

Expression and clinical significance of SARS-COV-2 receptor ACE2 in colon cancer

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Abstract

Background: Angiotensin-converting enzyme 2 (ACE2), a crucial cell entry receptor for severe acute respiratory syndrome coronavirus 2, has been identified as an oncogene in some tumour types. However, its role in colon cancer is poorly understood.

Methods: Integrative bioinformatics analyses were performed to uncover the role of ACE2 in colon cancer-associated immunology.

Results: The results showed that ACE2 was overexpressed in colon cancer tissues and correlated with poor survival. Moreover, ACE2 expression was closely associated with the immune-infiltrating levels of CD4+ T, CD8+ T, and neutrophils.

Conclusions: ACE2 is closely associated with colon cancer and may be involved in tumourigenesis and cancer-immune interactions, and could be a promising prognostic and therapeutic biomarker in colon cancer.

Background

In 2019, novel coronaviruses induced pneumonia outbreaks, causing a widespread global mortality. The pathogenesis and treatment of new coronavirus pneumonia have attracted a lot of attention from researchers [1, 2]. A large number of studies and analyses showed that severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) can specifically bind to the human angiotensin-converting enzyme 2 (ACE2) receptor, thus playing an important role in viral transmission [3, 4]. ACE, a metalloproteinase encoding 805 amino acids, is a type I transmembrane glycoprotein with a single extracellular catalytic domain [5]. ACE can transform inactive angiotensin (Ang) I into Ang II which regulates vasoconstriction. Ang II is the core effector component of the renin-angiotensin system (RAS) and has many biological functions through angiotensin receptors [6]. ACE2, a homolog of ACE, can split Ang II into Ang-(1-7), which indirectly participates in a variety of biological reactions in vivo [7, 8]. In addition, ACE2 regulates innate immunity and affects the composition of intestinal microbiota, and is directly involved in the initiation of inflammation [9]. ACE2 has been proven to be an essential molecule in the expression of neutral amino acid transporters on the surface of epithelial cells and also regulates insulin secretion and islet cell growth [10]. Recent studies have shown that ACE2 is involved in the occurrence and development of various cancers, including breast, lung, skin, stomach, thyroid, oral and colon cancers [11, 12, 13, 14, 15, 16, 17]. However, its specific functions and mechanisms in colon cancer are still poorly understood.

Colon cancer, and digestive malignant tumours occurring in the colon, is a common malignant tumour worldwide, and is ranked third among malignant tumours [18, 19, 20]. According to the different pathological types, colon cancer is mainly divided into adenocarcinoma, mucinous adenocarcinoma, and undifferentiated carcinoma [21]. The incidence of colon cancer varies from country to country. It also correlates with gender, age, and other factors [22]. Although the current level of medical technology in the treatment of colon cancer has improved greatly, many patients with colon cancer die [23]. The main

reason is that most patients with colon cancer are diagnosed at an advanced stage, and some patients show recurrence after surgery, which does not significantly prolong the overall survival rate of colon cancer [24]. Therefore, early diagnosis, identification, and treatment are of great significance for patients with colon cancer [25, 26].

Thus, the purpose of this study was to explore the role of ACE2, a novel coronavirus receptor, in colon cancer and its possible mechanism of action. Using the corresponding bioinformatics sites, we found that ACE2 was highly expressed in colon cancer tissues and cell lines. Survival analysis by MethSurv has shown that ACE2 is associated with colon cancer survival time, which could be used as a prognostic factor for colon cancer. From the mechanism, we found the expression levels of ACE2 protein and DNA methylation between patients with colon cancer and normal people on the UALCAN website, and properly drew a conclusion that their expression levels were significantly different, which can be used to evaluate the prognostic value of colon cancer. Furthermore, KEGG and gene ontology (GO) analyses revealed that the associated genes and signal pathways were co-expressed with the ACE2 gene, which demonstrates that ACE2 plays a vital role in the diagnosis and treatment of colon cancer.

Materials And Methods

Data were collected and reanalysed using different bioinformatics means.

Analysis of the expression levels of ACE2 in colon cancer tissues and cell lines was performed using a variety of bioinformatics network resources (Supplementary Table 1, Additional File 1).

Oncomine is a web-based data-mining platform and a cancer microarray database that contains 65 gene expression datasets, including nearly 48 million genes from over 4,700 microarray experiments [27]. The HPA database is a large-scale protein research project. The main purpose of this project is to map the location of proteins encoded by expressed genes in human tissues and cells [28, 29, 30]. The UALCAN website offers an electronic platform for the validation of target genes and the identification of tumour subgroup-specific candidate biomarkers [31]. GEPIA is a web server for human cancer gene expression and interaction analyses. It can provide researchers with interactive customisation functions such as differential expression analysis, patient survival analysis, and similar gene detection [32]. The Cancer RNA-Seq Nexus database, also known as CRN database, can directly provide information on gene expression [33]. After using these public bioinformatics platforms for analysis, we had a rudimentary understanding of the expression profile of ACE2 in human colon cancer tissues and cell lines.

MethSurv is a valuable and universal data platform that can be used for the preliminary evaluation of biomarkers of methylated cancer and rapid judgement of disease prognosis. The platform's data consist of 25 different human cancers, including 7358 methyl groups [34].

We downloaded two microarray datasets GSE56496 [35] and GSE34299 [36] from the GEO database, and further considered the connection between ACE2 expression and colon cancer-related drug therapy using SPSS software.

cBioPortal database integrates data from CCLE, TCGA, and several independent large-scale cancer research projects, which promotes the exploration, visualisation, and analysis of multidimensional cancer genomics data [37]. The convenient database makes it easy to obtain the co-expression genes of ACE2 in colon cancer tissues. Next, we constructed a protein-protein interaction (PPI) network with these co-expressed genes using the STRING database [38]. Then, a detailed visual analysis was carried out by the software of Cytoscape (version 3.7.2) and DAVID website [39, 40]. Next, we used WebGestalt to perform the enrichment analysis of GO [41]. At the same time, the Pathview Web algorithm was used to analyse the relevant KEGG paths, and the R language package was used to further analyse the signal paths [42].

