome expression data of the lung epithelial cells infected with SARS-CoV for 12, 24, and 48 hours were analyzed, and a total of 15 RAS family and 29 immune genes were found to be highly correlated with the exposure time to the virus in the studied groups.

**Conclusion:** RAS genes are important at the initiation of the infections caused by coronavirus family members and may have a strong relationship with the exchange of immune genes in due course following the infection.

## Keywords

RAS, ANPEP, ACE2, EGFR, IGF2R, SARS-CoV, SARS-CoV-2, immune system genes

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## Introduction

Coronaviruses (CoV) are the enveloped RNA viruses that can cause enzootic fatal infections primarily in birds and mammals.<sup>1–4</sup> Moreover, CoV has the ability to infect humankind.<sup>5–7</sup> The presenting symptoms and signs of the infection could be chills, night sweats, persistent cough, diarrhea, and fever. However, the viral infection could cause a wide variety of clinical presentations, ranging from upper respiratory infections (URI) to lower respiratory infections (LRI), including bronchitis, pneumonia, and severe acute respiratory syndrome (SARS). CoV affects the respiratory system and specifically the lungs.<sup>5,8,9</sup> Furthermore, the viral infection can also affect the gastrointestinal tract, as well as the neurological and hepatic systems.<sup>10</sup> The severe acute respiratory syndrome (SARS) epidemic in 2003 and the Middle East respiratory

syndrome (MERS) in 2012 revealed that CoV can be fatal when they cross the species barrier and infect humans.<sup>6</sup> A newly identified CoV called SARS-CoV-2 (formerly

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entitled nCoV) appeared within the last month of 2019 in the city of Wuhan, China.<sup>11</sup> Very early findings suggested that the virus passed from a specific animal species to humans. However, some studies then revealed that SARS-CoV-2 could also be passed from person to person, leading to serious respiratory diseases such as acute respiratory distress syndrome similar to that seen in the SARS-CoV infection.<sup>12,13</sup> SARS-CoV-2 is an enveloped, single-stranded, positive-sense RNA (beta-) CoV. The same as SARS-CoV and MERS-CoV, the SARS-CoV-2 genome can encode structural proteins such as the spike glycoprotein, non-structural proteins, and accessory proteins. The spike glycoprotein, in which this virus has encoding functions, plays an important role in the interactions of the virus and the cell receptor during viral entry.<sup>6</sup> More specifically, a spike protein that is associated with the envelope of SARS-CoV-2 binds first to the host receptor and then mediates the entry of the CoV into the host cells by fusing viral and host membranes.<sup>14–16</sup> Once the fusion has taken place, the RNA virus begins to replicate its genome in the cell and makes new virions that will be secreted to infect other cells.<sup>17–19</sup>

There is a clear pathobiological association between CoV and the renin–angiotensin system (RAS). SARS-CoV (a member of the CoV family that emerged in 2002), which is very similar to SARS-CoV-2, and angiotensin converting enzyme 2 (ACE2) interactions have been extensively studied.<sup>13,16</sup> There is a fundamental atomic interaction between the spike protein receptor binding domain of the virus and the host receptor of ACE2. SARS-CoV infections lead to ACE2 down-regulation by the binding of the SARS-CoV spike protein to ACE2.<sup>20</sup> Thus, the SARS-CoV family, including SARS-CoV-2, utilizes ACE2 as a critical receptor to enter target host cells.<sup>13,16</sup>

The main task of ACE2 is to hydrolyze angiotensin II (Ang II) to form angiotensin (1–7) (Ang-(1–7)) that binds to the G-coupled protein receptor MAS to antagonize Ang II-mediated cellular effects. This function is very important since the increased Ang II levels are estimated to cause increased ACE2 activity. ACE2 is one of the central RAS enzymes that regulate blood pressure, fluid/electrolyte balance, and systemic vascular resistance in the tissue RAS. ACE2 is also critical for the RAS, which functions for the regulation of cell growth/proliferation, inflammation/cytokine production, blood pressure, and as a homeostatic regulator of vascular function. The RAS is highly active within the lungs where SARS-CoV-2 is estimated to be the primary target organ. Ang II can cause pulmonary vasoconstriction in response to hypoxia, which is important in patients with pneumonia or lung injury. Locally increased Ang II production increases vascular permeability, which facilitates pulmonary edema. ACE2, which plays a role in regulating the Ang II as well as Ang-(1–7) levels, may be important for those pathobiological lung events.<sup>13,21,22</sup> ACE2 is an important negative directing

factor for the severity of lung injury, and SARS-CoV spike protein-associated ACE2 inhibition has been shown to contribute to the severity of lung pathologies.<sup>20</sup> In addition, numerous genes are involved within the interactions of ACE2, the RAS, and the pulmonary microenvironment.

