

A New Prognostic Biomarker for Clear Cell Renal Cell Carcinoma Associated With Immune Infiltration: Implications for COVID-19

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Abstract

SARS-CoV-2, the current severe acute respiratory syndrome coronavirus, has spread around the world and is posing a major danger to human life, particularly for cancer patients. Therefore, we aimed to ascertain the clinical functions of ACE2, the SARS-CoV-2 cell entry receptor, in clear cell renal cell carcinoma (ccRCC). The prognostic and clinicopathological features of ACE2 were ascertained using immunohistochemistry, western blot findings, and the TCGA, GEO, and TIP datasets. In the tissues and cell lines of ccRCC, ACE2 expression was down-regulated. Higher ACE2 expression was an independent predictor of prolonged OS (HR: 0.8259, 95%CI: 0.7734-0.8819, $P<0.0001$) and RFS (HR: 0.8023, 95%CI: 0.7375-0.8729, $P<0.0001$) in patients with ccRCC, according to the findings of the multivariate Cox regression analysis. Higher histological grade and pathological stage, metastasis, and advanced tumor stage were also linked to lower ACE2 expression. Additionally, there was a substantial positive correlation between ACE2 expression and CD4 Naïve infiltration and a negative correlation with CD4 Memory infiltration. Furthermore, prolonged OS and RFS as well as improved pathological characteristics were linked to greater CD4 Naïve and lower CD4 Memory infiltration levels. Additionally, the prognosis was good for the high ACE2 expression group in the enriched CD4 memory, reduced CD4 Naïve, and enriched CD4 Naïve cohorts. According to these results, AEC2 was much lower in ccRCC, and a lower ACE2 was linked to poorer clinical characteristics and a worse prognosis. Due to changing levels of immune cell infiltration, low ACE2 expression in ccRCC may have a partial impact on the prognosis.

Key words: Prognosis, ACE2, ccRCC, COVID-19, and immunological infiltration

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, previously known as 2019-nCoV) is the infectious agent that causes coronavirus disease 2019 (COVID-19) [1, 2]. COVID-19 was discovered in Wuhan City, Hubei Province, China, in the end of 2019 [2]. As of July 3, 2020, the World Health Organization (<https://covid19.who.int/>)

stated that there were 10662536 confirmed cases and 516209 COVID-19-related fatalities worldwide.

ACE2, or angiotensin-converting enzyme 2, is referred to as a severe acute respiratory syndrome coronavirus (SARS-CoV) host cell receptor [3]. SARS-CoV-2 and SARS-CoV have been shown to share 79.5% of their sequences, and they both use ACE2 as a cellular entrance receptor [4]. To promote SARS-CoV and SARS-CoV-2 entrance into target host cells, the structural Spike (S) protein attaches itself to the cellular receptor ACE2. As with the SARS-CoV infection, the S protein of SARS-CoV-2 interacts with the cellular ACE2 to facilitate the virus's attachment to the target cells [5]. Prior in vitro research have also shown that increased ACE2 expression increases vulnerability to infections caused by the S protein [6, 7]. Therefore, organs that produce high amounts of ACE2, such as the lung, heart, kidney, bladder, and esophagus, seem to be more vulnerable to SARS-CoV-2 infection in healthy persons [8–10].

Although fever and dry cough are the most common clinical signs of SARS-CoV-2 infection, additional symptoms include exhaustion, dyspnea, nasal congestion, nausea, or diarrhea [11,12]. Furthermore, it is believed that those who already have diabetes, hypertension, pulmonary, cardiac, or renal disorders are more likely to acquire severe illness [13–15]. More than 18 million new instances of cancer are diagnosed worldwide each year [16], and cancer patients are more susceptible to infection due to their poor health, coexisting chronic illnesses, immunosuppressive conditions brought on by tumors, and anti-tumor therapy [17]. In comparison to the prevalence of tumors in the general Chinese population (0.29%), Liang et al. found that 18 (1%) out of 1590 SARS-CoV-2 participants had a history of malignancies [18]. Miyashita et al. examined the clinical and demographic data of 5688 SARS-CoV-2 patients and discovered that 334 (6%) of them had tumors [19]. Twenty percent of SARS-CoV-2 individuals had received treatment for a malignancy during the preceding five years, according to an Italian investigation [20]. As a result, tumor patients infected with the SARS-CoV-2 coronavirus may fare worse than other participants.

In this study, we used the TCGA, GEO, and TIP databases to show the expression profile of ACE2 in clear cell renal cell carcinoma (ccRCC) and nearby normal tissues. We also used these data to determine the prognostic roles of ACE2 in ccRCC and its relationship to clinicopathological features and immune infiltration. Additionally, we evaluated the predictive and clinicopathological functions of ACE2 in ccRCC and confirmed the expression of ACE2 protein in 56 paired ccRCC tissues (ccRCC and neighboring normal tissue). Additionally, we examined the relationship between ACE2 and the expression of three immune cell markers (CD4, CD45RA, and CD45RO).

Materials and Methods

Ethics statement

The Peking University First Hospital's Biomedical Research Ethics Committee (IRB00001052-18004) gave its approval for this research. Additionally, all patients provided written informed permission. TCGA, GEO and TIP databases

TCGA was used to download RNA-sequencing and clinical follow-up (OS and RFS) data for 530 ccRCC samples. GEO provided the RNA-sequencing information for the GSE40435, GSE53757, GSE66272, GSE126964, and GSE73731 datasets. TIP—Tracking Tumor Immunophenotype—provided the information on immune cell infiltration in TCGA-KIRC.

Immunohistochemical examination

The protein expression of ACE2, CD45RA, and CD45RO was measured by immunohistochemical staining 54 pairs of matched paraffin section samples (ccRCC and nearby normal tissue). Anti-ACE2 (Affinity, AF5165, 1:100 dilution), anti-CD45RA (Abcam, ab755, 1:2000 dilution), and anti-CD45RO (Abcam, ab23, 1:1000 dilution) are the particular primary antibody details.

Western blot analysis

This research included a number of ccRCC cell lines, including as 786-O, OSRC2, and A498; it also used the normal human renal tubular epithelial cell line HK2. RIPA buffer was used to extract the protein from these cell lines, and the BCA technique was used to measure the amount of protein present overall. Following SDS-PAGE separation, the protein was transblotted onto PVDF membranes. After blocking the membranes with 5% skim milk powder, they were incubated with anti-ACE2 (Affinity, AF5165, 1:1000 dilution) and anti-GAPDH (Proteintech, Lot:00075847, 1:10000 dilution) for a whole night at 4 °C.