Immune infiltration analysis using the Tumour Immune Estimation Resource (TIMER)

The website tool TIMER is a comprehensive resource based on the TCGA database. We utilised TIMER gene modules for systematic analysis of the correlation of ACE2 expression with each immune infiltrating cell in colorectal cancers. The immune cells contained CD4+ T cells, CD8+ T cells, and neutrophils. For further investigation, we evaluated the associations between ACE2 expression and tumor-associated macrophage (TAM), monocyte, M1 macrophage, and M2 macrophage gene markers through the TIMER correlation modules. Log₂ RSEM was used to calculate expression levels of genes, supplemented with purity-corrected partial Spearman method to calculate the correlation.

Statistical analyses

The difference in ACE2 mRNA expression between cancer tissue and para-carcinoma tissue was investigated using Student's t-test and statistical software package SPSS (SPSS 23.0, IBM Analytics). At the same time, the chi-square test was used to determine the association between the expression of ACE2 and the clinicopathological characteristics of patients with colon cancer. Multivariate analysis used the Cox regression model and Pearson's correlation coefficient to estimate the correlations among genes. In the case of $p \leq 0.05$, the analysis results were statistically significant.

Results

ACE2 is overexpressed in colon cancer tissues

We analysed the expression levels of ACE2 in colon cancer tissues using various independent bioinformatics databases and found that the expression levels of ACE2 mRNA, protein, and methylation were significantly increased in colon cancer samples. First, in the HPA database, we found that ACE2 expression differs among various types of human cancers, and it was highly expressed in colon cancer tissues (Fig. 1A). Second, we downloaded the relevant data from the Hong colorectal group through the Oncomine database for further analysis, which contained 70 colon cancer samples and 12 normal samples. It was found that the increase in expression level of ACE2 mRNA in the colon cancer samples

was statistically significant ($p < 0.05$, Fig. 1B). Differential ACE2 mRNA expression was further confirmed using the GEPIA database (Fig. 1C). From the GEO dataset (GSE1737), we compared 40 pairs of colon cancer and adjacent normal tissues and found that the ACE2 mRNA expression in tumour tissues was higher than that in adjacent tissues, which was statistically significant (Fig. 1D). Moreover, to further explore the expression levels of ACE2 protein and methylation in colon cancer tissues, the UALCAN website was used to find outstanding between 97 colon cancer samples and 100 normal samples ($p < 0.01$, Fig. 2A). After grading and investigation, ACE2 expression was observed to be significantly elevated in stages 1 and 2 colon cancer samples compared with normal tissues ($p < 0.05$, Fig. 2B). To further verify these observations, we excavated the immunohistochemical map from HPA, and found that ACE2 protein expression was positive in colon cancer, but negative in normal tissue (Fig. 2C). Finally, we explored the methylation level of ACE2 in colon cancer tissues using UALCAN, and the results suggested that the ACE2 methylation in colon cancer tissues was much lower than that in normal tissues (Fig. 2D). The grading samples also showed significant differences in methylation between stages 1, 2, and 3 colon cancer tissues and normal tissues (Fig. 2E). Moreover, by accessing the Cancer RNA-Seq Nexus (CRN) database, we found that ACE2 expression in the pathological stages (I-IV) of colon was higher than that observed in normal tissues (Table I). These results demonstrate that ACE2 is overexpressed in colon cancer tissues.

Table I The expression of ACE2 in TCGA Colon adenocarcinoma (COAD) RNA-seq dataset were analyzed by the Cancer RNASeq Nexus

	ACE2 (Transcript ID: uc004cxb.2)		
Colon adenocarcinoma subset pair	Average expression in cancer	Average expression in normal	Cancer versus Normal p-value
Colon adenocarcinoma--Stage I versus Normal (adjacent normal)	2.18	0.62	p <0.05
Colon adenocarcinoma--Stage II versus Normal (adjacent normal)	1.05	0.62	
Colon adenocarcinoma--Stage IIA versus Normal (adjacent normal)	2.17	0.62	
Colon adenocarcinoma--Stage III versus Normal (adjacent normal)	1.38	0.62	
Colon adenocarcinoma--Stage IIIA versus Normal (adjacent normal)	2.45	0.62	
Colon adenocarcinoma--Stage IIIB versus Normal (adjacent normal)	4.20	0.62	
Colon adenocarcinoma--Stage IV versus Normal (adjacent normal)	1.80	0.62	

Note: The Cancer RNASeq Nexus (CRN, <http://syslab4.nchu.edu.tw/CRN>) is an open resource for intuitive data exploration, providing coding-transcript/lncRNA expression profiles that was contained alternative splicing to support researchers generating new hypotheses in cancer research and personalized medicine.

Fig.1 Expression of ACE2 in different tumor tissues. (A) The expression levels of ACE2 in different cancer tissues were provided by HPA database. (B) The expression levels of ACE2 mRNA in colon cancer in Hong data sets was downloaded from oncomine database. (C) From the GEPIA database, the expression levels of ACE2 mRNA in colon cancer tissues was detected. (D) ACE2 mRNA expression levels in colon cancer tissues and paired normal tissues were downloaded from the GEO database.

Fig. 2 Expression of ACE2 protein and its methylation in colon cancer from UALCAN database. (A) The expression level of ACE2 protein in colon cancer tissues was compared with that in normal tissues. (B) Difference of ACE2 protein expression and tumor grade in colon cancer. (C) The immunohistochemical results of ACE2 in colon cancer and adjacent tissues were downloaded from HPA public bioinformatics website. (D) Methylation expression of ACE2 in colon cancer and normal tissues. (E) The association between the methylation level of ACE2 and the grade of colon cancer. (Among them, * represents significant difference, i.e, P < 0.05)

Correlation of ACE2 expression with clinicopathological features

The correlation between expression of ACE2 and clinicopathological features of patients with colon cancer was assessed using clinical data from the TCGA database. These data included age, sex, T stage, N stage, M stage, TNM stage, and clinical stage, which showed that ACE2 expression is positively correlated with age and T stage ($p=0.003$ and $p=0.024$, respectively), while it was negatively correlated with sex, N stage, M stage, and clinical stage ($p>0.05$) (Table II). From the univariate analysis, a multivariate analysis was performed and confirmed that ACE2 expression was significantly correlated with age, T stage, and clinical stage. The correlation between ACE2 and clinicopathological features in colon cancer samples should be clarified in future studies (Table III).