The activation of the local pulmonary RAS can affect the genesis of lung damage through multiple mechanisms, such as increments in vascular permeability and alterations in alveolar epithelial cells. The pathogenesis of the CoV is a rather complicated process, which may also include local tissue RAS.<sup>13,21</sup>

The main purpose of this present in silico genomic study was to assess how the expressions of the RAS gene family changes after cellular infection with SARS-CoV in the lung epithelial cell culture. We hypothesized that CoV could affect pulmonary tissues via integration with the critical immune genes of the affected cellular microenvironment after their interaction with RAS elements. We also aimed to figure out whether these genomic alterations are related to the exposure time of the virus. In order to understand the pathobiology of SARS-CoV and SARS-CoV-2 infection better, since they share similarities within many aspects we examined the gene expression changes of other gene families as well. Elucidation of the interactions between SARS-CoV-2 and RAS genes and the description of the related genomic mechanisms are very important in the fight against this fatal viral global infection.

## Methods

### *Obtaining and normalizing whole-genome expression data of human bronchial epithelial cells infected with SARS-CoV*

Gene expression data of human bronchial epithelial cells treated with SARS-CoV for 12, 24, and 48 hours were obtained from Array Express (GSE17400).<sup>23</sup> These data were generated by Yoshikawa et al. to characterize the dynamic, spatial, and temporal changes of the gene expression induced by SARS-CoV using microarray technology.

In order to use the obtained data in other targeted analyses, the raw data were normalized by robust multi-sequence analysis in accordance with the procedure in the Affy package in R. These data consist of 23,344 genes (54,675 probe sets). In addition, each gene has three repeated expression data values for 12, 24, and 48 hours, respectively.

### *Comparison of RAS gene transcripts in 12-, 24- and 48-hour SARS-CoV-infected groups*

The whole normalized gene expression data of lung epithelial cells infected with SARS-CoV for 12, 24, and 48 hours were compared between different groups in order to

**Table 1.** The list of the RAS family genes whose expression shows a significant difference between 12-hour vs 24-hour (A) and 12-hour vs 48-hour (B) is depicted. Seven of the RAS signaling pathway genes showed different expression values in 12-hour vs 24-hour group, while 8 of the genes found significantly expressed in the 12-hour vs 48-hour group.

A			B		
Probe set ID	Gene symbol	p-Value	Probe set ID	Gene symbol	p-Value
202888_s_at	ANPEP	0.0008	208270_s_at	RNPEP	0.0110
222257_s_at	ACE2	0.0009	224999_at	EGFR	0.0112
209749_s_at	ACE	0.0040	202888_s_at	ANPEP	0.0116
219962_at	ACE2	0.0057	225943_at	NLN	0.0283
201392_s_at	IGF2R	0.0100	225944_at	NLN	0.0296
227463_at	ACE	0.0249	222257_s_at	ACE2	0.0371
203435_s_at	MME	0.0309	201392_s_at	IGF2R	0.0417
202834_at	AGT	0.0481	207904_s_at	LNPEP	0.0502
224999_at	EGFR	0.0501	200766_at	CTSD	0.0513

ANPEP: alanyl aminopeptidase; ACE2: angiotensin converting enzyme 2; ACE: angiotensin converting enzyme; IGF2R: insulin-like growth factor 2 receptor; MME: membrane metalloendopeptidase; AGT: angiotensinogen; EGFR: epidermal growth factor receptor; RNPEP: arginyl aminopeptidase; NLN: neurolysin; LNPEP: leucyl and cysteinyl aminopeptidase; CTSD: cathepsin D.

determine significantly and differentially expressed RAS family genes. The mean value of RAS gene transcripts was determined for each group infected with SARS-CoV for 12, 24, and 48 hours. The comparison was made between the 12- and 24-hour groups as well as between the 12- and 48-hour groups, based on the RAS gene transcripts. *p*-Values were calculated for each gene using the *t*-test. The genes belonging to the RAS family with a statistically significant difference among the groups were determined ( $p \leq 0.05$ ).

#### Linear regression analysis of the genes (other than the RAS genes) with the highest variance among the groups

In order to determine the genes whose expression is highly correlated with the exposure time to the virus, we performed linear regression analysis. Genes with a standard deviation  $>0.9$  were identified between the groups using Microsoft Excel (2016). Pearson product-moment correlation coefficient values (*r*) were calculated for these genes. Genes with a Pearson's *r*  $>0.95$  were selected for use in the subsequent analyses.

#### Hierarchical clustering, pathway analysis, and network analysis

In order to determine whether the identified genes could distinguish between the virus and infected groups, to identify the network connections between these genes and to demonstrate the pathways in which these genes were involved, we performed hierarchical clustering, pathway analysis, and network analysis.

