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

Mohsen Ahmadi [a,b,c,*], Salar Pashangzadeh [d], Pegah Mousavi [a,b,e,*], Negin Saffarzadeh [f], Mohammad Amin Habibi [g], Fatemeh Hajiesmaeili [c], Nima Rezaei [h,i,j,*]

[a] Student Research Committee, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
[b] Department of Medical Genetics, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
[c] Division of Medical Genetics, Booali Medical Diagnostic Laboratory, Qom, Iran
[d] Iranian Research Center for HIV/AIDS, Iranian Institute for Reduction of High-Risk Behaviors, Tehran University of Medical Sciences, Tehran, Iran
[e] Molecular Medicine Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
[f] Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[g] Student Research Committee, Qom University of Medical Sciences, Qom, Iran
[h] Research Center for Immunodeficiencies, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran
[i] Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[j] Network of Immunity in Infection, Malignancy and Autoimmunity (NIIMA), Universal Scientific Education and Research Network (USERN), Tehran, Iran

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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].

* Corresponding authors at: Division of Medical Genetics, Booali Medical Diagnostic Laboratory, Qom, Iran (M. Ahmadi). Research Center for Immunodeficiencies, Children's Medical Center Hospital, Dr. Qarib St, Keshavarz Blvd, Tehran 14194, Iran (N. Rezaei). Department of Medical Genetics, Faculty of Medicine, Hormozgan University of Medical Sciences, Imam Hossein Blvd, Bandar Abbas, Hormozgan, Iran (P. Mousavi).

E-mail addresses: Marajan@hums.ac.ir (M. Ahmadi), Salar71000@gmail.com (S. Pashangzadeh), pmousavi@hums.ac.ir (P. Mousavi), neginsaffarzade68@gmail.com (N. Saffarzadeh), mohammad.habibi1392@yahoo.com (M. Amin Habibi), hesmaeili1993@gmail.com (F. Hajiesmaeili), rezaei_nima@tums.ac.ir (N. Rezaei).

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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 infection can decrease the expression of *ACE2* on the infected cells, which may participate in the pathogenesis of SARS-CoV 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 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 severer 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 *ACE* 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/DatabaseList.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 $p\text{-value} < 0.05$ was considered statistically significant.