Table II Relationship between ACE2 expression and clinicopathological parameters in colon cancer

Parameter		Number	ACE2 mRNA expression	P value	
Low(n=272)	High(n=167)				
Age	<=80	370	218	152	0.003
	>80	69	54	15	
Gender	Male	231	140	91	0.539
	Female	208	132	76	
T stage	T1+T2+T3	385	231	154	0.024
	T4	54	41	13	
N stage	N0+N1	360	220	140	0.435
	N2	79	52	27	
M stage	Mx+M0	375	232	143	0.542
	M1	64	40	24	
Pathologic stage	Stage I	74	44	30	0.628
	Stage II+III+IV	365	228	137	

Table III Multivariate Cox regression analysis of ACE2 and clinic pathological characteristics

Covariate	HR	95% CI for HR	P value
Age (<=80 vs >80)	0.405	0.219-0.750	0.04*
Gender (Male vs Female)	1.104	0.743-1.640	0.625
T (T1+T2+T3 vs T4)	0.537	0.346-0.833	0.006*
N (N0+N1 vs N2)	0.729	0.480-1.107	0.138
M (Mx+M0 vs M1)	0.709	0.433-1.160	0.171
Stage (Stage I vs Stage II+III+IV)	1.642	1.032-2.611	0.036*

From the GEO database, we downloaded a microarray dataset associated with chemotherapy to further determine the biological role of ACE2 in the treatment of patients with colon cancer. From the dataset of GSE56496, it was found that rosemary diterpenes have an anti-tumour effect in colon cancer SW620 cell lines, and expression of ACE2 correlated with the dose of rosemary diterpenes ($p=0.004$) [35] (Fig. 3A). The dataset from GSE34299 demonstrated that colon 205RC PLX4720 cell lines presented high ACE2 expression, and the expression levels differed in different colon cancer cell lines. The differences among cell lines were statistically significant ($p<0.05$) [36] (Fig. 3B). Finally, we found from MethSurv, a survival analysis website, that a high expression of ACE2 correlated with a shorter survival time ($p=0.05$) (Fig. 3C).

Fig. 3 Analysis of ACE2 gene on the treatment of colon cancer from GEO database. (A) Dose effect of supercritical rosemary extract on SW620 colon cancer cells downloaded from GEO database. (B) Relationship between acquired resistance to BRAF inhibitors and expression of ACE2 in colon cancer cell lines. (C) To explore the relationship between ACE2 expression and survival time in colon cancer using by MethSurv database.

Analysis of the co-expression network and pathway enrichment of ACE2

From the above-mentioned general analysis of ACE2 expression, we further explored the biological functions of ACE2. From the cBioportal database, we downloaded ACE2-related co-expressed genes, performed primary screening based on the condition of $\text{Log}|FC|>0.7$ and $p<0.05$, and identified 217 differentially expressed genes (Supplementary Table 2, Additional File 2). We conducted a corresponding PPI network using the STRING site and Cytoscape software, and found that the trefoil factor 3 (TFF3) gene had the largest relevance. At the same time, the RAS signalling pathway was found to be the most correlated pathway using the functional annotation tool of DAVID for KEGG pathway enrichment analysis (Fig. 4B; Supplementary Table 3, Additional File 3). Furthermore, we used the WebGestalt tool to perform GO biological analysis of these 217 differentially expressed genes and obtained the following results. On biological process analysis, we found that biological regulation and the metabolic process were strongly enriched in these co-expressed genes, and on molecular function analysis, the main related process turned to protein binding. As for the cellular component, the membrane was mainly enriched (Fig. 4C).

Fig. 4 Analysis of ACE2 co-expression network. (A) Using STRING database, DAVID website and Cytoscape software to construct the network of co-expression gene with ACE2. (B) The KEGG enrichment pathway of co-expressed genes was analyzed by DAVID biological website, and bubble diagram was drawn by R language package. (C) The analysis of biological processes, molecular functions and cell components is from WebGestalt website.

Correlation between ACE2 expression and immune infiltration in colon cancer

To investigate the association between ACE2 and the immune response in the tumour microenvironment (TME), TIMER provided high-throughput immune cell infiltration data. Significant correlations were found between expression of ACE2 and the infiltrating levels of immune cells. Among them, CD8+ T cells ($r=-0.207$, $p=5.38e-04$) and neutrophils ($r=-0.15$, $p=1.27e-02$) showed a negative correlation, while CD4+ T cells ($r=0.14$, $p=2.02e-02$) showed a positive correlation (Fig. 5). These results suggest the potential inhibitory effect of ACE2 expression on immune infiltration in colon cancer.

Correlations with immune signature markers of typical immune infiltrating cells were further characterised using the TIMER database. We used signature TAM, monocyte, M1 macrophage and M2 macrophage markers to evaluate their association with ACE2 expression, and the results are illustrated in Fig. 6. Although there was no significant association between ACE2 expression and the TAM gene marker CCL2 ($p>0.05$), while the TAM gene marker CD68 and IL10 show significant correlations (all $p<0.05$, Fig. 6A). We also found that ACE2 expression significantly correlated with monocyte (CD86, C3AR1, and CSF1R), M1 macrophage (IRF5, PTGS2, and NOS2), and M2 macrophage (MS4A4A, CD163, and VSIG4) expressions (all $p<0.05$, Fig. 6B-D). Therefore, ACE2 may participate in restraining the monocyte-macrophage system and regulating macrophage polarisation. These results indicate that ACE2 plays an important role in the immune response in TME by affecting immune cell infiltration in colon cancer.

Fig. 5 Correlation between the expression of ACE2 and the level of immune infiltration in colon cancer. (A) There was a positive correlation between the expression of ACE2 and the immune infiltration of CD8 + T cells in colon cancer. (B) The expression of ACE2 was positively correlated with the immune infiltration of CD4 + T cells. (C) The infiltration of Neutrophil cells was negatively correlated with the expression of ACE2.