Genes with the highest variation and Pearson's *r*-value between groups were hierarchically clustered using the

similarity metric parameter and the Euclidean distance Gene Cluster v3.0 program as a full link.<sup>24</sup> Using the DAVID online tool, the pathways related to the genes were determined to elaborate on the pathway data resulting from the analysis further.<sup>25,26</sup>

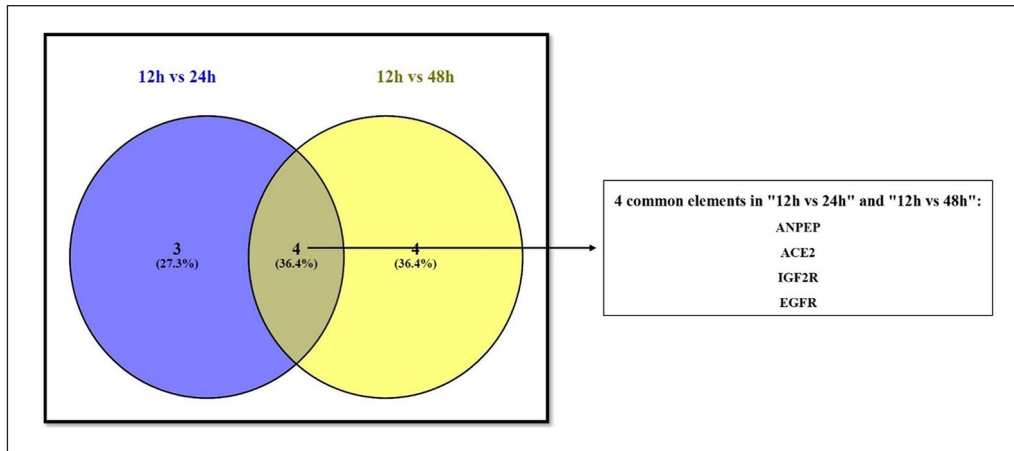
In addition, the GeneMANIA application in Cytoscape was used to indicate the network and pathway relationship between the selected genes and other genes.<sup>27,28</sup>

## Results

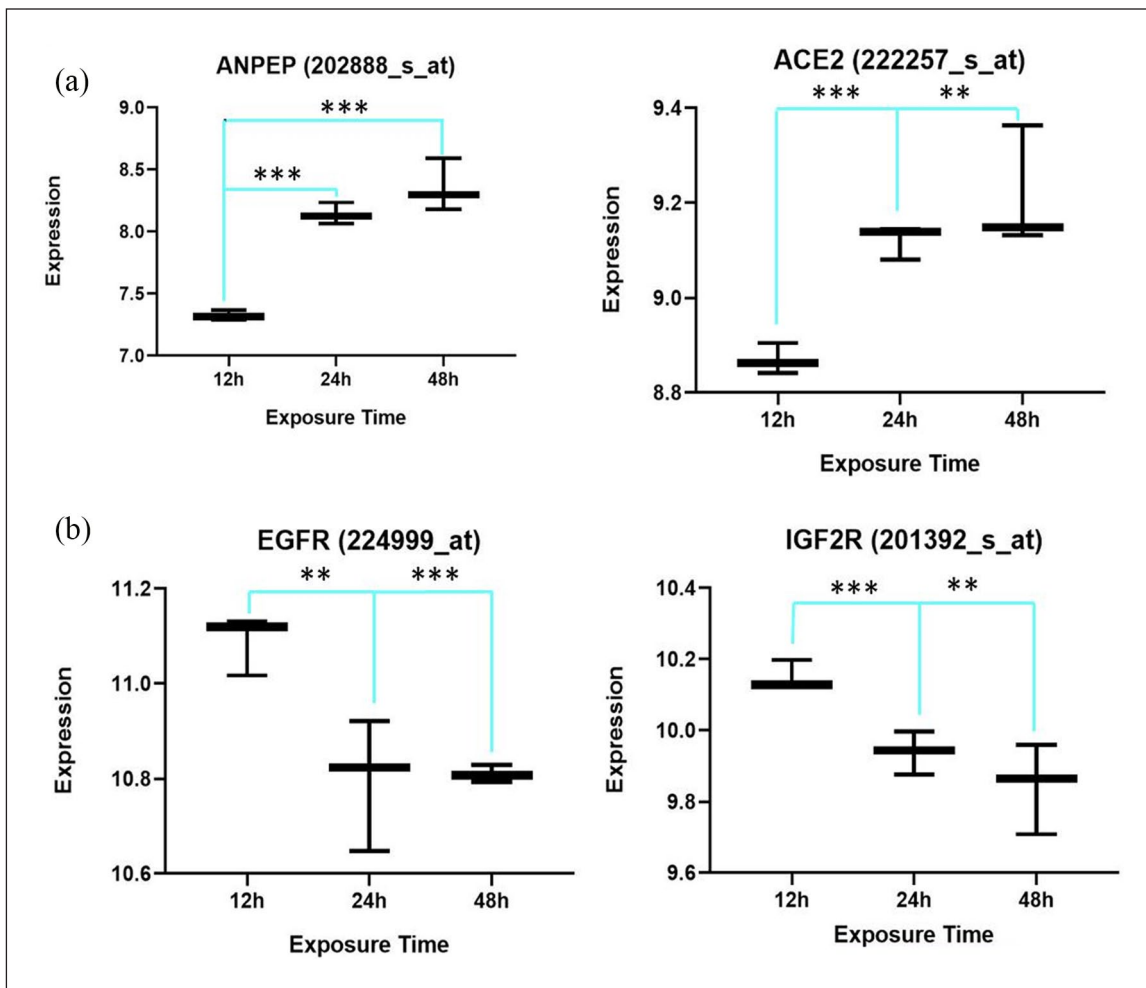
Among the three groups of human bronchial epithelial cells with different exposure times to SARS-CoV (12-, 24- and 48-hour groups), we identified the changes in expression of the RAS gene transcripts as well as the trend in expression of the most differentially expressed genes among groups other than the RAS family.

