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ACE2 correlates with immune infiltrates in colon adenocarcinoma: Implication for COVID-19

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ABSTRACT

Novel coronavirus disease (COVID-19) pandemic has become a global health emergency. It has been reported that a few conditions, including cancer, predispose individuals to SARS-CoV-2 infection and severe form of COVID-19. These findings led us to evaluate the susceptibility of colon adenocarcinoma (COAD) patients to SARS-CoV-2 infection by investigating ACE2 expression in their tumor tissues. The expression analysis revealed that both mRNA and protein levels of ACE2 had increased in colon cancer samples than normal group. Next, the prognosis analysis has indicated that the upregulation of ACE2 was not correlated with patient survival outcomes. Further assessment displayed the hypomethylation of the ACE2 gene promoter in COAD patients. This methylation status has a strong negative correlation with ACE2 gene expression. The functional enrichment analysis of the genes that had similar expression patterns with ACE2 in colon cancer tissues demonstrated that they mainly enriched in Vitamin digestion and absorption pathway. Finally, we found that ACE2 gene expression had a significant association with the immune cell infiltration levels in COAD patients. In conclusion, it has plausible that COAD patients are more likely to be infected with SARS-CoV-2 and experience severe injuries. Moreover, COVID-19 would bring unfavorable survival outcomes for patients with colon cancer by way of immune cell infiltration linked process. The present study highlights the importance of preventive actions for COAD patients during the COVID-19 pandemic.

1. Introduction

Following the first report of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in China in December 2019, coronavirus disease (COVID-19) has affected more than 109 million confirmed cases around the world by the middle of February 2021 [1–4]. Several pieces of evidence have ascertained that SARS-CoV-2 could interact with angiotensin-converting enzyme 2 (ACE2) to infect the host cells [5–7]. Others have indicated that lung cells are the major target of SARS-CoV-2 [8,9]. However, the wide range of COVID-19 symptoms, such as fever, cough, tiredness, headache, diarrhea, and cardiac injury, implying that SARS-CoV-2 infects not only the respiratory tract but also various human organs [2,10–14]. This could reflect that the potential receptor of SARS-CoV-2 also is widely distributed in other parts of the body [15].

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Interestingly, it has been determined that the expression of ACE2 is high in many human tissues, including the lung, heart, liver, kidney, stomach, and colon [16,17]. It has been proposed that cell-free SARS-CoV-2 probably spread from the lungs to other different organs through blood circulation [15]. Noteworthy, previous reports have suggested that SARS-CoV-2 infection can decrease the expression of ACE2 on the infected cells, which may participate in the pathogenesis of SARS-CoV-2 infection and its organ damages. Considering this regard that SARS-CoV-2 has high homology with SARS-CoV and utilizes ACE2 for its entry, the ACE2 and its subsequent decreased expression have been introduced to have a pivotal role in the pathogenesis of COVID-19 and its related injuries [15,18–20]. Therefore, some researchers have tried to estimate the potential vulnerability of various organs to SARS-CoV-2 infection by evaluating the existence of ACE2 in different kinds of human organs through published single-cell RNA sequencing data. They have uncovered that susceptible organs with a high risk of infection are lung, heart, esophagus, kidney, bladder, and ileum [9,21]. Although a higher prevalence of COVID-19 has been reported in patients with pre-existing conditions like diabetes, hypertension, and cardiovascular diseases [22,23], multiple studies have suggested that cancer patients have a higher risk of being infected by SARS-CoV-2 and developing severe symptoms, mainly because of their compromised immune systems and the malignancy state [24,25]. B Wang et al. have indicated that among cancer patients with COVID-19, the prevalence of lung, colorectal, and breast cancer patients were 24.7%, 20.5%, and 13.0%, respectively, demonstrating that SARS-CoV-2 poses an additional risk to these patients [26]. Another research has shown that cancer patients infected with SARS-CoV-2 had an elevated risk of experiencing severe/critical disease and mortality than non-cancer patients [27].