Fig. 6 Correlation between ACE2 expression and tumor markers of macrophages, monocytes, M1 macrophages, M2 macrophages. (A) Correlation between ACE2 expression and tumor associated gene markers CCL2, CD68 and L10 in macrophages. (B) ACE2 expression is associated with CD86, C3AR1 and CSF1, which are gene markers related to mononuclear . (C) ACE2 expression is associated with RF5, PTGS2 and NOS2, which are gene markers related to M1 macrophages. (D) ACE2 expression is associated with MS4A4a, CD163A and VSIG4, which are gene markers related to M2 macrophages.

Discussion

The main purpose of this investigation was to study the potential role of ACE2 in the development of human colon cancer. In this study, public datasets were first used to analyse the expression levels of ACE2 in colon cancer. At the same time, some co-expressed genes and signalling pathways that may be of significance in tumour progression were found. After analysing the GEO and TCGA databases, we found that ACE2 expression was significantly upregulated in colon cancer tissues. In addition, we also found a correlation between ACE2 expression and clinicopathological features such as age and T stage.

Ang II is a multifunctional bioactive peptide in the RAS, and ACE2 is an important member of the RAS. ACE2 is known to be the SARS-CoV-2 receptor necessary to complete the infection process [43]. SARS-CoV-2 infection can induce ACE2 shedding and cause multiple multi-organ dysfunction and poor prognosis in a RAS-dependent manner by promoting vasoconstriction, inflammatory response, hypertension, oxidation, and fibrosis [44]. The absorption of amino acids by small intestinal epithelial cells and proximal renal tubular cells is mainly dependent on the neutral amino acid transporter B(0)AT1 (SLC6A19). It was found that the expression of this transporter mainly depends on ACE2, which proved that ACE2 may also have a regulatory role in amino acid absorption [45]. ACE2 can also inhibit the transport of intestinal amino acids in a non-RAS dependent manner to impair the synthesis of antibacterial peptides, resulting in gut microbiota dysbiosis, elevated intestinal permeability, and immune response dysfunction [46]. Through bioinformatics analysis, we found that ACE2 plays a corresponding biological function by regulating the TFF3 gene and RAS signalling pathways. TFF peptides are a family of secreted molecules associated with mucus, which play an important role in gastrointestinal mucosal damage and inflammatory response [47]. TFF3 has been reported to play an important role in a variety of human cancers. For example, in the human colorectal cancer cell line HCT8/S11, TFF3 can activate the STAT3 signalling pathway by phosphorylation of STAT3 α/β [48]. Subsequently, Sun et al. [49] found that the STAT3 binding site was essential to the self-induction action of hTFF3 through site-directed mutagenesis and that the hTFF3 promoter specifically binds to STAT3 to induce the transcription of its own promoter. RAS signalling pathways are abnormally activated in malignancies, such as colon cancer, where the EGFR/RAS/RAF/MEK/ERK signalling pathway is activated by the upstream gene HOXA3 to promote tumour cell growth [50]. The RAS signalling pathway also plays an important role in regulating the invasion of colon cancer cells [51]. In recent years, many studies have found that the key protein kinase in the RAS signalling pathway plays a regulatory role in the immune response of malignant tumours and other processes [52]. Abnormal activation of the RAS/MEK pathway has also been established to mediate the IRF1 expression and exert a certain role in tumor-associated immune responses [53]. However, the specific role of ACE2 in the RAS pathway and whether it further mediates tumour immune infiltration through this signalling pathway has not been fully elucidated. The role of ACE2 in the pathogenesis, development and prognosis of colon cancer needs further exploration.

Immune cells are important constituents of the TME and participate in tumour immune escape and tolerance [54, 55]. A large number of studies have shown that there are significant differences in the infiltration ratio of immune cells between colon cancer tumour tissues and corresponding adjacent tissues, and is characterised by extensive heterogeneity. In the study of cell subpopulations, it was found that the difference in TME infiltration of M1 macrophages, M2 macrophages, eosinophils, neutrophils,

and other immune cells was associated with poor prognosis and clinical stage of the tumour [56, 57]. There are two types of blocking antibodies of programmed death 1 currently used in the treatment of colon cancer with microsatellite instability high (MSI-H): nivolumab and pembrolizumab. Nivolumab plus ipilimumab therapy for colon cancer has a progression-free survival rate of 76% (9 months), overall survival at 87%, and objective response rate at 55%, which indicates a good prospect [58, 59, 60]. However, this immunotherapy approach is not effective in most colon cancers that have not been mutated, namely MS stable or MSI-low colon cancers [61]. Therefore, additional research is needed to explore these associations among immune cells, immune-related genes, and colon cancer tumour progression, to discuss novel therapies targeting TME, and to complement immune checkpoints. ACE2 is an important molecule that participates in TME regulation. Previous studies have shown that ACE2 expression is negatively correlated with immune cell infiltration, such as neutrophils and macrophages [62, 63, 64]. Cheng et al. [65] demonstrated that ACE2 overexpression could inhibit the synthesis of vascular endothelial growth factor in TME and inhibit tumour invasion and inflammatory response. In this study, we further analysed the strong correlation between ACE2 expression and colon cancer tumour-immune infiltration. The TIMER analysis revealed that ACE2 expression was negatively correlated with CD8+ T cells and neutrophil infiltration in tumour tissues, but positively correlated with CD4+ T cells. Correlation analysis with immune signature marker genes also suggested that ACE2 expression plays a regulatory role in monocytes/macrophage functions. Compared with M1 macrophages, ACE2 and M2 gene markers have a stronger correlation, suggesting that ACE2 may be involved in the control of macrophage polarity.

Conclusions

We can conclude that ACE2 expression plays an important role in the regulation of the TME in colon cancer, suggesting that ACE2 can be a novel molecular target and a new immune checkpoint in tumour immune escape and tolerance.

Our study indicates that ACE2 may serve as a potential biomarker for colon cancer and is intricately associated with TME. Further analysis using the public colon cancer data will provide a meaningful method to screen key genes associated with the onset of human malignant diseases.