A total of seven RAS signaling pathway genes (nine probe sets; alanyl aminopeptidase (ANPEP), ACE2, angiotensin converting enzyme (ACE), insulin-like growth factor 2 receptor (IGF2R), angiotensinogen (AGT), epidermal growth factor receptor (EGFR) and membrane metalloendopeptidase (MME)) showed significantly different expression values between the 12-hour group and the 24-hour group. Comparing the 12-hour and 48-hour groups, eight genes (nine probe sets; arginyl aminopeptidase (RNPEP), epidermal growth factor receptor (EGFR), ANPEP, neurolysin (NLN), ACE2, IGF2R, leucyl and cysteinyl aminopeptidase (LNPEP) and cathepsin D (CTSD)) were statistically significantly expressed (Table 1). Four common genes (IGF2R, ANPEP, ACE2, and EGFR) in these two separate groups were identified (Figure 1).

Figure 2 shows the correlation of the expression and exposure time to the virus of the statistically significant



**Figure 1.** Venn diagram showing common genes between the 12-hour and 24-hour groups and the 12-hour and 48-hour groups. Four genes were found to be common to both groups. As mentioned, these genes consistently showed statistically meaningful expression differences according to exposure time to the virus.



**Figure 2.** Two out of four common differentially expressed renin-angiotensin system (RAS) genes show a positive correlation with exposure time to the virus (a), while the other common genes, epidermal growth factor receptor (EGFR) and insulin-like growth factor 2 receptor (IGF2R), show a negative correlation (b). \*\* $p \leq 0.05$ ; \*\*\* $p \leq 0.01$ .

**Table 2.** The most variant genes between all groups. 29 genes (36 Probe Sets) found to have  $>0.9$  standard deviation value. All of these genes are highly correlated with exposure time to viruses as most of them are immune genes.