Statistical analysis

Non-parametric Continuous variable differences were found using the Mann-Whitney test. The relationship between ACE2 expression and immune cell infiltration and immune cell marker expression was investigated using the Pearson's correlation test. The Kaplan-Meier curve was used to analyze the predictive value of ACE2, and the log-rank test was used to illustrate the importance of the variation in survival curves. ACE2 was subjected to both univariate and multivariate Cox regression analysis. Statistical significance was indicated by a P-value < 0.05.

Results

ACE2 expression was down-regulated in ccRCC tissues and cell lines

The expression of ACE2 mRNA in ccRCC and nearby normal tissues was first examined using four GEO datasets (GSE40435, GSE53757, GSE66272 and GSE126964), and discovered that the expression of ACE2 mRNA was down-regulated in tissues with ccRCC as compared to nearby normal tissues (Figure 1A). We looked studied ACE2 protein expression in 54 ccRCC tissues and the equivalent neighboring normal tissues in order to better understand the expression pattern of ACE2 protein in ccRCC. Table 1 lists the specific clinical characteristics of the 54 patients. Immunohistochemistry results demonstrated that ACE2 protein expression was considerably reduced in ccRCC tissues compared to nearby normal tissues, which is consistent with the findings of GEO data analysis (Figure 1B). Additionally, the ccRCC

cell lines' reduced expression of the ACE2 protein was validated by the western blot data (Figure 1C).

Decreased ACE2 expression was associated with worse prognosis

We acquired the RNA-sequencing and clinical follow-up data of 530 ccRCC samples in order to ascertain the predictive significance of ACE2 in ccRCC. from TCGA. Based on the median critical value of CLDN10 expression, the 530 patients were split into two groups. The results of the log-rank test revealed that the group with high ACE2 expression had significantly longer OS and RFS than the group with low ACE2 expression (Figure 2A). Furthermore, the findings of a univariate Cox regression analysis indicated that although greater ACE2 expression was associated with improved OS, advanced tumor stage (T3/T4), metastasis (M1), higher pathological stage (Stage III/IV), and histological grade (Grade 3/4) were possible risk factors for poor OS. The findings of the multivariate Cox regression analysis then showed that whereas higher ACE2 expression alone predicted longer OS (HR: 0.8259, 95%CI: 0.7734-0.8819, $P < 0.0001$), M1, Stage III/IV, and Grade 3/4 were separately associated with shorter OS (Table 2). Regarding While higher ACE2 expression was linked to improved RFS, RFS, T3/T4, M1, Stage III/IV, and Grade 3/4 were possible risk factors for poor RFS. According to the findings of the multivariate Cox regression analysis, M1, Stage III/IV, and Grade 3/4

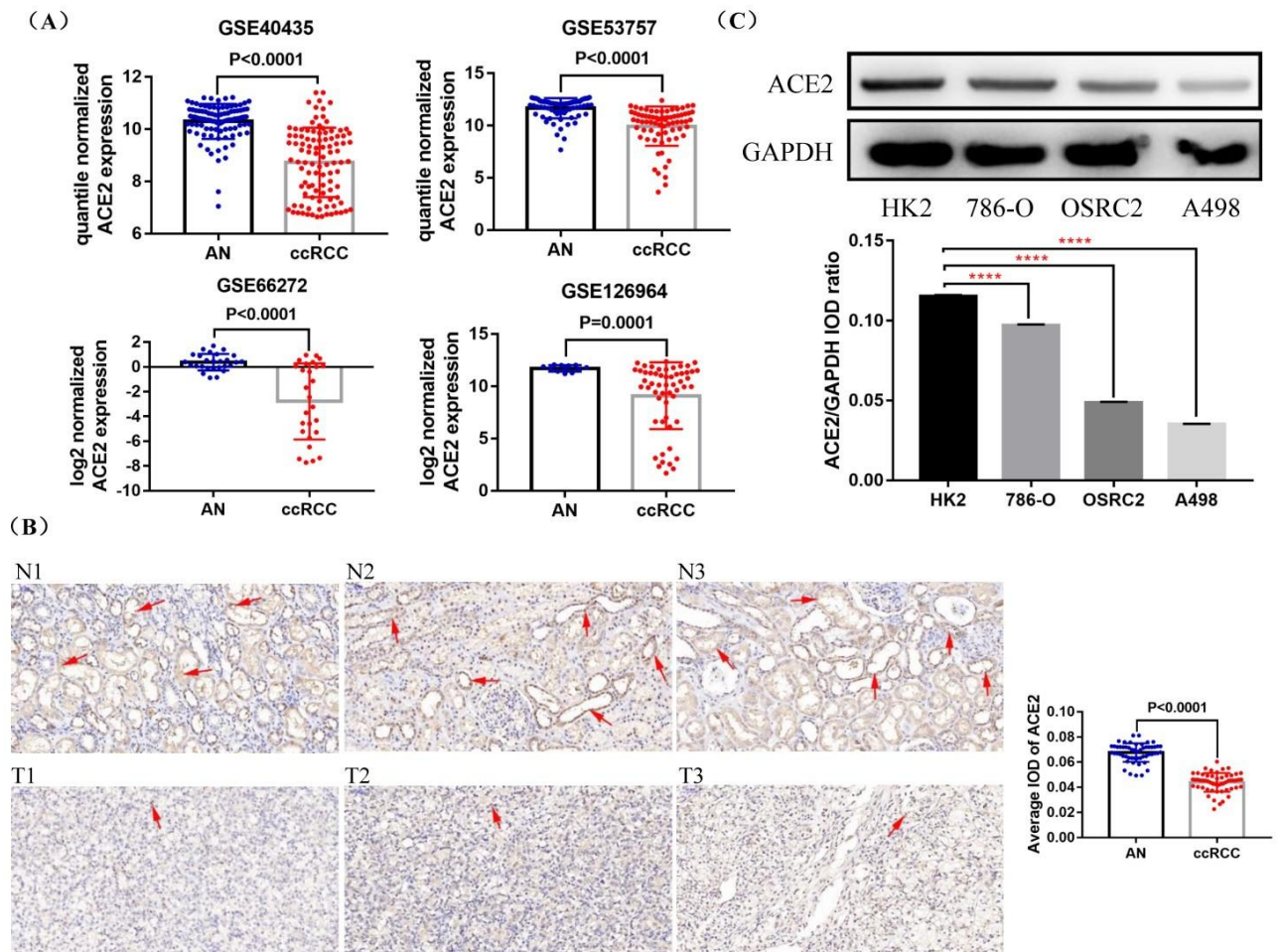


Figure 1. Expression status of ACE2 in ccRCC tissues and cell lines. (A) Comparison of ACE2 mRNA expression in ccRCC and adjacent normal tissues (AN) based on GSE40435, GSE53757, GSE66272 and GSE126964 datasets. (B) Comparison of ACE2 protein expression in 54 pairs of matched paraffin section samples (ccRCC and adjacent normal tissue) using immunohistochemistry (C) Comparison of ACE2 protein expression in HK2 cell line

and three ccRCC cell lines (786-O, OSRC2 and A498). **** $p < 0.0001$. In patients with ccRCC, elevated ACE2 expression (HR: 0.8023, 95%CI: 0.7375-0.8729, $P < 0.0001$) was an independent predictor of longer RFS, whereas were independent predictors of shorter DFS (Table 3). Additionally, we combined the OS and RFS data with the ACE2 protein expression data of 54 clinical ccRCC samples to confirm the predictive significance of ACE2 in ccRCC (Figure 2B).