Given the ACE2 roles in the pathogenesis of COVID-19, and the literature has reported that abnormal expression of ACE2 is a significant regulator of carcinogenesis in several cancers, some scientists have attempted to predict the susceptibility of cancer patients to SARS-CoV-2 infection and disease outcome by way of assessment of ACE2 expression in cancer tissues and related bioinformatic analyzes [28–32]. For instance, H Zhang et al. have revealed that patients with non-small cell lung cancer are probably susceptible to COVID-19 since the ACE2 has upregulated in tumor tissues [33]. Y Zhou et al. also have discovered that ACE2 expression has increased in uterine corpus endometrial carcinoma and kidney renal papillary cell carcinoma tissues, and upregulated ACE2 has positively associated with survival outcomes. They then declared that these patients have a high risk of SARS-CoV-2 infection, and COVID-19 may aggravate their clinical outcomes by immune-related processes [34].

Herein, we choose the Colon Adenocarcinoma (COAD) as the fourth most common cancer by incidence and the second leading cause of cancer-related deaths worldwide for our research. Besides, as we mentioned earlier, colorectal cancer is the second prevalent malignancy among cancer patients with SARS-CoV-2 infection [35,36]. Moreover, Haoyan Chen et al. recently have assessed the mRNA expression of ACE2 in the colon cancer tissues and healthy counterparts and have determined that ACE2 has markedly expressed in colon cancer epithelial cells. They then have indicated that ACE2 expression has elevated in colorectal tissues compared to normal ones [37]. Increasing data have shown the correlation of the immune state in colon cancer with the patient’s prognosis [38]. These findings led us to investigate the vulnerability of COAD patients to SARS-CoV-2 infection by analyzing the expression levels of the ACE2 in these patients as a fundamental component for the SARS-CoV-2 pathogenesis. Indeed, we checked the possibility of changing the COAD patients’ prognosis through the immune cell infiltration dependent mechanism after infection by COVID-19.

In the present study, we used several databases to identify the expression, prognostic merit, the methylation status of the promoter of ACE2, and its co-expressed genes in COAD. We also conducted an enrichment analysis of ACE2 co-expressed genes. Finally, we examined the association of ACE2 expression with immune cell infiltration level in COAD patients.

2. Material and methods

2.1. Expression analysis

The expression levels of the human ACE2 gene in the COAD and corresponding control tissues in The Cancer Genome Atlas (TCGA)-COAD cohort were assessed by Gene Expression Profiling Interactive Analysis 2 (GEPIA2) database (http://gepia2.cancer-pku.cn/#analysis) [39]. The TCGA provides total-gene expression, which includes all identified isoforms of the ACE2 gene. A p-value < 0.01, and log2 fold change [log2fc] > 1 were set as cut of criterion. Besides, we applied the protein expression analysis for ACE2 in normal tissues and colon cancer, utilizing the data from Clinical Proteomic Tumor Analysis Consortium (CPTAC) Confirmatory/Discovery dataset through the UALCAN database (http://ualcan.path.uab.edu/analysis-prot.html) [40].

2.2. Survival analysis

We introduced four online databases to perform the survival analysis for the ACE2 gene in COAD. We first evaluated the impact of ACE2 expression on the overall survival (OS) outcome of COAD patients, using the OncoLnc database (http://www.oncolnc.org/) [41], and the meta-survival analysis option in the Gene Expression database of Normal and Tumor tissues 2 (GENT2) database (http://gent2.appex.kr/gent2/) [42]. Then, we utilized the LOGpc database (http://bioinfo.henu.edu.cn/DatasetList.jsp) to assess the impact of ACE2 expression and several clinical features of COAD patients, including TNM staging, sex, lymph node, and race on their OS outcome. In addition, we used “Gene Outcome module” from Tumor Immune Estimation Resource2.0 (TIMER2.0) web tool (http://timer.cistrome.org/) [43] to investigate the relevance of ACE2 gene expression and clinicopathological characteristics of COAD patients (age, gender, race, and stage) with OS outcome. To conduct these analyses, a p-value < 0.05 was set as a cut-off, and COAD patients were divided into high expression and low expression groups based on the median of ACE2 expression levels in their tumor tissues.