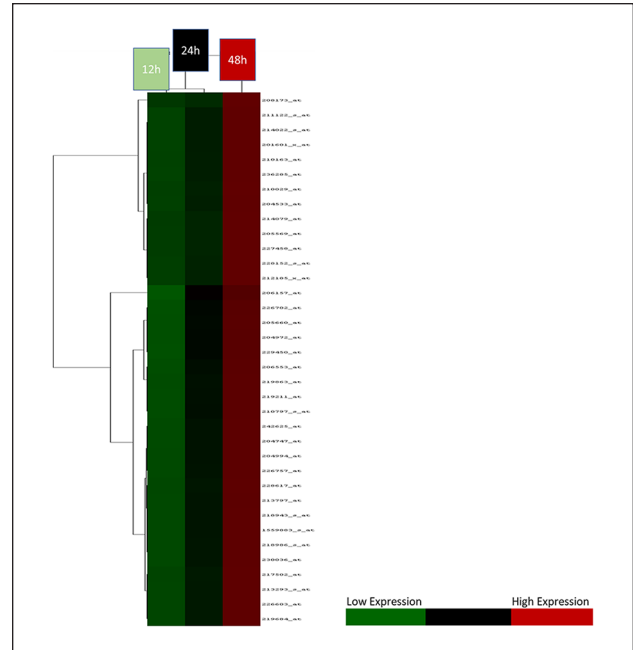
Probe set ID	Gene symbol	SD	Pearson's <i>r</i>
211122_s_at	<i>CXCL11</i>	1.68295417	0.99402069
210163_at	<i>CXCL11</i>	1.67995636	0.99308306
213293_s_at	<i>TRIM22</i>	1.64689117	0.99812764
214079_at	<i>DHRS2</i>	1.38291569	0.98065023
242625_at	<i>RSAD2</i>	1.3619956	0.99998811
208173_at	<i>IFNB1</i>	1.32200238	0.96674605
213797_at	<i>RSAD2</i>	1.29016111	0.99987245
210029_at	<i>IDO1</i>	1.28419322	0.98922768
226702_at	<i>CMPK2</i>	1.23620496	0.99368587
204972_at	<i>OAS2</i>	1.20086416	0.99494266
205660_at	<i>OASL</i>	1.18935939	0.99589149
214022_s_at	<i>IFITM1</i>	1.17991713	0.99401632
201601_x_at	<i>IFITM1</i>	1.17122332	0.99420957
206553_at	<i>OAS2</i>	1.14649914	0.99866159
204533_at	<i>CXCL10</i>	1.11355998	0.99132404
204994_at	<i>MX2</i>	1.10795097	0.99999559
228152_s_at	<i>DDX60L</i>	1.08126568	0.98604577
206157_at	<i>PTX3</i>	1.06159153	0.97359778
228617_at	<i>XAF1</i>	1.04957699	0.99912443
219863_at	<i>HERC5</i>	1.0436716	0.99976953
204747_at	<i>IFIT3</i>	1.04191315	0.99999404
217502_at	<i>IFIT2</i>	1.01930351	0.99727623
236285_at	<i>KLHDC7B</i>	1.01815991	0.99341293
205569_at	<i>LAMP3</i>	1.01338642	0.98242768
226757_at	<i>IFIT2</i>	1.0132658	0.99994894
229450_at	<i>IFIT3</i>	1.00446864	0.99482924
219211_at	<i>USP18</i>	0.99619048	0.9993661
218943_s_at	<i>DDX58</i>	0.94794454	0.99979879
218986_s_at	<i>DDX60</i>	0.94081343	0.99961176
210797_s_at	<i>OASL</i>	0.94028093	0.99930186
1559883_s_at	<i>SAMHD1</i>	0.93726862	0.9997964
219684_at	<i>RTP4</i>	0.93351655	0.99821178
226603_at	<i>SAMD9L</i>	0.93102808	0.99815238
212185_x_at	<i>MT2A</i>	0.92870941	0.98679396
227458_at	<i>CD274</i>	0.92087529	0.98185747
230036_at	<i>SAMD9L</i>	0.90113608	0.99960499

SD: standard deviation.

four common members of RAS genes found in the two groups (Figure 2).

In order to figure out the role of the non-RAS genes, we determined the most variant genes among these three groups. The standard deviation values of 29 genes (36 probe sets) were found to be  $>0.9$ . Linear regression analysis revealed that all these genes had a Pearson's  $r > 0.95$  and were highly correlated with the exposure time to the virus (Table 2).

Likewise, all of these genes showed a positive correlation with exposure time to the virus. The correlation figure for each gene that was found to be highly correlated to



**Figure 3.** Hierarchical cluster of most variant genes between three groups. As shown, determined genes were able to classify the groups clearly. In the 12-hour group, these genes show low expression, while in the 48-hour group, they all show high expression.

virus infection exposure time is presented in the Supplemental Material.

In addition, as shown in Figure 3, these genes were able to be clustered for the 12-, 24- and 48-hour groups separately.

Table 3 shows the pathway analysis of all the significant genes. With the aim of discovering the relationship of these genes with each other and with other genes, a network analysis was performed (Figure 4).

## Discussion

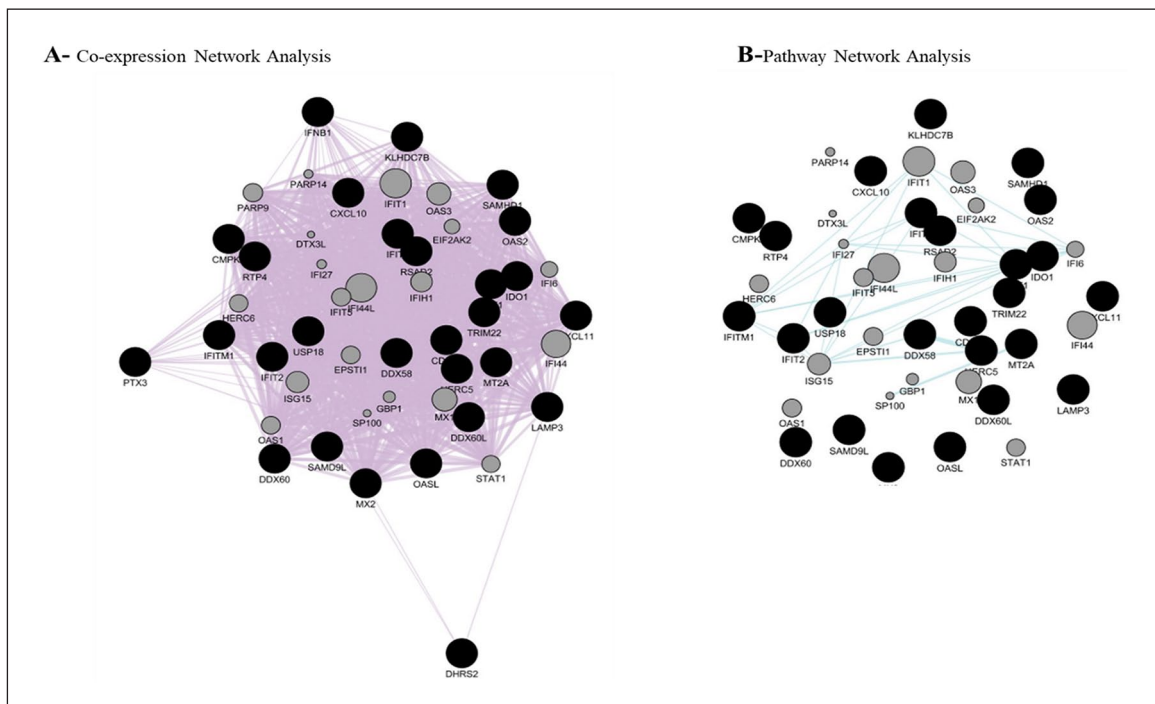
SARS-CoV-2 was found to be mostly similar to the SARS-CoV at the amino-acid level, despite some differences. Based on the phylogenetic analysis on all genomes of various viruses, SARS-CoV-2 is in the same beta-CoV clone as SARS-CoV, SARS-like bat CoV, and MERS-CoV. SARS-CoV-2 has the highest similarity with SARS-like bat CoV and is less associated with MERS-CoV.<sup>29</sup>