Lower ACE2 expression was associated with worse pathological features

The relationship between ACE2 expression and the pathological characteristics of ccRCC patients was investigated using the GSE40435, GSE73731, and GSE66272 datasets, TCGA RNA-sequencing, and clinical data. Grade 3/4 groups had lower levels of ACE2 expression, according to the analytical findings of the GSE40435 and GSE73731 datasets (Figure 3A), whereas T3/T4, Grade 3/4, and M1 ACE2 expression was similarly reduced in these individuals (Figure 3B). Furthermore, the findings of the TCGA data analysis showed that metastasis, higher histological grade and pathological stage, and advanced

tumor stage were all linked to reduced ACE2 expression (Figure 3C). Furthermore, the reduced ACE2 expression in the T3/T4, Grade 3/4, and M1 groups was validated by our immunohistochemical data (Figure 3D). Additionally, we examined the predictive value of ACE2 in many subgroups (T3/T4, Grade 3/4, Stage III/IV, and Metastasis subgroups) using the Kaplan Meier curve. High ACE2 expression groups were linked to longer OS in T3/T4, Grade 3/4, Stage III/IV, and Metastasis subgroups (Figure 4A), and they were also linked to longer RFS in T3/T4, Grade 3/4, and Stage III/IV subgroups (Figure 4B), according to the findings of the log-rank test.

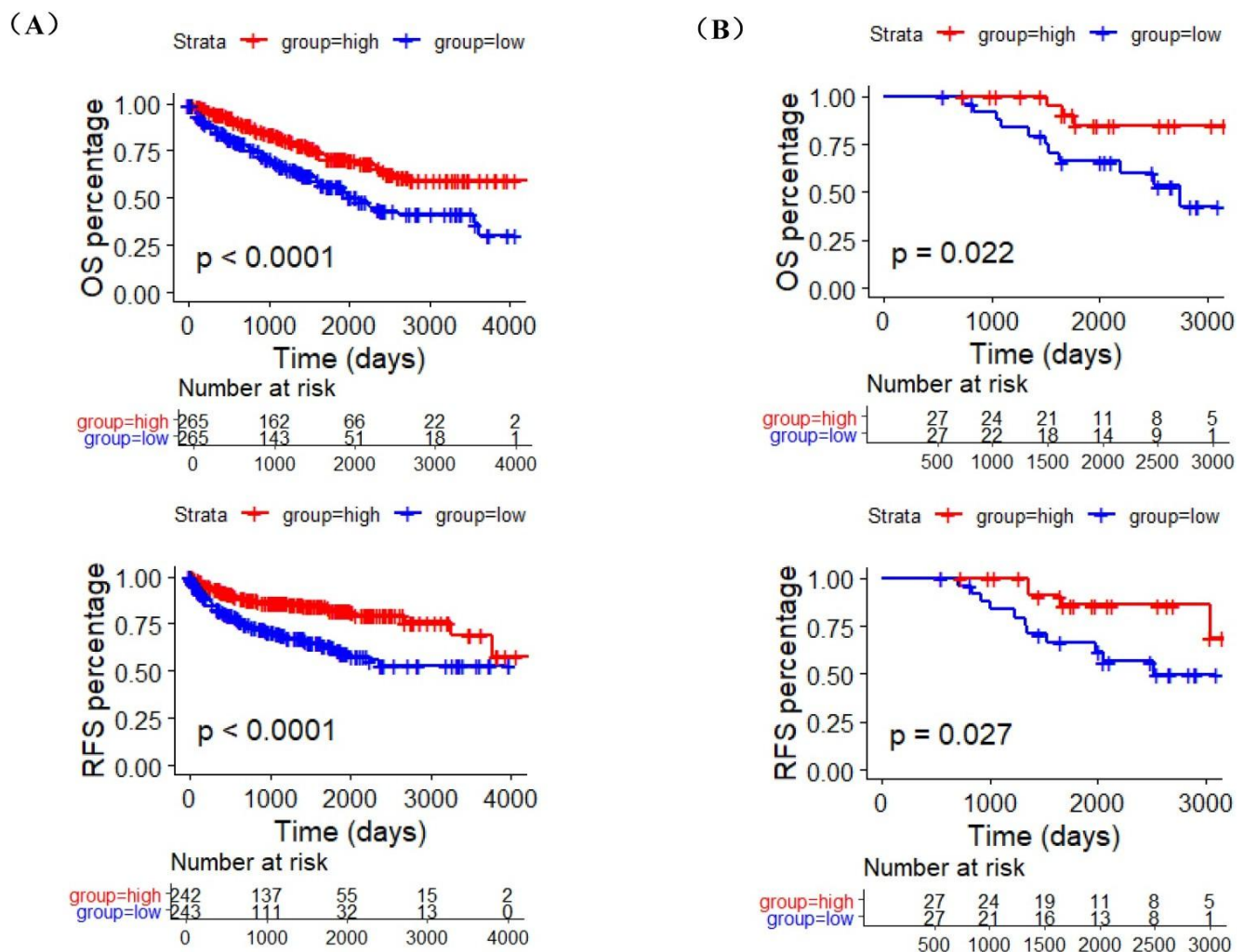


Figure 2. The prognostic value of ACE2 in ccRCC patients. (A) K-M curves of OS and RFS in 530 and 485 TCGA ccRCC patients, respectively. (B) K-M curves

of OS and RFS **Table 1.** The clinicopathological characteristics of 54 ccRCC paraffin section samples

Clinicopathological characteristics	n(%)
Gender	
Male	32 (59.3)
Female	22 (40.7)
Tumor size	