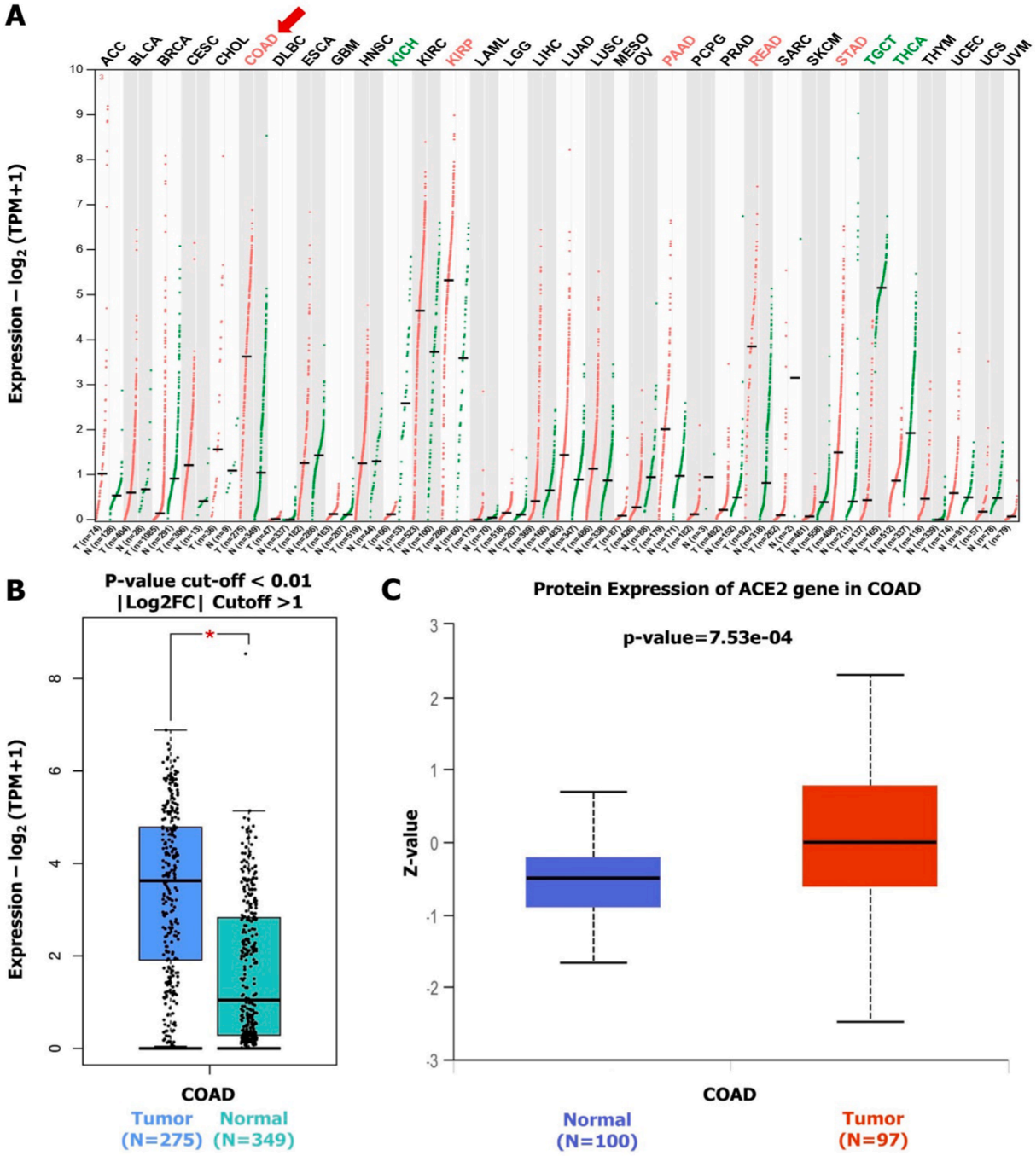

Fig. 1. The expression analysis of the ACE2 in colon cancer. We demonstrated the expression level of the *ACE2* gene in multiple cancers (A), especially in COAD tissues and corresponding normal tissues (B), using the GEPIA2 database. The protein expression levels of ACE2 in normal tissue and colon cancer 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 overexpression 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, $p = 7.53e\text{-}04$).