The mechanism of SARS-CoV and its association with the renin-angiotensin pathway had been defined in previous studies. The first genetic proof of the ACE2 and SARS-CoV receptor relationship was reported by Kuba et al.<sup>20</sup> Our results showed that in lung epithelial cells, ACE2 gene expression increased between 12 and 24 hours and remained at the same level between 24 and 48 hours after infection with SARS-CoV. Complementary to our findings, Kuba et al. demonstrated a decrement in the

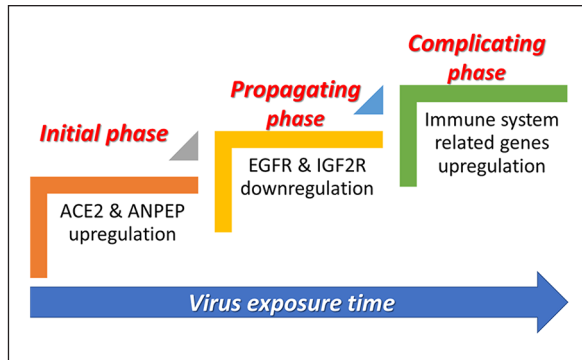


**Table 3.** Pathways related to non-RAS differentially expressed genes. Most of non-RAS differentially expressed genes were found to be related immune the system and pathways which could be involved during the course of the viral infectious disease.

<b>OAS2</b> 2'-5'-oligoadenylate synthetase 2 (OAS2)	Related genes	Homo sapiens
<b>KEGG_PATHWAY</b> hepatitis C, measles, influenza A, herpes simplex infection		
<b>CXCL10</b> C-X-C motif chemokine ligand 10 (CXCL10)	Related genes	Homo sapiens
<b>KEGG_PATHWAY</b> cytokine–cytokine receptor interaction, chemokine signaling pathway, Toll-like receptor signaling pathway, RIG-I-like receptor signaling pathway, cytosolic DNA-sensing pathway, TNF signaling pathway, influenza A		
<b>CXCL11</b> C-X-C motif chemokine ligand 11 (CXCL11)	Related genes	Homo sapiens
<b>KEGG_PATHWAY</b> cytokine–cytokine receptor interaction, chemokine signaling pathway, Toll-like receptor signaling pathway		
<b>CD274</b> CD274 molecule (CD274)	Related genes	Homo sapiens
<b>KEGG_PATHWAY</b> cell adhesion molecules (CAMs)		
<b>DDX58</b> DExD/H-box helicase 58 (DDX58)	Related genes	Homo sapiens
<b>KEGG_PATHWAY</b> nuclear factor kappa B signaling pathway, RIG-I-like receptor signaling pathway, cytosolic DNA-sensing pathway, hepatitis C, hepatitis B, measles, influenza A, herpes simplex infection, Epstein–Barr virus infection		
<b>CMPK2</b> cytidine/uridine monophosphate kinase 2 (CMPK2)	Related genes	Homo sapiens
<b>KEGG_PATHWAY</b> pyrimidine metabolism, metabolic pathways		
<b>IDO1</b> indoleamine 2,3-dioxygenase 1 (IDO1)	Related genes	Homo sapiens
<b>KEGG_PATHWAY</b> tryptophan metabolism, metabolic pathways, African trypanosomiasis		
<b>IFNB1</b> interferon beta 1 (IFNB1)	Related genes	Homo sapiens
<b>KEGG_PATHWAY</b> cytokine–cytokine receptor interaction, PI3K-Akt signaling, osteoclast differentiation, Toll-like receptor signaling pathway, RIG-I-like receptor signaling pathway, cytosolic DNA-sensing pathway, Jak-STAT signaling pathway, natural killer cell-mediated cytotoxicity, Chagas disease (American trypanosomiasis), tuberculosis, hepatitis C, hepatitis B, measles, influenza A, herpes simplex infection		
<b>LAMP3</b> lysosomal associated membrane protein 3 (LAMP3)	Related genes	Homo sapiens
<b>KEGG_PATHWAY</b> lysosome		
<b>MT2A</b> metallothionein 2A (MT2A)	Related genes	Homo sapiens
<b>KEGG_PATHWAY</b> mineral absorption		
<b>RSAD2</b> radical S-adenosyl methionine domain containing 2 (RSAD2)	Related genes	Homo sapiens