Prognostic and clinicopathological roles of immune cells in ccRCC

and the prognosis of individuals with RCC was associated with tumor infiltration [23, 24]. Nevertheless, it is still unclear how immune cells specifically affect the prognosis of individuals with ccRCC and how they relate to the disease's clinical features. We compared the infiltration levels of 14 different types of immune cells (B cells, CD4 Naïve, CD4 Memory, CD8 Naïve, CD8 Memory, CD8 Effector, Treg cell, Th cell, Monocytes CD16, Monocytes CD14, DC, pDC, NK, and Plasma) in ccRCC and nearby normal tissues using data from TIP and TCGA. We also tested the relationship between these infiltration levels and the prognostic and clinicopathological roles of ccRCC patients. According to the results, ccRCC had lower infiltration levels of CD4 Naïve, CD8 Memory, Monocytes CD16, and pDC than adjacent normal tissues, while ccRCC had higher infiltration levels of CD4 Memory, CD8 Effector, Th cell, DC, NK, and plasma than adjacent normal tissues (Figure 5A and 5B). The predictive functions of these differently dispersed immune cells in ccRCC were examined using the Kaplan-Meier curve, and the findings of the log-rank test showed that the only factors associated with both OS and RFS were the infiltration levels of CD4 Naïve, CD4 Memory, and DC cells. Longer OS and RFS were linked to higher CD4 Naïve infiltration levels, while longer OS and RFS were linked to lower CD4 Memory and DC infiltration levels (Figure 6A). However, the infiltration levels of CD8 Memory, pDC, and plasma were only associated with RFS, and there was no difference in OS or RFS between the groups with high and low infiltration levels of CD8 Effector, Th cell, Monocytes CD16, and NK

(Supplementary 1). Additionally, CD4 Naïve was lower and CD4 Memory and DC infiltration were greater in the T3/T4, G3/G4, Stage III/IV, and Metastasis (M1) groups (Figure 6B).

Table 3. Univariate and multivariate Cox regression analysis of RFS in 467 TCGA ccRCC patients

Parameters	Univariate analysis				Multivariate analysis			
	P	HR	95%CI		P	HR	95%CI	
			Lower	Upper			Lower	Upper
Tumor stage								
T1/T2 (66.2%)		1.0000				1.0000		
T3/T4 (33.8%)	<0.0001	4.5180	3.0690	6.6510	0.4445	0.7727	0.3989	1.4966
Metastasis								
M0 (88.7%)		1.0000				1.0000		
M1 (11.3%)	<0.0001	12.0300	8.0760	17.9100	<0.0001	5.2895	3.2799	8.5303
Pathological stage								
I/II (63.2%)		1.0000				1.0000		
III/IV (36.8%)	<0.0001	6.8300	4.4650	10.4500	0.0006	4.0809	1.8324	9.0887
Histological grade								
Grade 1/2 (46.9%)		1.0000				1.0000		
Grade 3/4 (53.1%)	<0.0001	3.3350	2.1480	5.1760	0.0418	1.6327	1.0183	2.6177
ACE2	<0.0001	0.8075	0.7485	0.8712	<0.0001	0.8023	0.7375	0.8729