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

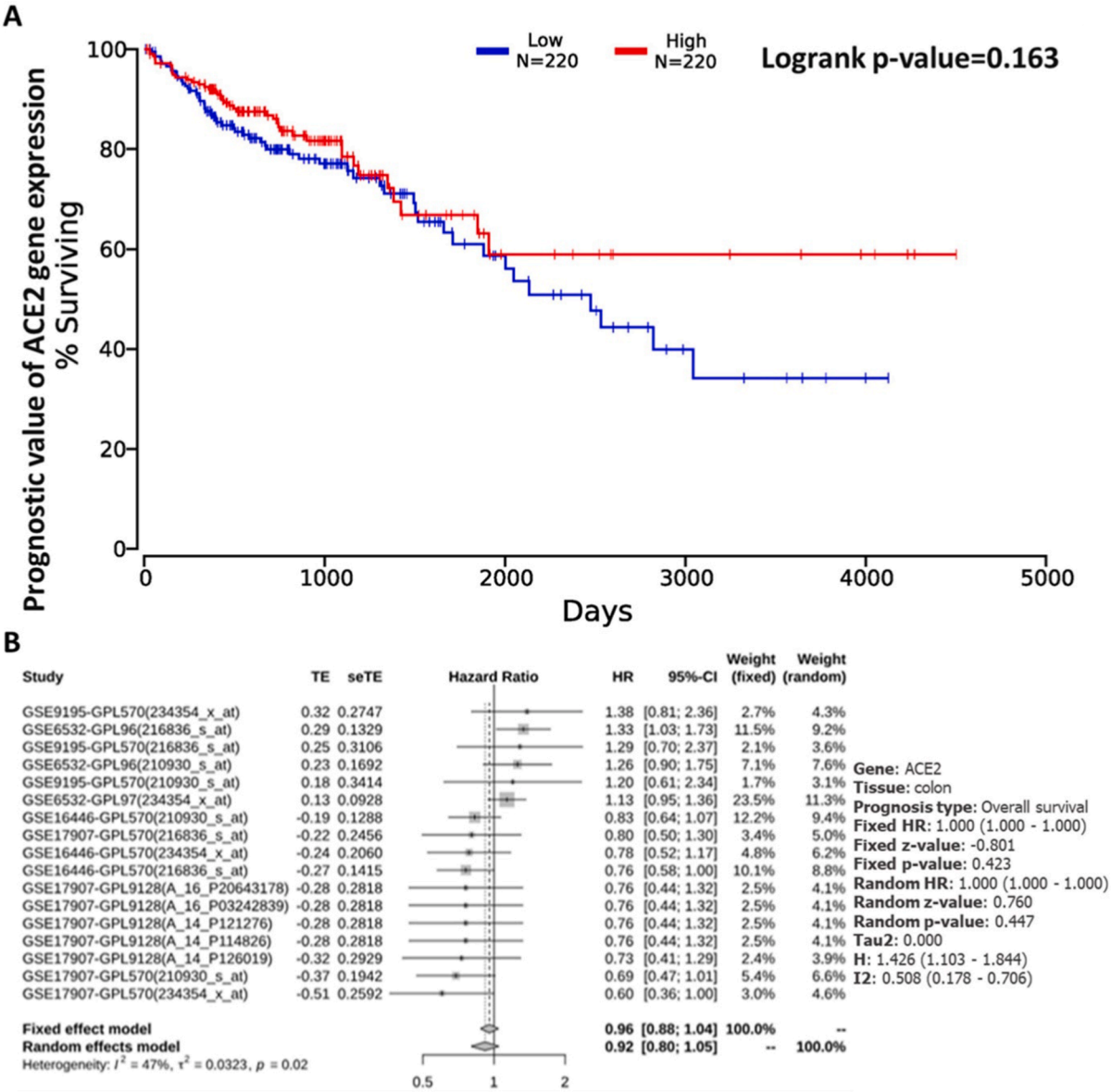

Fig. 2. Survival analysis of colon cancer patients based on ACE2 expression. We used the OnoLnc, and GENT2 databases to assess the overall survival analyses Based on the TCGA-COAD cohort data (A) and survival data of multiple GEO datasets (B), respectively. A log rank *p*-value < 0.05 was considered 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, sulfurtransferase 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 hutu80, 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 DISEASES 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.

	HR	95% CI of HR	*P*-value*
Stage IV	6.583	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
Stage II	1.182	0.392–3.560	0.766
ACE2	1.006	0.874–1.159	0.930

* 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 exploited 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 ARCN1 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, Ran role 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-444257, 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, tRNA processing in the mitochondrion Homo sapiens R-HSA-6785470; 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 isoform of *ACE2*, known as delta*ACE2* (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