2.3. DNA methylation analysis

We investigated the DNA methylation status of the ACE2 gene in the COAD cases of the TCGA-COAD cohort through the UALCAN database. Furthermore, the association between gene expression and promoter methylation of the ACE2 gene in primary COAD tissues was explored by the DNA Methylation Interactive Visualization Database (DNMIVD) database (http://1193.41.228/dnmivd/) [44]. The CpG sites that have been used for these analyses are listed in Table S1. A p-value < 0.05 was considered statistically significant.

2.4. Functional enrichment analysis

Based on the data of the TCGA-COAD, we employed the GEPIA2 database to find those genes that have a similar expression pattern with ACE2 gene in COAD. Then, by utilizing the data obtained from the Enrichr database (http://amp.pharm.mssm.edu/Enrichr) [45], we collected the top five Biological Process, Cellular Component, and Molecular Function terms in Gene Ontology (GO) analysis, the top five terms in Jensen TISSUES, Jensen COMPARTMENTS, and Jensen DISEASES, and the five top enriched pathway in Kyoto Encyclopedia of Genes and Genomes (KEGG) 2019 Human, WikiPathways 2019 Human, BioPlanet 2019, Reactome 2016, Panther 2016, and BioCarta 2016 databases, for these co-expressed genes. The collected terms were imported to Microsoft Excel 2019, and after removing those terms with a p-value greater than 0.05, they were sorted based on the combined score. This
value was provided by the Enrichr database and is the result of multiplying the log (Fisher’s exact test p-value) by z-score deviation from the expected rank. The bar chart was depicted using the Microsoft Excel 2019.

2.5. Immune cell infiltration analysis

We utilized the CIBERSORT algorithm through the TIMER2.0 database to evaluate the correlation of the ACE2 expression with immune cells infiltration in COAD, including CD8+ T cell, activated Memory CD4+ T cell, resting Memory CD4+ T cell, Naive CD4+ T cell, Regulatory T cell (Tregs), Follicular helper T cell, Gamma delta T cell, Memory B cell, Naive B cell, Plasma B cell, Neutrophils, Monocytes, Eosinophils, M0 Macrophages, M1 Macrophages, M2 Macrophages, activated Myeloid Dendritic Cell (DC), resting Myeloid DC, activated Natural Killer (NK) cell, resting NK cell, activated Mast cell, and resting Mast cell. The association between mRNA expression levels of ACE2 and immune infiltration levels were analyzed by tumor purity-adjusted partial Spearman’s correlation. A p-value < 0.05 was considered statistically significant.

Fig. 1. The expression analysis of the ACE2 in colon cancer. We demonstrated the expression level of the ACE2 gene in multiple cancers (A), especially in COAD tissues and corresponding normal tissues (B), using the GEPIA2 database. The protein expression levels of ACE2 in normal tissue and colon cancer tissue were also obtained by the UALCAN database (C). ACE2: angiotensin-converting enzyme 2; COAD: Colon Adenocarcinoma; GEPIA: Gene Expression Profiling Interactive Analysis.
3. Results

3.1. Expression analysis

First, we conducted the expression analysis using GEPIA2 database. As depicted in Fig. 1, pan-cancer analysis of the human ACE2 gene revealed that mRNA levels of ACE2 increased in several tumors, especially in colon cancer (Fig. 1A). Box plot analysis detected an over-expression level of ACE2 in the tissues of COAD (Fig. 1B, \( p < 0.01 \)), compared with the normal tissues. The protein expression analysis results further showed that the expression level of ACE2 protein was higher in the cancer tissues than that in the controls (Fig. 1C, \( p = 7.53\times10^{-4} \)).