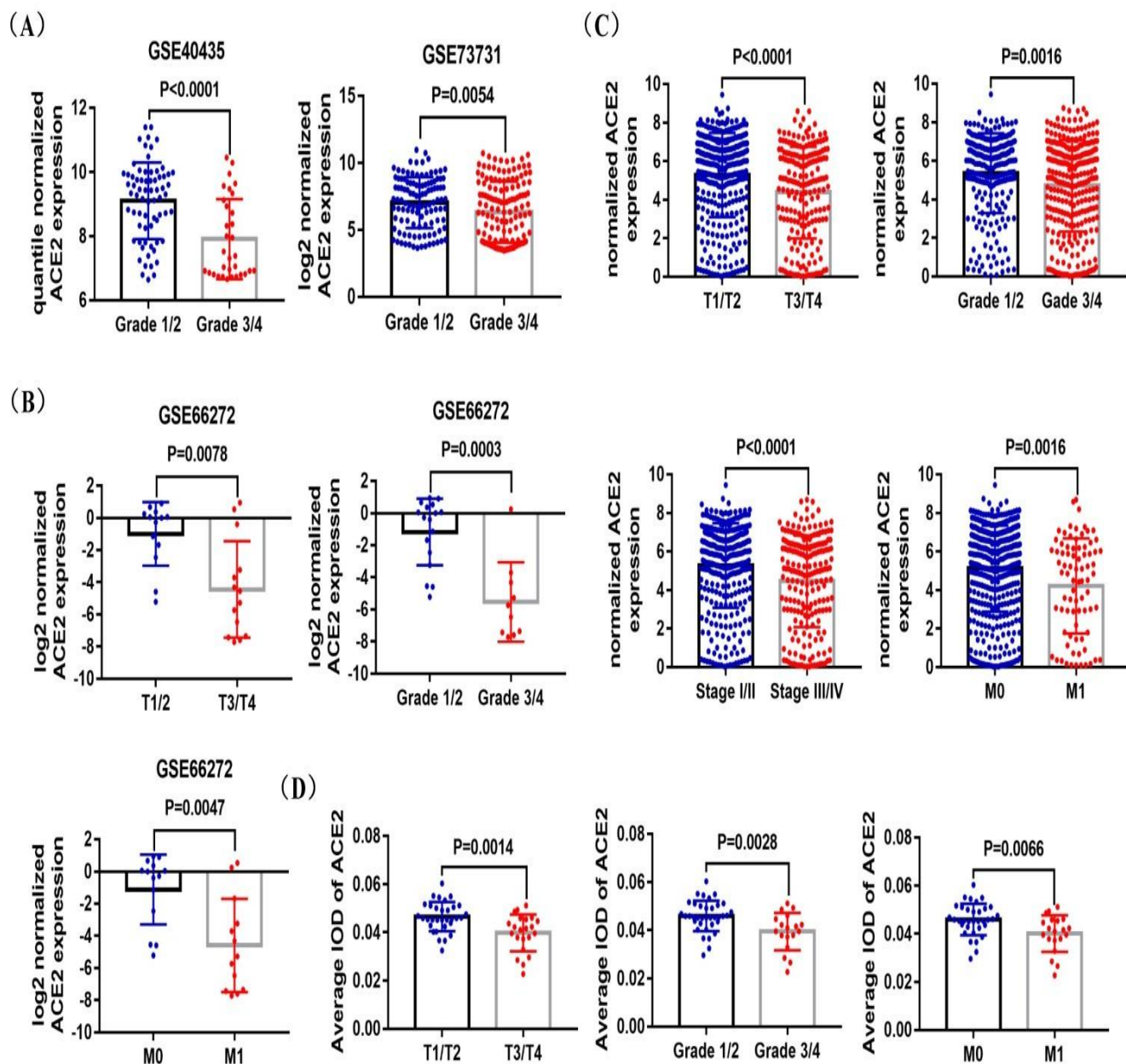
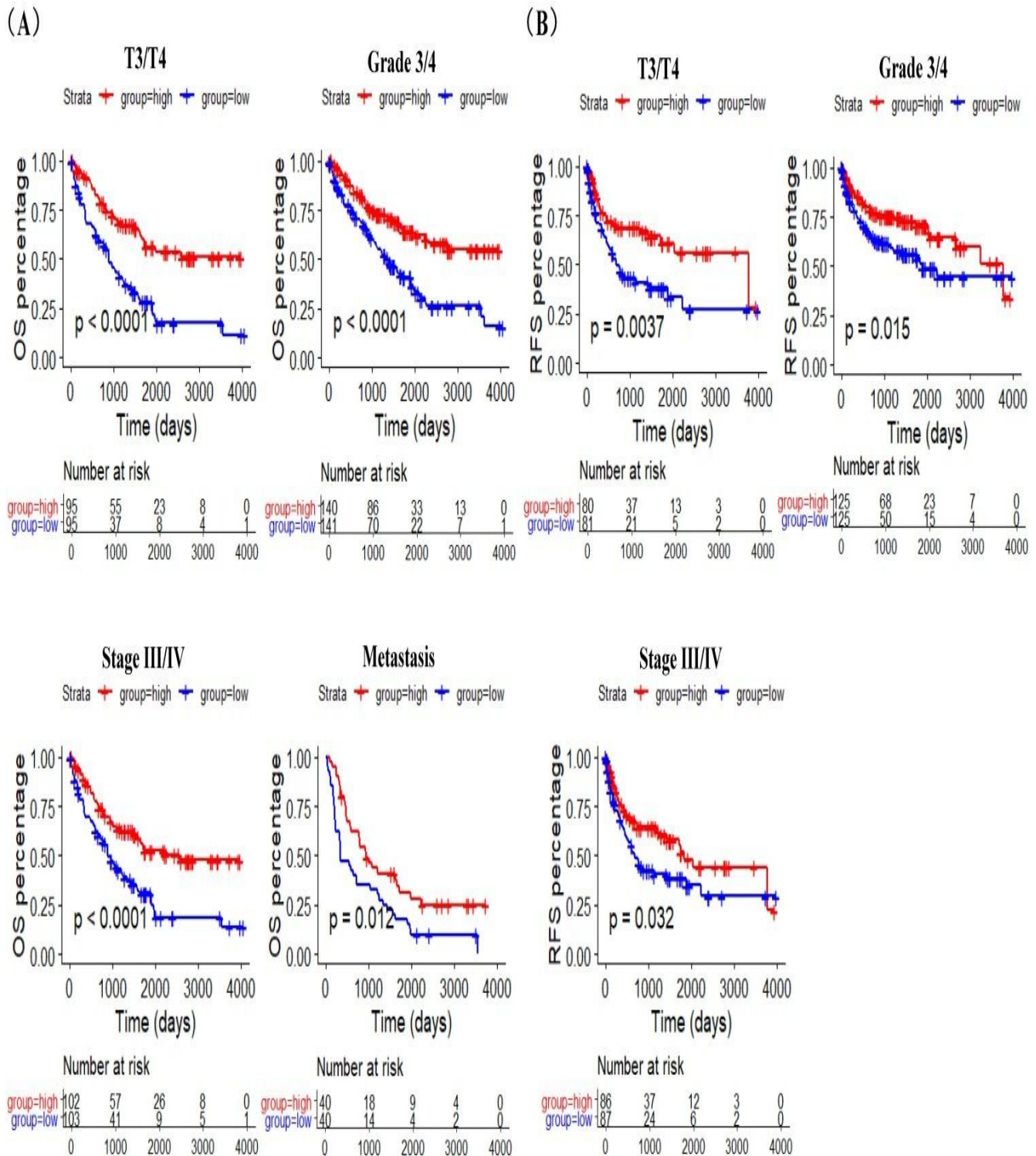


Figure 3. Correlation between ACE2 expression and the pathological features of ccRCC patients. (A) Comparison of ACE2 mRNA expression in Grade 1/2 and Grade 3/4 patients based on GSE40435 and GSE73731 datasets. (B) Comparison of ACE2 mRNA expression in T1/T2 and T3/T4 patients, Grade 1/2 and Grade 3/4 patients, and M0 and M1 patients based on GSE66272 dataset. (C) Comparison of ACE2 mRNA expression in T1/T2 and T3/T4 patients, Grade 1/2 and Grade 3/4 patients, Stage I/II and Stage III/IV patients, and M0 and M1 patients based on TCGAKIRC data. (D) Comparison of ACE2 mRNA expression in T1/T2 and T3/T4 patients, Grade 1/2 and Grade 3/4 patients, and M0 and M1 patients based on the immunohistochemistry results of 54



ccRCC paraffin section samples.

Figure 4. The prognostic value of ACE2 in T3/T4, Grade 3/4, Stage III/IV and Metastasis subgroups. (A) K-M curves of OS in TCGA T3/T4, Grade 3/4, Stage III/IV and metastasis ccRCC patients respectively. (B) K-M curves of RFS in TCGA T3/T4, Grade 3/4 and Stage III/IV ccRCC patients respectively. Patients were separated into two groups according to the median cutoff of ACE2 expression.

Figure 5. The infiltration levels of 14 kinds of immune cells in ccRCC and adjacent normal tissues. Heatmap (A) and statistical comparison (B) of the difference in the infiltration levels of 14 kinds of immune cells (B cells, CD4 Naïve, CD4 Memory, CD8 Naïve, CD8 Memory, CD8 Effector, Treg cell, Th cell, Monocytes CD16, Monocytes CD14, DC, pDC, NK and Plasma) in ccRCC and adjacent normal tissues. All the data were from TIP.