**Figure 4.** Co-expression and pathway analysis of non-RAS differentially expressed genes. The figure shows that 28/29 genes create a strong co-expression network. On the other hand, the program was able to connect some of the genes and predict some related genes in pathway network analysis.



**Figure 5.** Three important phases associated with SARS-CoV infection. At the initial and propagating phases of infection, some RAS family genes were up-regulated while some others were down-regulated. At the complicating phase, there is an increase in the expression of some key immune system genes.

ACE2 protein level in the lungs of SARS-CoV-infected mice on day 2. They also reported that the addition of SARS-CoV spike into mice exacerbates acute lung failure in vivo that can be reduced by inhibiting the renin-angiotensin pathway.<sup>20</sup>

Moreover, SARS-CoV-2 uses a similar mechanism as SARS-CoV. The structural analyses showed that like SARS-CoV, SARS-CoV-2 also utilizes ACE2 as the host receptor.<sup>16</sup>

Based on the evidence with regard to the similarity between SARS-CoV and SARS-CoV-2, we used data from Yoshikawa et al. In their study, all the genome expression data of the confluent 2B4 cells which were infected with SARS-CoV and grown in T-75 flasks for 12, 24 and 48 hours were used to characterize the dynamic, spatial and temporal changes of the gene expressions caused by SARS-CoV. The study was performed three times at each time point in order to meet the minimum number required for the application of statistical algorithms, and a total of nine arrays were given for SARS-CoV.<sup>23</sup>

Our current study was carried out to understand the biological mechanism of SARS-CoV-2 infection better by focusing on the similarity of SARS-CoV-2 and SARS-CoV in terms of structure, biological function, and the pathology of the infection. We proposed a hypothesis based on the genomic results obtained in the present study under three key headings (Figure 5).

### Initial phase

According to the results of the analysis, there was a significant up-regulation of ACE2 and ANPEP genes in human bronchial epithelial cells within 12 and 24 hours of infection. Many previous studies indicated that SARS-CoV also uses ACE2 as a receptor to enter host cells, as in SARS-CoV-2.<sup>30</sup> The results of our present study showed the additional possible function of ANPEP (Figure 5).

The ANPEP gene acts as a receptor, especially for HCoV-229E, another member of the CoV family. This virus is predicted to induce the infection by triggering conformational changes in the spike glycoprotein, which interacts with the host ANPEP receptor and activates membrane fusion.<sup>31</sup> Moreover, ANPEP (CD13) can act as a receptor in SARS-CoV and induce growth inhibition and apoptosis by infecting hematopoietic stem/progenitor cells.<sup>32</sup> These findings are in concordance with our results demonstrating the up-regulation in the ACE2 and ANPEP as exposure time to the virus increased.

As suggested in many previous studies, ACE2 forms Ang-(1-7) from Ang II and binds to the MAS receptor, the specific receptor of Ang-(1-7), and then inhibits inflammatory, vascular and cellular growth mechanisms.<sup>33</sup> In this case, it may be useful to use the agonistic peptides of Ang-(1-7) to change the functioning of ACE2 in favor of the infected host in the initial phase of infection.

### Propagating phase

Based on our results, in this phase, as the exposure time to SARS-CoV increases, EGFR and IGF2R, two receptors with key roles in the RAS signaling pathway, were significantly down-regulated in the infected human bronchial epithelial cells.

Habib et al. proposed that apoptosis occurs in various cell types that require an active tyrosine kinase but do not require EGFR autophosphorylation sites by experimentally increasing the level of EGFR expression. The expression of a predominant negative RAS mutant in cells that over-express the EGFR leads to significantly enhanced EGFR-induced apoptosis.<sup>34</sup> On the other hand, IGF2R, acting as a G-coupled protein receptor, could lead to the activation of the mitochondria-mediated apoptosis pathway, when insulin-like growth factor-II (IGF2) binds to this receptor.<sup>35</sup> Thus, those two receptors, which are associated with cellular apoptosis, are expected to be down-regulated as the infection time of virus-infected cells progresses. This event could give the chance for the virus to have sufficient time to continue replication and increase its copy number. This phase of the viral infection might be important, particularly for hypertensive subjects.