Abbreviations

ACE2: Angiotensin converting enzyme 2; SARS-CoV-2: Severe Acute Respiratory Syndrome coronavirus 2; ACE: Angiotensin converting enzyme; AngI: Angiotensin I; AngII: Angiotensin II; RAS: Renin-angiotensin system; CCLE: Cancer Cell Line Encyclopedia; TCGA: The Cancer Genome Atlas; PPI: protein-protein interaction; GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; TIMER: Tumor Immune Estimation Resource; HPA: Human Protein Atlas; STAT: Signal transducer and activator of transcription; TME: Tumor microenvironment; TAMs: Tumor-associated macrophages; TFF: Trifoliolate factor; PD-1: Programmed death 1; MSI-H: Microsatellite instability high; MSS: Microsatellite stable; MSI-L: Microsatellite instability low; VEGF: Vascular endothelial growth factor.

Declarations

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Authors' contributions

YTW and HN wrote this article. JHZ and CLO designed, organized and reviewed this article. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

All data generated or analyzes during this study are included in this published article and its supplementary information files.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

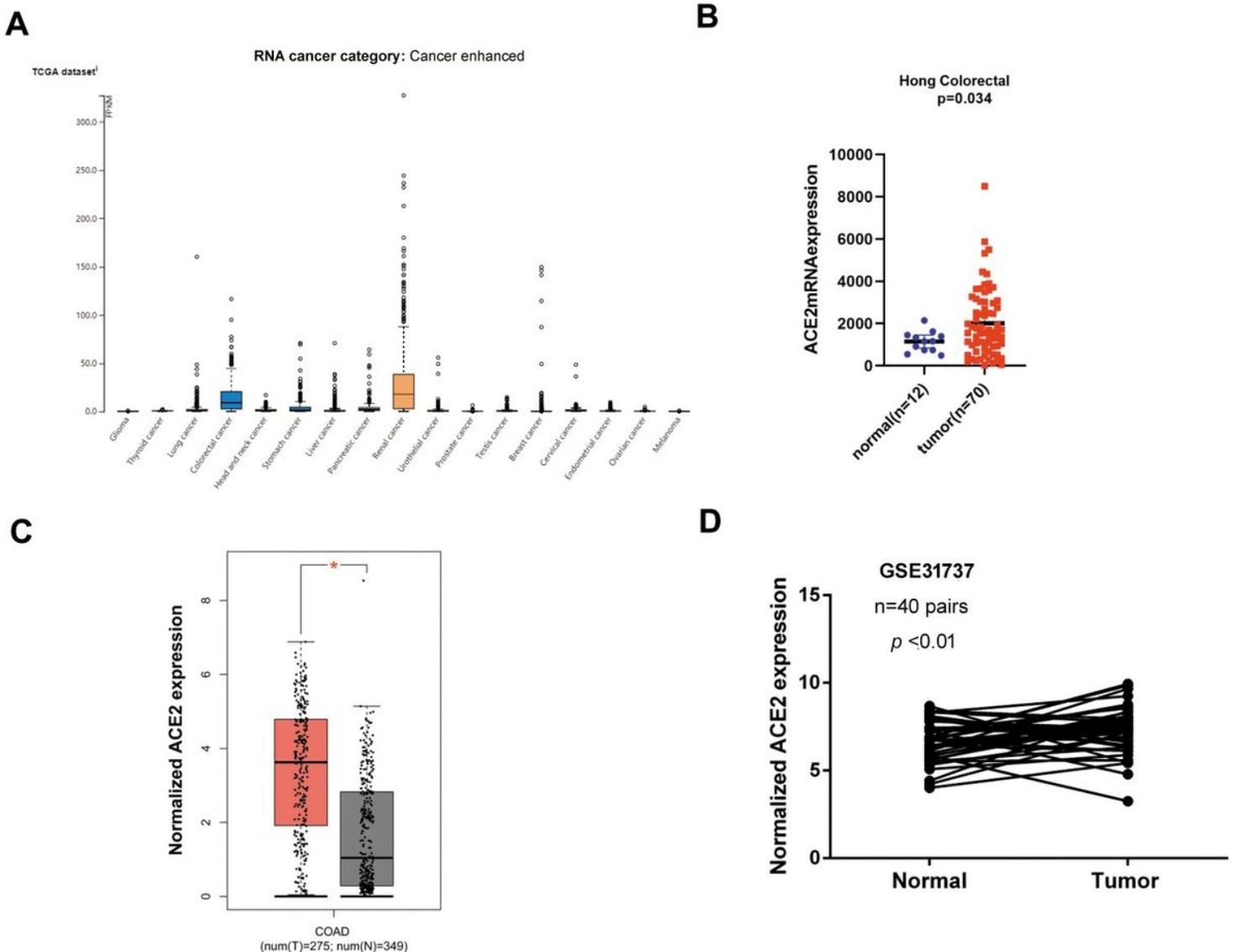


Figure 1

Expression of ACE2 in different tumor tissues. (A) The expression levels of ACE2 in different cancer tissues were provided by HPA database. (B) The expression levels of ACE2 mRNA in colon cancer in Hong data sets was downloaded from oncomine database. (C) From the GEPIA database, the expression levels of ACE2 mRNA in colon cancer tissues was detected. (D) ACE2 mRNA expression levels in colon cancer tissues and paired normal tissues were downloaded from the GEO database.