ACE2 expression was associated with CD4 Naïve and CD4 Memory infiltration

According to a recent research, ACE2 expression is a predictive biomarker in a variety of cancers and is connected with immune infiltration [25]. The association between ACE2 expression and the levels of CD4 Naïve, CD4 Memory, and DC infiltration was investigated using linear regression analysis to determine if ACE2 is connected to immune cell infiltration in ccRCC. The findings indicated that although there was no significant correlation between ACE2 expression and DC infiltration, it was considerably favorably connected with CD4 Naïve infiltration and negatively correlated with CD4 Memory infiltration (Figure 7A). We also looked at the relationship between ACE2 expression and CD4 and CD45 expression since CD4 and CD45RA were used to screen CD4 Naïve cells and CD4 and CD45RO were used to identify CD4 Memory cells. The findings demonstrated a strikingly strong correlation between ACE2 expression and CD4 and CD45 expression (Figure 7B). Furthermore, We used immunohistochemistry to examine the relationship. 54 ccRCC samples were examined for the relationship between ACE2 protein levels and CD45RA and CD45RO protein levels. Our findings showed that ACE2 expression was negatively connected with CD45RO expression and favorably correlated with CD45RA expression (Figure 7C). Additionally, we examined the prognosis based on the degree of ACE2 expression in pertinent immune cell subgroups. According to the findings, the groups with higher ACE2 expression had longer OS and RFS in the enhanced CD4 memory, reduced CD4 Naïve, and enriched CD4 Naïve cohorts (Figure 8A-D).

Discussion

The safety of people worldwide is now in grave danger because to COVID-19. Due to immunosuppression brought on by tumors and anti-tumor medications, poor health state, and comorbid chronic disorders, cancer patients are more vulnerable to infection [17]. Additionally, prior data suggest that tumor patients infected with the SARS-CoV-2 coronavirus may have a poorer prognosis than other patients [18–20]. The 79.5% shared by SARS-CoV-2 and SARS-CoV has been verified.

homologous sequences and use ACE2 as a cell entry receptor [4]. Therefore, determining the expression profile of ACE2 in ccRCC and its relationship with the prognosis of ccRCC patients can help to further understand the impact of COVID-19 on ccRCC patients.

In this study, we found that ACE2 mRNA was down-regulated in ccRCC compared with adjacent normal tissues by analyzing four GEO datasets, and our western blot and immunohistochemistry results also identified that ACE2 protein expression was decreased in ccRCC tissues and cell lines. Using the RNA-sequencing and clinical data from TCGA, survival analysis results showed that the high ACE2 expression group had remarkably longer OS and RFS than the low ACE2 expression group, and the multivariate Cox regression analysis results indicated that increased ACE2 expression independently predicted longer OS and RFS of ccRCC patients. es, we verified the prognostic roles of ACE2 in ccRCC by combining the ACE2 protein expression data of 54 clinical ccRCC samples and their OS and RFS information. In addition, lower ACE2 expression was associated with advanced tumor stage, higher pathological stage and histological grade, and metastasis. Moreover, our immunohistochemistry results confirmed that T3/T4, Grade 3/4 and M1 groups had lower ACE2 protein expression than that in T1/T2, Grade 1/2 and M0 groups, respectively. Furthermore, survival analysis results also demonstrated that higher ACE2 expression was associated with better prognosis in T3/T4, Grade 3/4, Stage III/IV and Metastasis subgroups. Taken together, the above analysis and experimental results suggested that ACE2 was low expressed in ccRCC, and higher ACE2 expression was related to better prognosis and pathological features of ccRCC patients.

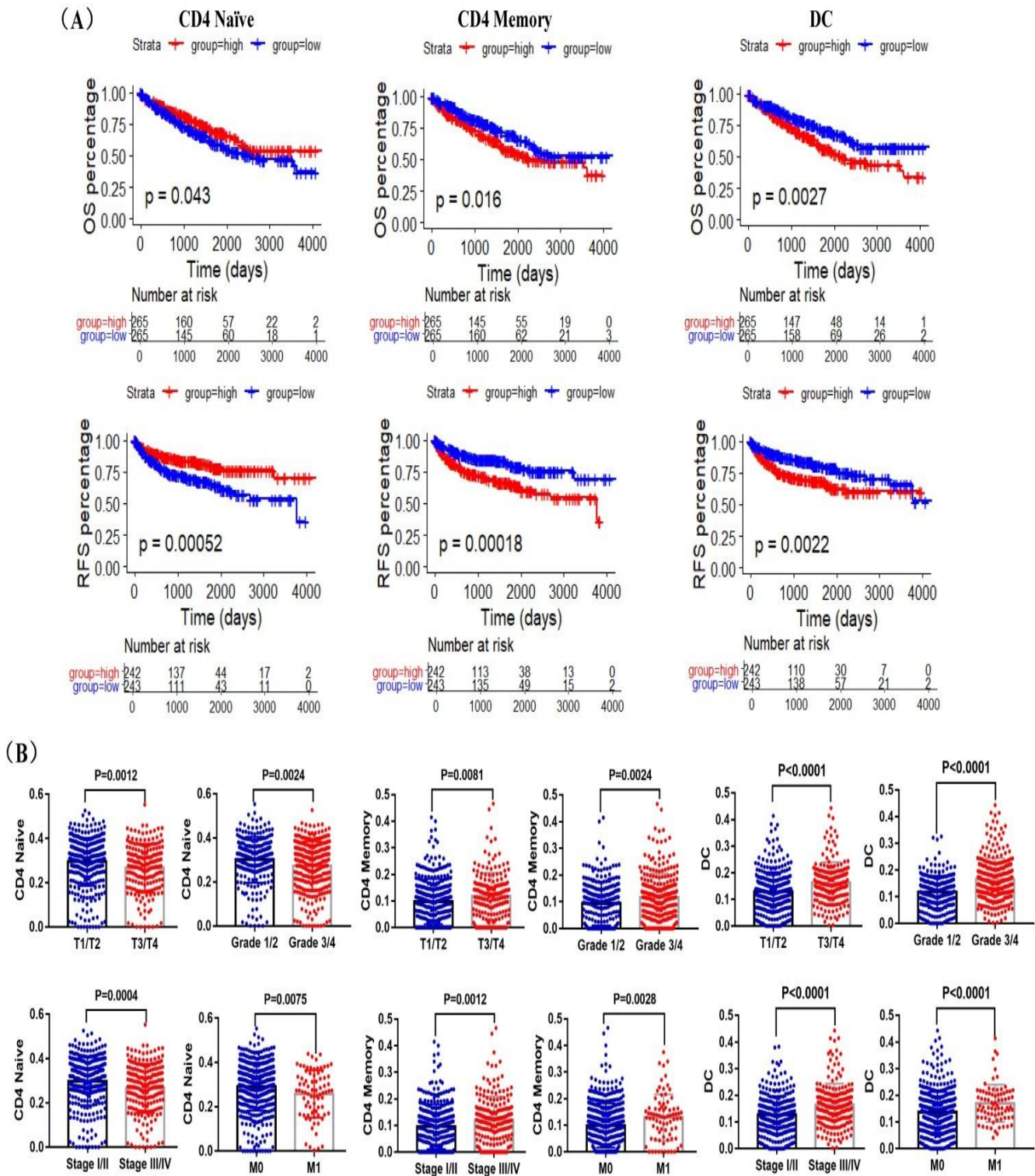


Figure 6. Prognostic and clinicopathological roles of immune cells in ccRCC. (A) K-M curves of OS and RFS in TCGA ccRCC patients. Patients were separated into two groups according to the median cutoff of ACE2 CD4 Naïve, CD4 memory and DC infiltration levels. (B) Comparison of CD4 Naïve, CD4 memory and DC infiltration levels in T1/T2 and T3/T4 patients, Grade 1/2 and Grade 3/4 patients, Stage I/II and Stage III/IV patients, and M0 and M1 patients based on TCGA data.