### Complicating phase

In this in silico genomic study, 29 genes (36 probe sets) were detected which showed noteworthy up-regulation in 24- versus 48-hour groups. Almost all of these are genes associated with the immune system, innate immune response, and adaptive immune response.

The relevant biological analyzers of these genes whose expressions are concordant with cell exposure time to the virus were explored via the pathway analyzers of the 29 genes. The genes involved in specific pathways related to

the critical pathological events of CoV infection, including SARS-CoV and SARS-CoV-2, such as the Toll-like receptor (TLR) signaling pathway.

TLRs are essential sensor molecules of the host innate immune system. Various TLRs are involved in early interactions of invasive viruses and host cells, which affect viral pathogenesis and regulate viral replication as well as host responses. Initiation of antiviral immune responses with TLR agonists has been shown to provide protection from many different viruses, including hepatitis B virus, influenza virus, some HIV strains, and CoV. Furthermore, non-structural protein 3 of the SARS-CoV interacts with IRF3 through the papain-like protease domain. After that, the associations of the TLR and RLR pathways occur, and this binding prevents the nuclear translocation as well as the phosphorylation of IRF3.<sup>36</sup>

It has previously been observed that the TLR signal through the TRIF adapter protein protects mice from lethal SARS-CoV. Thus, a balanced immune response that works both in TRIF and MyD88-guided pathways provides the most effective host-cell intrinsic antiviral defense responses against severe SARS-CoV, and the absence of any branch of TLR signaling leads to SARS-CoV being fatal.<sup>37</sup> Another important pathway is associated with the interferon beta 1 (IFNB1) gene identified as a result of pathway analysis is the natural killer (NK) cell-mediated cytotoxicity pathway. Interferons are considered as the screams of the affected cells during the active virus attack. IFNB1 deficiency results in partial suppression of the sterol pathway in macrophages during viral infections, thereby associating the regulation of the lipid metabolism pathway with interferon antiviral defense responses.<sup>38</sup> Furthermore, NK cells are important in immune defense against virus infections. Enlarged virus replication and more serious diseases during encephalomyocarditis virus, Coxsackie virus, and Theiler's murine encephalitis virus infections are linked with the reduction of NK cells or low levels of NK cell cytolytic function. NK cells are correspondingly associated with direct inhibition of virus replication and stimulation of liver damage during mouse hepatitis virus (MHV) infection. It is not known whether NK cells play a direct antiviral role in human infections with picornaviruses or CoV such as SARS.<sup>39</sup>

Lysosome, which has an important path in CoV infection, is another pathway related to the pathway analysis performed. Enveloped viruses must fuse with a host-cell membrane so as to transport their genomes to the host cell. Although some viruses fuse with the plasma membrane, many viral infections are associated with endocytosis before fusion. In the endosomal microenvironment, a particular marker induces conformational changes in viral fusion proteins, leading to viral and host membrane fusion.<sup>40</sup> According to the De Haan et al. study, the proteins known to be important for late endosomal maturation and endosome-lysosome fusion deeply promoted infection of cells with mouse hepatitis CoV (MHV).<sup>41</sup> In another study of SARS-CoV, SARS-CoV accessory protein open reading

frames (SARS 3a) were oligomerized by dynamically inserting them into late endosomal, lysosomal and trans-Golgi network membranes.<sup>42</sup> Furthermore, the chemotactic cytokines are engaged in a great deal of biological processes. Besides microbial infection, the infected cells have strong chemokine signals. Those signals could play significant roles in both innate and adaptive immune responses that control the growth of the invading pathogen.<sup>43</sup> Therefore, the presence of chemokine signaling pathways parallels the other results obtained. The main limitation of this study is the lack of uninfected control data, which is subject to further experimental studies.

## Conclusion

In conclusion, if the results obtained in the current study are validated by in vitro experiments and clinical samples, it can be suggested that the disruption of RAS genes may be important for the initial management of the CoV infections, particularly SARS-CoV-2. The results of the current study help us to understand better the pathobiology of SARS-CoV and other similar CoV family members such as SARS-CoV-2. Based on our results, the interactions between the SARS-CoV-2 and RAS genes, resulting in immune-related genomic disruption, lead to acquired immune deficiency states. It is hoped that critical local RAS-affecting drugs such as MAS agonists, soluble ACE2 and Ang-(1-7) will be used for the modulation of SARS-CoV-2 and RAS pathological interactions for the improvement of immune genomic states in patients infected with SARS-CoV-2 in future trials.

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