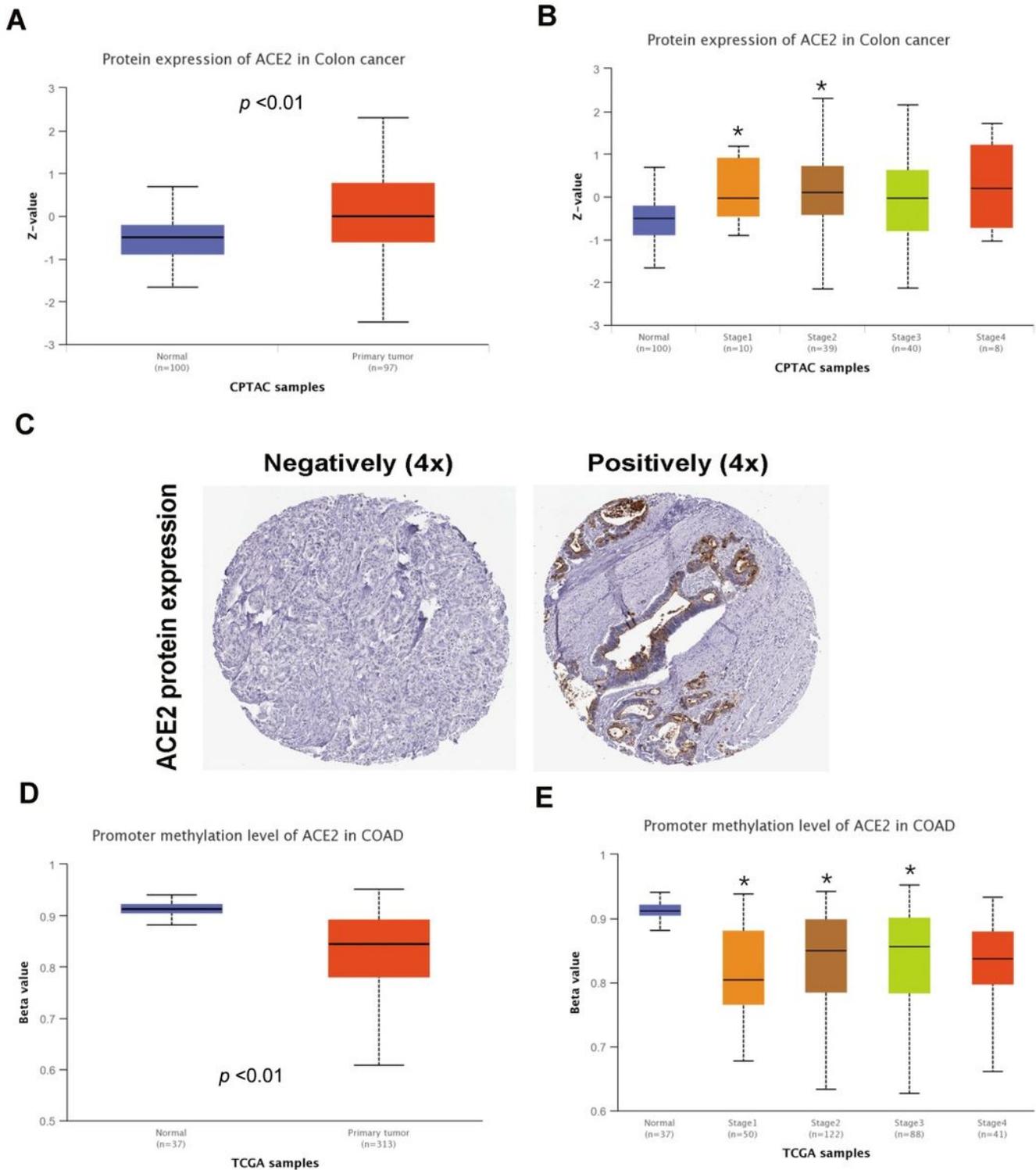
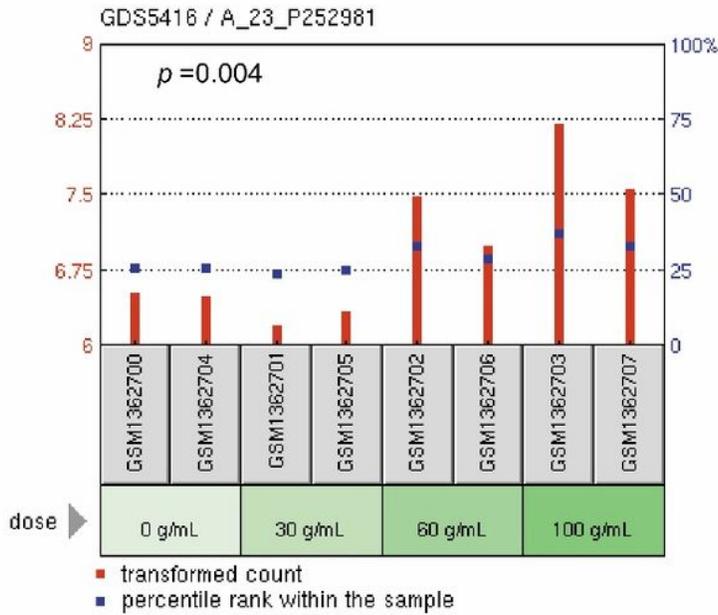


Figure 2

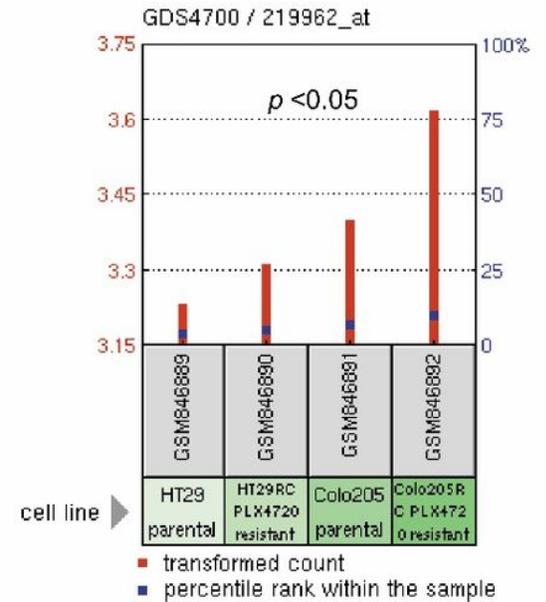
Expression of ACE2 protein and its methylation in colon cancer from UALCAN database. (A) The expression level of ACE2 protein in colon cancer tissues was compared with that in normal tissues. (B) Difference of ACE2 protein expression and tumor grade in colon cancer. (C) The immunohistochemical results of ACE2 in colon cancer and adjacent tissues were downloaded from HPA public bioinformatics website. (D) Methylation expression of ACE2 in colon cancer and normal tissues. (E) The association

between the methylation level of ACE2 and the grade of colon cancer. (Among them, * represents significant difference, i.e, $P < 0.05$)