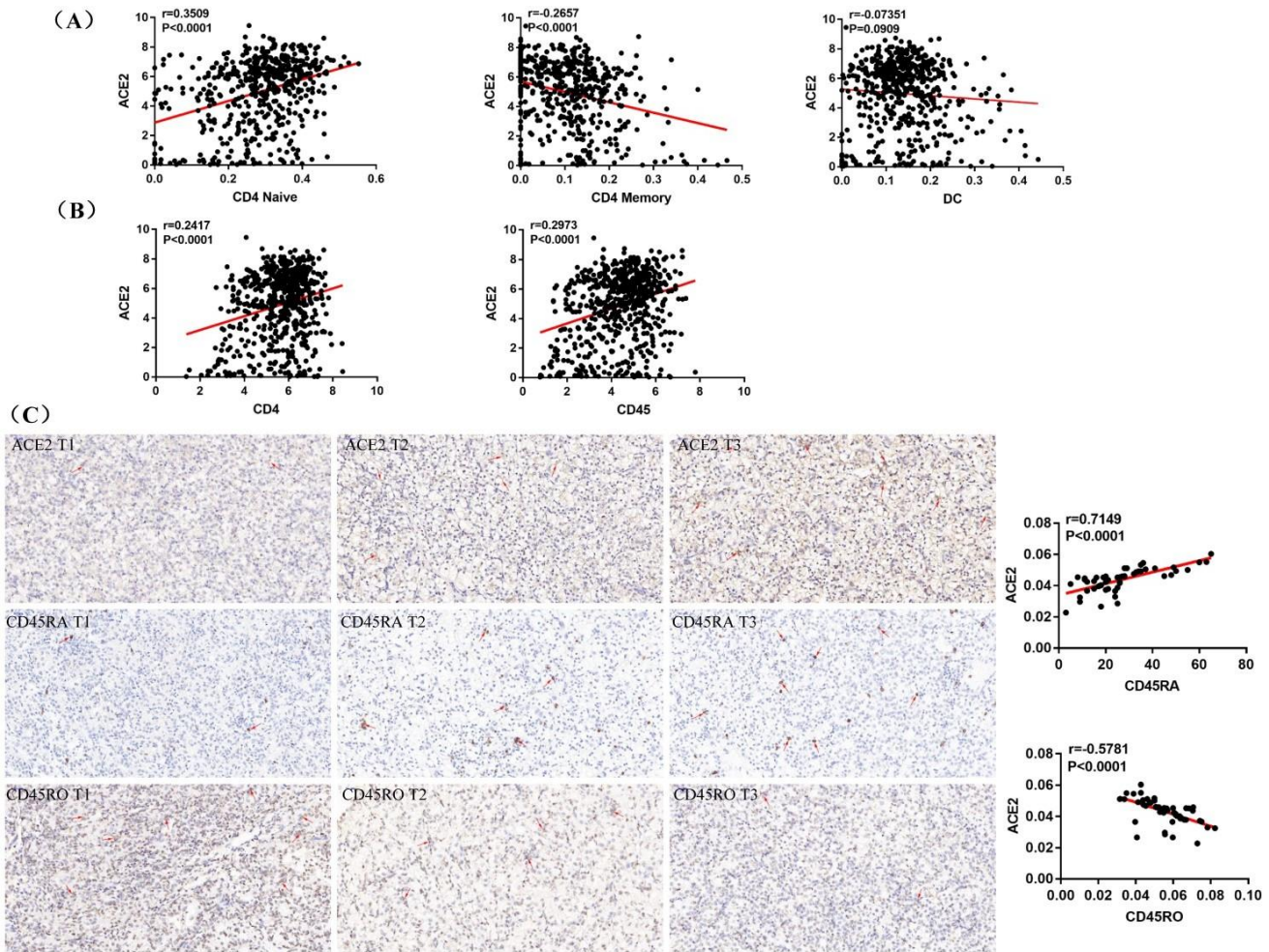


Figure 7. ACE2 expression was associated with CD4 Naïve and CD4 Memory infiltration. (A) Correlation between ACE2 expression and CD4 Naïve, CD4 Memory and DC infiltration levels based TCGA and TIP data. (B) Correlation between ACE2 expression and CD4 and CD45 expression based on TCGA data. (C) Correlation between ACE2 protein expression and CD45RA and CD45RO protein expression based on the immunohistochemistry staining results of 54 clinical ccRCC samples.

Several recent studies have shown that tumor-associated immune cells in the tumor associated microenvironment (TAM) play an important role in regulating multiple important biological processes of tumor cells. For example, CD8+ cytotoxic T lymphocytes (CTLs) in TAM have been shown to inhibit tumor progression by targeting tumor-related antigens, and activation of CTLs predicts a better prognosis in various tumors [26]. A recent study through the analysis of various databases indicates that ACE2 expression in Uterine Corpus Endometrial Carcinoma and Kidney Renal Papillary Cell Carcinoma was significantly increased, which was positively correlated with immune infiltration and prognosis [25]. However, the correlation between ACE2 expression and the infiltration of immune cells in ccRCC remains unclear.

During this study, using data from TIP and TCGA, we compared the infiltration levels of 14 kinds of immune cells (B cells, CD4 Naïve, CD4 Memory, CD8 Naïve, CD8 Memory, CD8 Effector, Treg cell, Th cell, Monocytes CD16, Monocytes CD14, DC, pDC,

NK and Plasma) in ccRCC and adjacent normal tissues. Our results indicated that the infiltration levels of CD4 Naïve, CD8 Memory, Monocytes CD16 and pDC were decreased in ccRCC compared with adjacent normal tissues, and the infiltration levels of CD4 Memory, CD8 Effector, Th cell, DC, NK and Plasma were increased in ccRCC than that in adjacent normal tissues. Survival analysis results showed that higher CD4 Naïve infiltration level was associated with longer OS and RFS, while lower infiltration levels of CD4 Memory and DC were connected with longer OS and RFS. Besides, lower CD4 Naïve and higher CD4 Memory and DC infiltration levels were associated with advanced tumor stage, higher pathological stage and histological grade, and metastasis. Interestingly, ACE2 expression was significantly positively correlated with CD4 Naïve infiltration and negatively correlated with CD4 Memory infiltration.