3.2. Survival analysis

Based on the TCGA-COAD cohort data from the OncoLnc database and survival data of multiple GEO datasets in the GENT2 platform, we explored the prognostic merit of ACE2 mRNA expression in terms of OS for COAD patients. The results demonstrated the absence of association between elevated expression of the ACE2 gene and the OS of COAD patients (Fig. 2).

In addition, we used the LOGpc database to explore the effect of different clinical features of COAD patients, such as TNM staging, gender, lymph nodes, and race. The obtained data indicated that the high expression levels of ACE2 in tumor tissues were not associated with OS in COAD patients with stage I (\( p = 0.4806; \) Figure S1A), COAD patients with stage II (\( p = 0.294; \) Figure S1B), COAD patients with stage III (\( p = 0.2892; \) Figure S1C), COAD patients with stage IV (\( p = 0.8448; \) Figure S1D), male COAD patients (\( p = 0.7614; \) Figure S2A), female COAD patients (\( p = 0.0689; \) Figure S2B), COAD patients with lymph nodes (\( p = 0.5428; \) Figure S3A), COAD patients with no-lymph nodes (\( p = 0.7714; \) Figure S3B), White COAD patients with (\( p = 0.1779; \) Figure S4A), and in Black or African-American COAD patients (\( p = 0.9633; \) Figure S4B). We also employed the using the TIMER2.0 database to investigate the correlation between mRNA expression levels of ACE2 and overall survival in COAD patients.

Fig. 2. Survival analysis of colon cancer patients based on ACE2 expression. We used the OncoLnc, and GENT2 databases to assess the overall survival analyses based on the TCGA-COAD cohort data (A) and survival data of multiple GEO datasets (B), respectively. A log rank \( p \)-value \(< 0.05 \) was considered statistically significant. ACE2: angiotensin-converting enzyme 2; GENT2: Gene Expression database of Normal and Tumor tissues 2; TCGA: The Cancer Genome Atlas Program; COAD: Colon Adenocarcinoma.
ACE2 gene and OS outcome of COAD patients in a multivariate context (Table 1). The results showed that higher expression of ACE2 were not correlated with OS in these patients (p = 0.930). However, older patients (p = 0.012) and those with stage IV (p = 0.001) probably had increased risk and worse prognosis. Therefore, it is convincing that the overexpression of ACE2 mRNA was not correlated with the OS of COAD patients.

3.3. DNA methylation analysis data

We further studied the potential etiology of the elevated ACE2 expression in COAD patients. So, we used UALCAN database to determine the methylation status of ACE2 promoter in COAD. Interestingly, the methylation status of the ACE2 promoter in COAD was significantly reduced when compared with that in normal tissue (Fig. 3A). Meanwhile, DNMIVD database analysis indicated that the promoter region of the ACE2 gene was hypomethylated in COAD tumor tissues (Fig. 3B). Notably, the Pearson and Spearman correlation analysis revealed a significant negative correlation between the expression of the ACE2 mRNA and methylation status of the ACE2 gene in COAD (Fig. 3C-D). Thus, DNA methylation may be implicated in the molecular mechanism of the ACE2 gene overexpression in tumor tissues and colon cancer’s pathogenesis.

3.4. Functional analysis of co-expressed genes of ACE2 in COAD

The co-expression analysis using the GEPIA2 database provided a total of 100 associated genes for ACE2 in COAD (Table S2). The results revealed the top five related biological process (vitamin transmembrane transport, cellular triglyceride homeostasis, molybdopterin cofactor biosynthetic process, molybdopterin cofactor metabolic process, regulation of systemic arterial blood pressure by renin-angiotensin), cellular component (histone acetyltransferase complex, MLL1 complex, MLL1/2 complex, cortical cytoskeleton, spindle microtubule), and molecular function (ribosomal protein S6 kinase activity, sulfurtansferase activity, coreceptor activity involved in Wnt signaling pathway, planar cell polarity pathway, azole transmembrane transporter activity, N6-methyladenosine-containing RNA binding) in GO enrichment analysis (Fig. 4A-C).