A



B



C

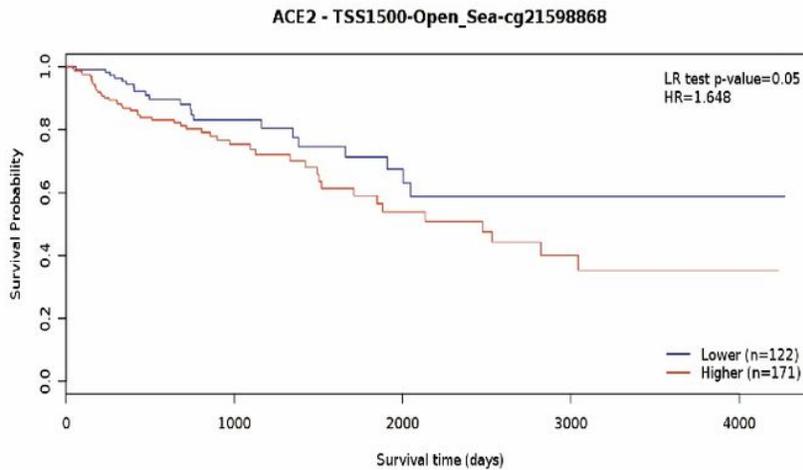


Figure 3

Analysis of ACE2 gene on the treatment of colon cancer from GEO database. (A) Dose effect of supercritical rosemary extract on SW620 colon cancer cells downloaded from GEO database. (B) Relationship between acquired resistance to BRAF inhibitors and expression of ACE2 in colon cancer cell lines. (C) To explore the relationship between ACE2 expression and survival time in colon cancer using by MethSurv database.

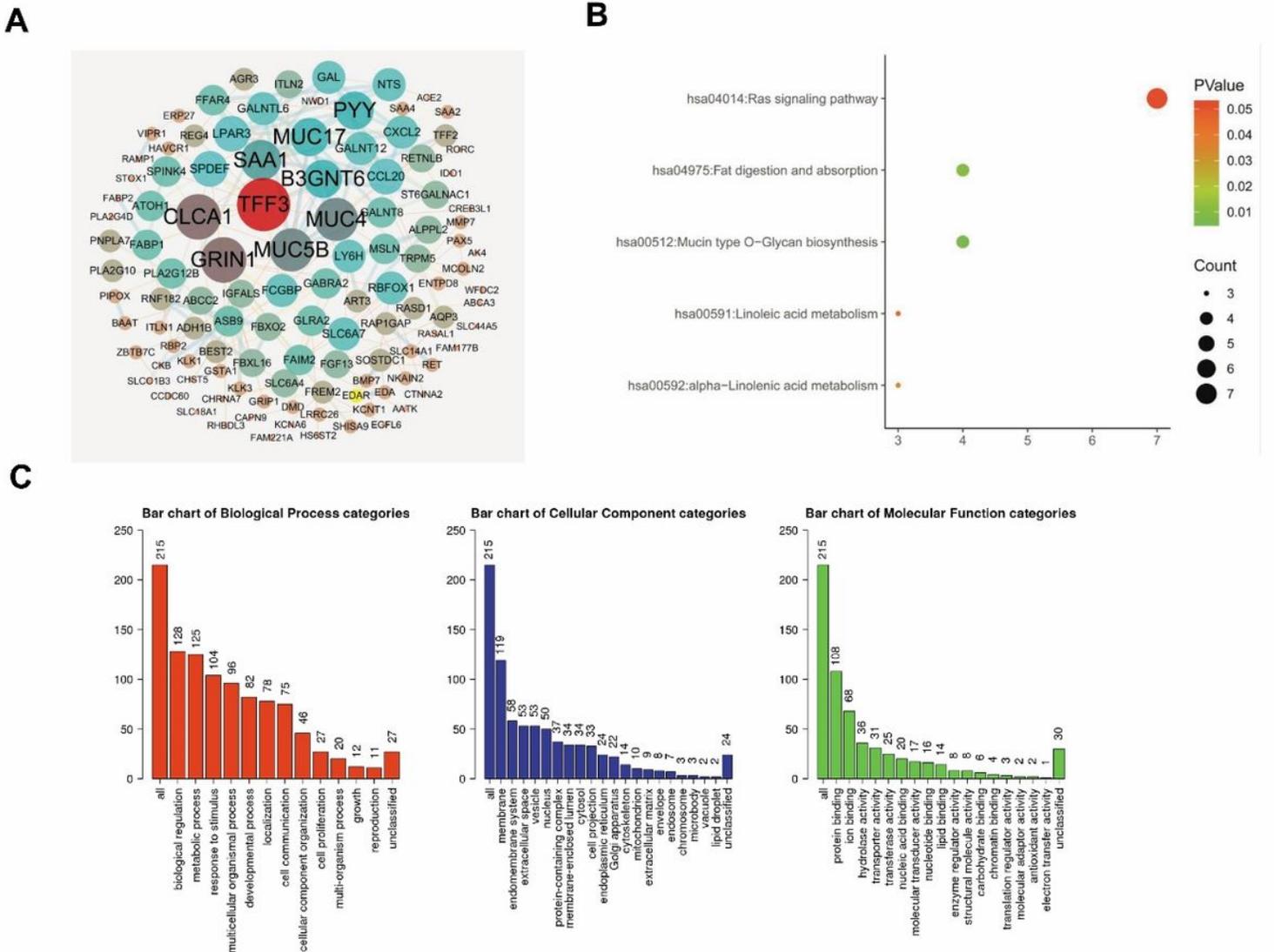


Figure 4

Analysis of ACE2 co-expression network. (A) Using STRING database, DAVID website and Cytoscape software to construct the network of co-expression gene with ACE2. (B) The KEGG enrichment pathway of co-expressed genes was analyzed by DAVID biological website, and bubble diagram was drawn by R language package. (C) The analysis of biological processes, molecular functions and cell components is from WebGestalt website.