Moreover, our immunohistochemistry results determined that ACE2 expression was also positively correlated with CD45RA (a CD4 Naïve cell marker) expression and

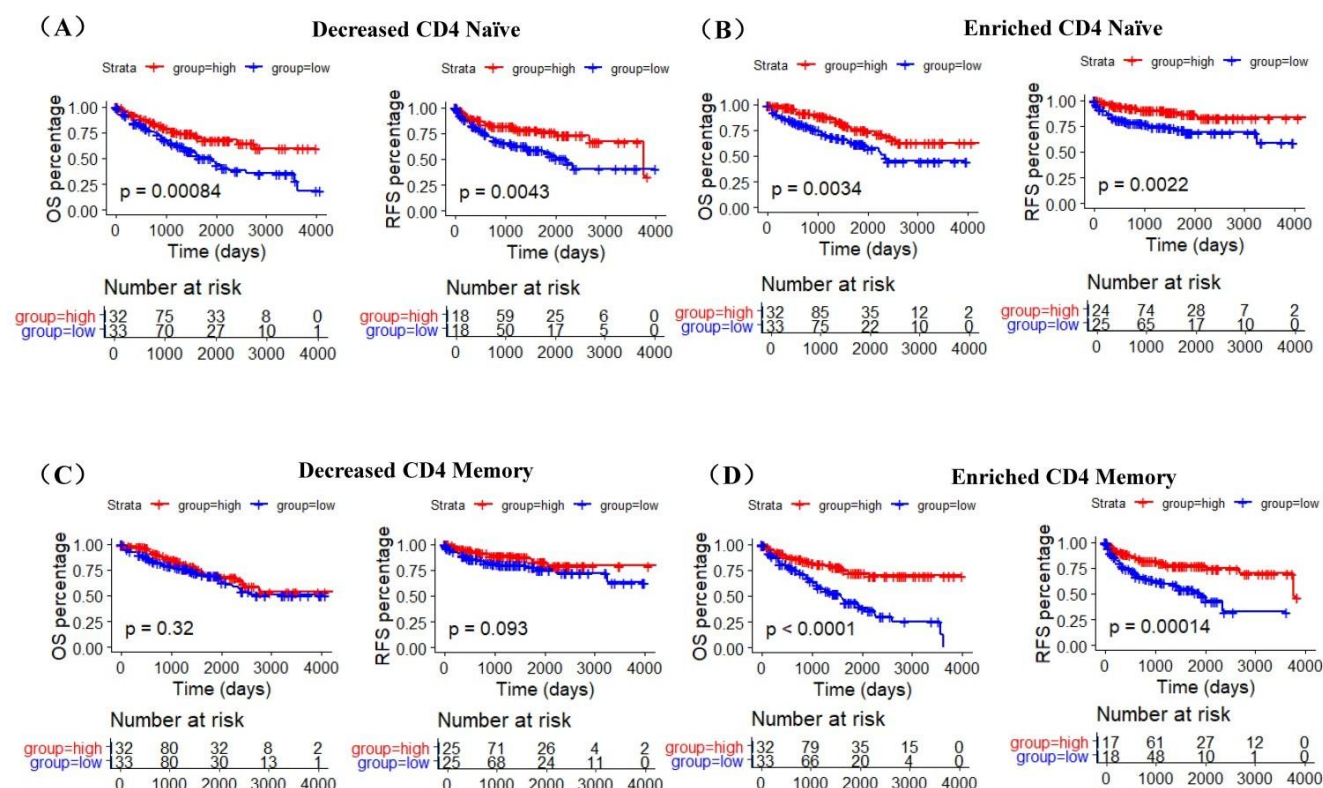


Figure 8. The prognostic roles of ACE2 in relevant immune cell subgroups. K-M curves of OS and RFS in decreased CD4 Naïve (A), enriched CD4 Naïve (B), decreased CD4 memory (C) and enriched CD4 memory (D) cohort. Patients were separated into two groups according to the median cutoff of ACE2 expression.

negatively correlated with CD45RO (a CD4 Memory cell marker) expression. Furthermore, we analyzed the prognosis according to the expression level of ACE2 in relevant immune cell subgroups, and results confirmed that high ACE2 expression group in decreased CD4 Naïve, enriched CD4 Naïve and enriched CD4 memory cohort had favorable prognosis. The above results suggested that the low expression of ACE2 in ccRCC may partially affect the prognosis of ccRCC patients due to altered immune cells infiltration levels.

Consistent with our results, previous study also suggested that ACE2 expression was reduced in breast cancer [27], non-small cell lung cancer [28], pancreatic cancer [29] and hepatocellular carcinoma [30], and healthy individuals have increased serum ACE2 activity [31]. All these finding demonstrated that lower ACE2 expression levels are frequently associated with the presence of cancer. Besides, recent studies indicated that tumor patients with SARS-CoV-2 coronavirus infection may have a worse outcome than other subjects [18-20]. Although ACE2 is the cellular entry receptor of SARS-CoV-2, it is not clear whether ACE2 is involved in the process by which SARS-COV-2 coronavirus infection affects the prognosis of cancer patients. Therefore, the impact of SARS-CoV-2 coronavirus infection on the prognosis of ccRCC patients and the role of ACE2 in this process need to be further studied.

In addition, our study still has some limitations. First, the number of patients enrolled to verify the prognostic value of ACE2 in ccRCC patients needs to be expanded. Second, more in vitro experiments such as immunofluorescence and flow cytometry are needed to screen a kind of specific immune cell to further determine the correlation of ACE2 expression with CD4 naïve T and CD4 memory cells infiltration. Nevertheless, our study is the first to determine the clinical roles of ACE2 in ccRCC through various databases and laboratory experiments, and these findings may contribute to a better understanding of the impact of COVID-19 on ccRCC patients.

Conclusions

In conclusion, our research verified that ccRCC had considerably lower AEC2, and that a lower ACE2 was linked to poorer pathological characteristics and a worse prognosis. Furthermore, our work showed that one of the reasons of poor prognosis resulting from lower ACE2 expression may be changed immune cell infiltration levels.

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