Jensen TISSUES showed that these genes were mainly enriched in hutub80, CACO-2 cell, Mature stage, Choroid plexus papilloma cell, and Cuboid bone (Table S3). Jensen COMPARTMENTS also demonstrated that co-expressed genes with ACE2 mostly existed in Axon hillock, MPT synthase complex, Prefoldin complex, tetraspanin-enriched microdomain, and BRCA1-A complex (Table S4). Finally, the Jensen DIS-EASES determined that Coffin-Lowry syndrome, Biotin deficiency, Purine nucleoside phosphorylase deficiency, Hypohidrosis, and Congenital bile acid synthesis defect, were the top five diseases that correlated with the top 100 genes with similar expression pattern with ACE2 in COAD (Table S5).

Table 1

| Multivariate analysis of ACE2 expression and overall survival in COAD. The data was acquired from the TIMER2.0 database. |
|---------------------------------|
| Stage  | HR  | 95% CI of HR | P-value |
|-------|-----|--------------|---------|
| IV    | 6.83 | 2.225–19.478 | 0.001   |
| Age   | 1.029 | 1.006–1.053 | 0.012   |
| Stage III | 2.251 | 0.767–6.606 | 0.140   |
| Race (White) | 0.542 | 0.123–2.384 | 0.418   |
| Race (Black) | 0.603 | 0.124–2.922 | 0.530   |
| Gender | 1.174 | 0.688–2.004 | 0.555   |
| ACE2  | 1.006 | 0.874–1.159 | 0.920   |

P-value was calculated utilizing the Wald test. A p-value < 0.05 was defined as a threshold for this analysis. HR: hazard ratio; CI: confidence interval; ACE2: angiotensin-converting enzyme 2; COAD: Colon Adenocarcinoma.

The pathway enrichment analysis data explored the top five associated pathways with ACE2 correlated genes in COAD were Vitamin digestion and absorption, Sulfur relay system, Fat digestion and absorption, Renin-angiotensin system, Bladder cancer in KEGG 2019 Human database (Fig. 4D). The data from WikiPathways 2019 Human database also revealed that these genes probably were enriched in Thiamine metabolic pathways WP4297, miR-517 relationship with ARG1N1 and USP1 WP3596, ACE Inhibitor Pathway WP554, Robo4, and VEGF Signaling Pathways Crosstalk WP3943, let-7 inhibition of ES cell reprogramming WP3299 pathways (Table S6). Besides, according to the BioPlanet 2019 database, RSK activation, Molybdenum cofactor biosynthesis, Run in mitotic spindle regulation, Metabolism of vitamins and cofactors, and Angiotensin-converting enzyme 2 regulation of heart function were the most potential pathways in which these genes exerted their roles in COAD (Table S7).

Further enrichment analysis data unveiled the top five related pathways in the Reactome 2016 database (RSK activation Homo sapiens R-HSA-44257, Molybdenum cofactor biosynthesis Homo sapiens R-HSA-947581, Recycling pathway of L1 Homo sapiens R-HSA-437239, Scavenging by Class B Receptors Homo sapiens R-HSA-3000471, RNA processing in the mitochondrion Homo sapiens R-HSA-675470; Table S8) and Panther 2016 database (Pyruvate metabolism Homo sapiens P02772, TCA cycle Homo sapiens P00051, Insulin/IGF pathway-mitogen activated protein kinase kinase/MAP kinase cascade Homo sapiens P00032, Parkinson disease Homo sapiens P00049, Interleukin signaling pathway Homo sapiens P00036; Table S9) and BioCarta 2016 dataset (Role of Ran in mitotic spindle regulation Homo sapiens h ranMsPathway, Eukaryotic protein translation Homo sapiens h eifPathway, Pelp1 Modulation of Estrogen Receptor Activity Homo sapiens h pelp1Pathway, Erk1/Erk2 Mapk Signaling pathway Homo sapiens h erkPathway, CBL mediated ligand-induced downregulation of EGF receptors Homo sapiens h cblPathway; Table S10).