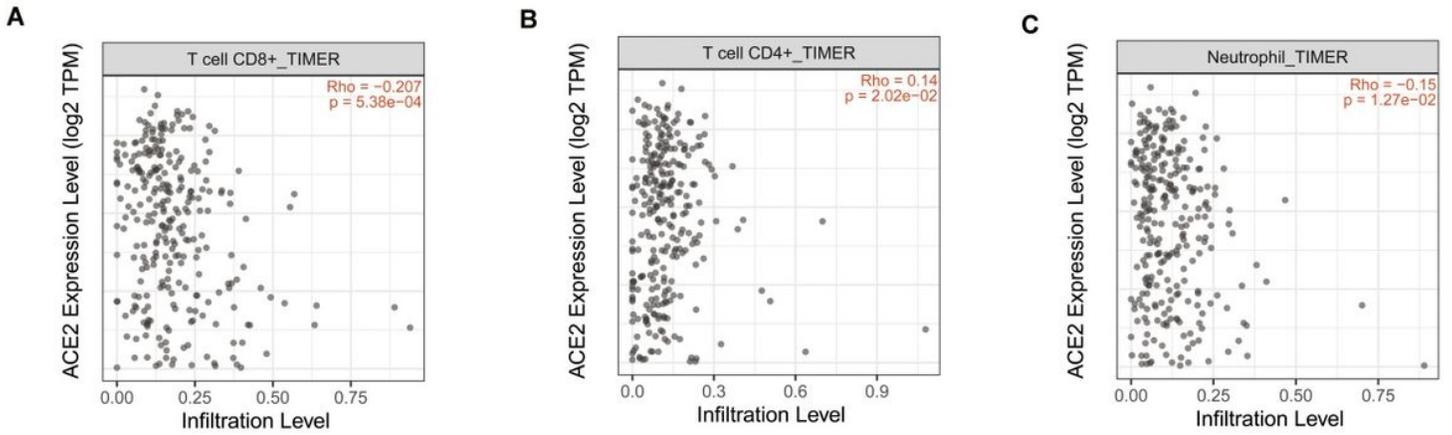


Figure 5

Correlation between the expression of ACE2 and the level of immune infiltration in colon cancer. (A) There was a positive correlation between the expression of ACE2 and the immune infiltration of CD8 + T cells in colon cancer. (B) The expression of ACE2 was positively correlated with the immune infiltration of CD4 + T cells. (C) The infiltration of Neutrophil cells was negatively correlated with the expression of ACE2.

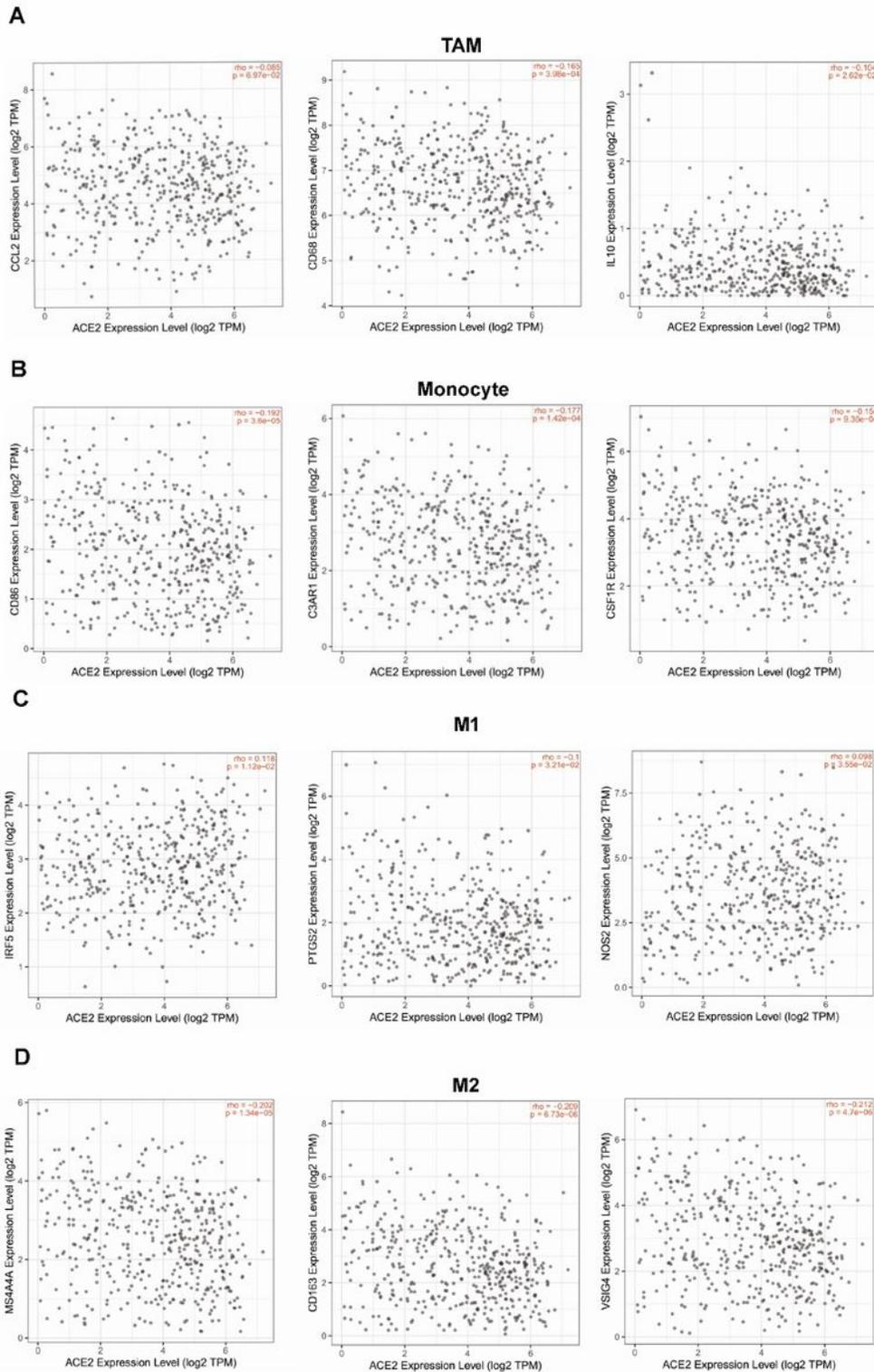


Figure 6

Correlation between ACE2 expression and tumor markers of macrophages, monocytes, M1 macrophages, M2 macrophages. (A) Correlation between ACE2 expression and tumor associated gene markers CCL2, CD68 and L10 in macrophages. (B) ACE2 expression is associated with CD86, C3AR1 and CSF1, which are gene markers related to mononuclear. (C) ACE2 expression is associated with RF5, PTGS2 and NOS2,

which are gene markers related to M1 macrophages. (D) ACE2 expression is associated with MS4A4a, CD163A and VSIG4, which are gene markers related to M2 macrophages.

Supplementary Files

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