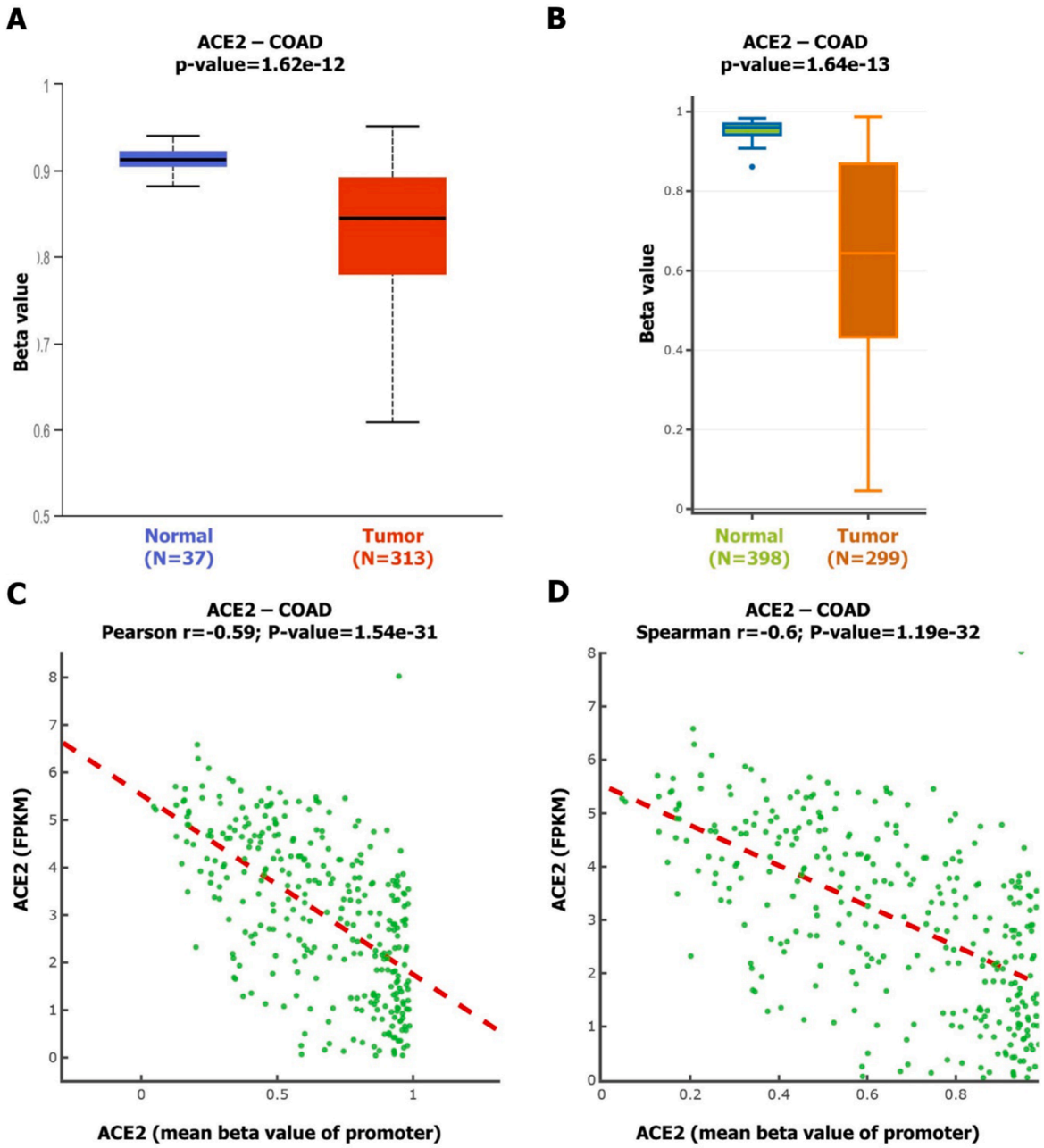

Fig. 3. The promoter methylation status of the *ACE2* gene in COAD. DNA methylation analysis by the UALCAN showed a lower methylation level for the *ACE2* gene in COAD (A). We also detected the hypomethylation of the *ACE2* promoter in COAD (B), and a strong negative correlation between the mRNA expression 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.

expression of d*ACE2* 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

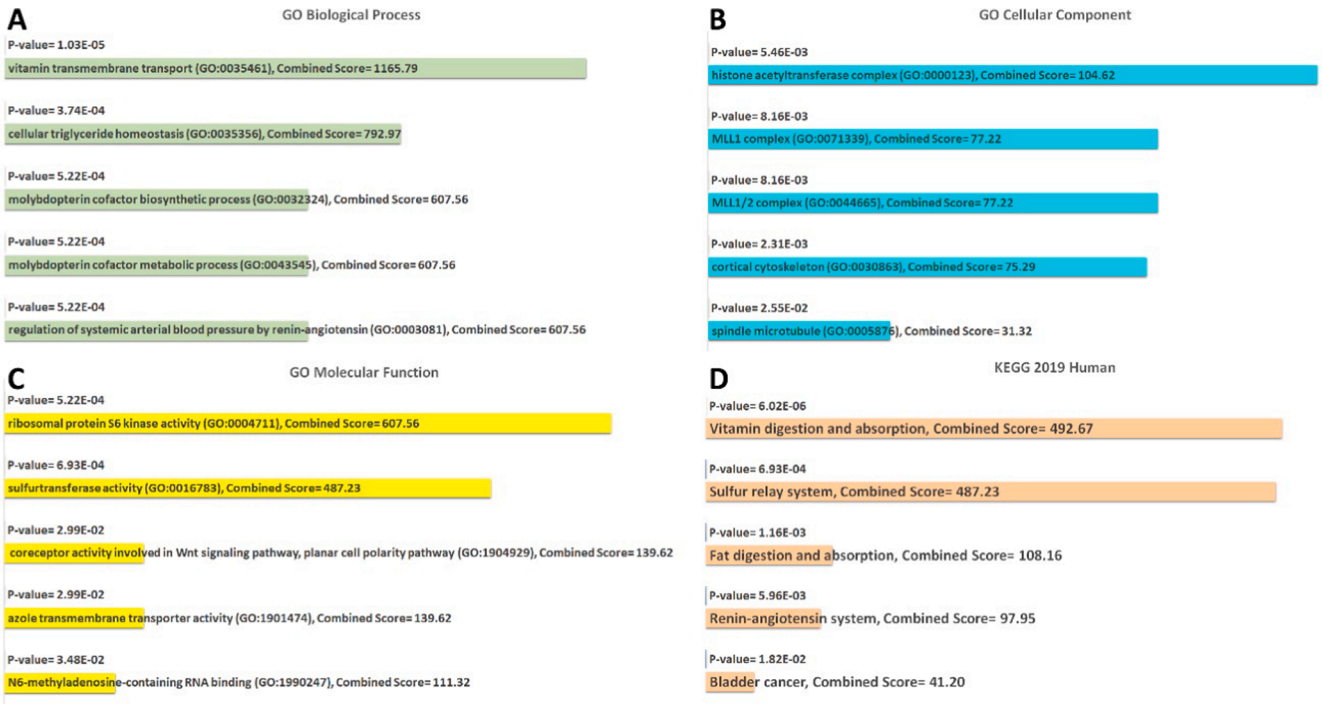

Fig. 4. Data of GO and KEGG enrichment analysis. The top 100 genes with similar expression pattern with *ACE2* in TCGA-COAD, the enriched information of biological process (A), cellular component (B), and molecular function (C) in GO analysis and KEGG pathway (D) were obtained using the Enrichr database. *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.

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	0.00013
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
Naïve 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	0.62009
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.
** A p-value < 0.05 was considered statistically significant. ACE2: angiotensin-converting enzyme 2; COAD: Colon Adenocarcinoma.

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

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 https://doi.org/10.1016/j.intimp.2021.107568.

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