3.5. Immune cell infiltration analysis data

We investigated whether the transcription levels of the ACE2 gene play a role in the etiology of colon cancer through immune cell infiltration. As listed in Table 2, the expression of the ACE2 gene in colon cancer positively correlated with the infiltration levels of the following immune cells: Plasma B cell, Memory B cell, and activated myeloid DC. Moreover, the infiltration levels of CD8+ T cells, and activated NK cells negatively correlated with the ACE2 gene expression levels (Figure S5).

4. Discussion

The present report intended to seek the biological links of the human the ACE2 gene expression with SARS-CoV-2 in colon cancer patients from different perspectives.

We first used the GEPIA2 database and detected the overexpression of the ACE2 mRNA in primary tissues when compared with controls. Hao Xu et al. showed that ACE2 expression was upregulated in the mucosa of the oral cavity and significantly enriched in epithelial cells of the tongue [46]. The protein expression analysis by the UALCAN database displayed that the protein levels of ACE2 were increased in COAD patients. Yongyi Chen et al. also proved the higher expression of ACE2 in colon cancer versus other tissues through immunohistochemistry analysis [47]. Considering that ACE2 probably acts as a gate for SARS-CoV-2 entrance to the target cells like the colon, and basically the expression of the ACE2 gene in colon tissues is high [7,16], the observed upregulation of the ACE2 gene in tumor tissues of COAD patients may increase their risk for COVID-19 infection. Nonetheless, a current study that was done by Onabajo et al. has introduced a novel isof orm of ACE2, known as deltaACE2 (d-ACE2), which seems to act distinctively and does not serve as a tool for viruses to enter the host cell. They suggested interferons (type I, II, and III) and viruses induce this truncated version’s expression instead of full-length ACE2. They also proposed the higher
expression of dACE2 in squamous neoplasms of the respiratory, urogenital, and particularly gastrointestinal tract (the interested organ of our research), which are expected to be targeted by SARS-CoV-2 [48]. Since the GEPIA2 uses the data from TCGA for its expression analysis, and TCGA represents total-gene expression, consisting of full-length and short (d-ACE2) isoforms, we have to note that all the findings presented in our research probably are influenced by both isoforms. Therefore, we cannot deny that only the full-length isoform may increase the infection risk. However, the association between ACE2 isoforms and COVID-19 in COAD patients needed to be further explored in future experiments.

We further examined the prognostic significance of the ACE2 gene expression in patients with COAD utilizing the OnoLnc, GENT2, and LOGpc databases. The survival analysis showed no apparent association between mRNA levels of the ACE2 gene and the OS of COAD patients, in contrast with findings that proposed ACE2 could be used as a prognostic indicator in both kidney cancer and hepatocellular carcinoma [34,49]. It has been proposed that ACE2 may play opposite roles in the development of different cancers. For instance, while some reports have demonstrated that the elevated expression of ACE2 suppresses cell growth and angiogenesis in several cancers such as lung and colon cancer. Others have suggested that the upregulation of ACE2 probably promotes the migration and invasion of human kidney cancer. Therefore, the exact role of ACE2 in cancer development and progression is intricated and needed to be investigated [50]. Subsequently, using co-expression analysis of the GEPIA2 database, we obtained the ACE2 co-expressed genes in colon cancer tissues. Then carried out a series of functional annotation and enrichment analyses. Our data demonstrated that the ACE2 and its associated genes mediate COAD through the

Fig. 3. The promoter methylation status of the ACE2 gene in COAD. DNA methylation analysis by the UALCAN showed a lower methylation level for the ACE2 gene in COAD (A). We also detected the hypomethylation of the ACE2 promoter in COAD (B), and a strong negative correlation between the mRNA expression and the methylation status of the ACE2 gene in COAD (C, D), using the DNMIVD database. A p-value < 0.05 was defined as a threshold for this analysis. ACE2: angiotensin-converting enzyme 2; COAD: Colon Adenocarcinoma; DNMIVD: DNA Methylation Interactive Visualization Database.
various pathways in which RSK activation, Metabolism of vitamins and cofactors, and mitotic spindle regulation are the most probable pathway. It has been documented that the RSK signaling pathway regulates various carcinogenic events through mediating multiple processes such as protein synthesis, transcription factors, and other cellular modulators, thereby, takes its part in a variety of human cancers [51]. Finally, we evaluated the correlation of ACE2 gene expression with immune cell infiltration in patients with COAD. The data retrieved from the TIMER2.0 database indicated a considerable correlation between the ACE2 gene expression and the infiltration level of CD8+ T cell, Plasma B cells, memory B cell, activated myeloid DCs, and activated NK cells in patients with colon cancer. This result was consistent with an observation that indicated elevated expressions of the ACE2 mRNA in endometrium cancer were clearly correlated with immune infiltration levels [34,48].

The detected upregulation of the ACE2 gene in tumor tissues suggested that COAD patients were more vulnerable to SARS-CoV-2 infection. Several pieces of evidence highlighted the role of tumor-infiltrating immune cells in colon cancer patients’ survival [52,53]. For example, Wu D et al. have uncovered the remarkable association of memory B cells with colon cancer survival risk [38]. Our data also unearthed a considerable correlation between ACE2 expression and immune cell infiltration in COAD. It has indicated that the SARS-CoV genome sequence has high homology with SARS-CoV-2 [54]. Moreover, Kuba et al. showed the SARS-CoV decreased the expression of ACE2 in the lung cells [55]. These findings unveiled that the levels of ACE2 in colon tumor tissues similarly be reduced after COVID-19 infection. If so, the prognosis of colon cancer patients may be influenced by SARS-CoV-2 infection since we found a positive association between ACE2 expression and Immune cell infiltration in colon cancer patients.

It is necessary to note that during the severe phase of SARS-CoV-2 infection, cytokine storm results in systemic inflammation, various organ dysfunction, decreased number of natural killer cells and T cells, and dysregulated activation of neutrophils, monocytes, and macrophages. These neutrophils produce several products such as protein and DNA web-like structures, which are also known as neutrophil extracellular traps (NETs). It has been reported that the massive proinflammatory cytokine production and NET can activate the dormant cancer cells (DCCs) and cause metastasis by some processes; A) extensive NF-κβ activation in both immune and non-immune cells is the

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**Table 2**

Correlation between ACE2 gene expression and infiltration level of immune cells in COAD using TIMER2.0 database.

| Infiltrates                                      | Rho value | P-value |
|-------------------------------------------------|-----------|---------|
| activated Natural Killer (NK) cell              | -0.24844  | 0.00003 |
| Plasma B cell                                   | 0.22851   | 0.00113 |
| CD8+ T cell                                     | -0.20265  | 0.00072 |
| activated Myeloid Dendritic Cell (DC)           | 0.18477   | 0.00209 |
| Memory B cell                                   | 0.13652   | 0.02356 |
| resting Myeloid Dendritic Cell (DC)             | 0.11675   | 0.05312 |
| Gamma delta T cell                              | -0.10833  | 0.07288 |
| resting Memory CD4+ T cell                      | 0.10799   | 0.07379 |
| resting Mast cell                               | 0.09759   | 0.10633 |
| M1 Macrophages                                  | -0.09723  | 0.10764 |
| resting NK cell                                 | 0.08939   | 0.13925 |
| Eosinophils                                     | 0.08462   | 0.16169 |
| M2 Macrophages                                  | -0.07558  | 0.21152 |
| Monocytes                                       | 0.06902   | 0.25399 |
| Naive CD4+ T cell                               | 0.05555   | 0.35875 |
| M0 Macrophages                                  | 0.04913   | 0.41711 |
| activated Mast cell                             | -0.03626  | 0.54931 |
| Neutrophils                                     | -0.03356  | 0.57945 |
| Follicular helper T cell                        | -0.03299  | 0.58593 |
| Regulatory T cell (Tregs)                       | 0.03002   | 0.62099 |
| Naive B cell                                    | -0.02774  | 0.64698 |
| activated Memory CD4+ T cell                    | 0.00056   | 0.99261 |

* rho value of Spearman’s correlation, rho value was adjusted by purity.

**Fig. 4.** Data of GO and KEGG enrichment analysis. The top 100 genes with similar expression pattern with ACE2 in TCGA-COAD, the enriched information of biological process (A), cellular component (B), and molecular function (C) in GO analysis and KEGG pathway (D) were obtained using the Enrichr database. A p-value < 0.05 was defined as a threshold for this analysis. The terms were sorted according to the combined score computed by the Enrichr database. ACE2: angiotensin-converting enzyme 2; TCGA: The Cancer Genome Atlas; COAD: Colon Adenocarcinoma; GO: Gene Ontology; KEGG: Kyoto Encyclopedia Of Genes And Genomes.
consequence of elevated proinflammatory cytokines such as interleukin 6 (IL-6), which results in cancer cell proliferation and pro-metastatic microenvironment induction, B) it has been reported that lipopolysaccharide-induced lung infection causes not only epithelia-to-mesenchymal (EMT), but also reactivation and metastasis of breast DCCs, C) hypoxia and respiratory distress upon COVID-19 account for DCCs generation, drug resistance, EMT, and stemness, and D) NET-related proteases destruct laminin and activate integrin signaling in lung-resident DCCs, subsequently and therefore cause proliferation and lung metastasis [56–59]. Moreover, numerous studies have proposed that ACE2 affects tumor metastasis. For example, it has been reported that downregulation of ACE2 may promote metastasis in breast cancer, and lower expression of ACE2 was correlated with lymph node metastasis in squamous cell carcinoma of the gallbladder [60–63]. Therefore, it is plausible that COVID-19 could trigger metastasis in COAD patients through cytokine storm-related mechanisms and probable down-regulation of ACE2 in their tumor tissues. However, further studies focusing on the impact of COVID-19 on the metastasis event of COAD patients are crucial.

All data together, the ACE2 levels had increased in colon cancer tissues. Hence, these patients appear to be more susceptible to COVID-19. Furthermore, the SARS-CoV-2 infection presumably aggravates clinical outcomes and brings a poor prognosis for patients with COAD through metastasis and altered immune cell infiltration levels. Our findings highlight the value of protective actions for COAD patients during the COVID-19 pandemic era.

Data availability statement

All data generated/examined for the present research are presented in the manuscript context and the supplementary file.

CRediT authorship contribution statement

Mohsen Ahmadi: Conceptualization, Formal analysis, Methodology, Visualization, Software, Writing - original draft, Writing - review & editing. Salar Pashangzadeh: Writing - original draft, Writing - review & editing. Pegah Mousavi: Funding acquisition, Validation, Writing - original draft, Writing - review & editing. Negin Saffarzadeh: Writing - review & editing. Mohammad Amin Habibi: Writing - original draft, Writing - review & editing. Fatemeh Hajiesmaeili: Writing - original draft, Writing - review & editing. Nima Rezaei: Project administration, Supervision, Validation, Writing - original draft, Writing - review & editing.

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.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at http://doi.org/10.1016/j.intimp.2021.107568.

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