The impact of ACE2 and co-factors on SARS-CoV-2 infection in colorectal cancer

SARS-CoV-2 is the novel coronavirus leading to COVID-19. Patients with cancer show a higher risk of infection with SARS-CoV-2 than patients without cancer, and two co-factors, TMPRSS2 and FURIN, could be differentially expressed in various tissues involved in the susceptibility of cancer patients to SARS-CoV-2 infection. However, the functional role of these genes in colorectal cancer with COVID-19 is not clear. This study is the first report to explore the expression pattern of ACE2 and its co-factors in colorectal cancer, as well as their effects on SARS-CoV-2 infection.

To assess the mRNA and protein levels of ACE2 and two co-factors in colorectal cancer, we performed RNA-sequencing and proteomics analysis in both colorectal cancer tissues and adjacent normal tissues. ACE2 was higher in colorectal tumour tissues than in normal tissues (Figure 1A–C), whereas TMPRSS2 and FURIN were lower in tumour tissues compared to normal tissues (Figure 1D–H). The mRNA levels of TMPRSS2 and FURIN gradually decreased with malignant progression in the course of normal, adenoma, and tumour (Figure 1I–L). However, no significant increase in ACE2 mRNA was observed during malignant progression (Figure S1). The mRNA level of ACE2 positively correlated with TMPRSS2 and negatively correlated with FURIN (Figure S2). Stratification analysis showed that no significant differences in the age of onset, tumour site, or stage were related to the expression of these three genes (Figure S3).

Based on single-cell RNA-sequencing profiling, ACE2 was primarily expressed in enterocyte cells (Figures 1M–R and S4). TMPRSS2 was primarily expressed in enterocytes and Paneth cells, and FURIN was expressed in all types of colorectal epithelial cells (Figure 1M–R). These results provide the co-expression pattern of ACE2 with its co-factors in colorectal tissues.

Due to the low mRNA and protein expressions of ACE2 in colon cells (Figure S5A and Table S1), we constructed colon cells with a stable overexpression of ACE2 (Figure 2A–C). We transfected cells with the SARS-CoV-2 pseudovirus at an appropriate dilution (Figure 2D). Significantly increased luciferase activity and immunofluorescence were observed in ACE2-positive colon cells compared to ACE2-negative cells (Figure 2E,F). Because the level of TMPRSS2 could influence SARS-CoV-2 infection, we further analysed the expression patterns of TMPRSS2 and ACE2. We found a positive correlation between the levels of ACE2 and TMPRSS2 in multiple colorectal cell lines (Figure 2G). The mRNA and protein levels of TMPRSS2 were increased with the increased mRNA and protein levels of ACE2 (Figure S5B). Therefore, ACE2 and TMPRSS2 may play crucial roles in influencing SARS-CoV-2 infection in colorectal cells.

Moreover, we compared the expression levels of ACE2 and its co-factors in different tissues. The ACE2 and FURIN protein expressions showed moderate-to-strong immunoreactivity, and the protein immunoreactivity of TMPRSS2 was low in most tumours (Figure S6). Notably, the ACE2 and TMPRSS2 mRNAs were expressed higher in colorectal tumour tissues than in many other tumours, such as lung, breast, and liver (Figure S7). These data indicate that even if ACE2 and TMPRSS2 are expressed at low levels in colorectal cancer, colorectal tissues may be particularly susceptible to SARS-CoV-2.

Previous studies demonstrated that ACE2 correlated with immune infiltration. The mRNA expressions of ACE2 and its co-factors were associated with immune infiltration in colorectal cancer (Figure S8, Table S2). Immune and stromal cells dominate the tumour microenvironment (TME) in cancer development. Therefore, we analysed the correlation between immunity-related scores and the expression of ACE2 and its co-factors. The expression of ACE2 and TMPRSS2 negatively correlated with the immune score and stromal score (Figure 3A,B), and a positive correlation was detected between FURIN mRNA expression and these scores. Higher mRNA levels of ACE2 and TMPRSS2 were associated with lower overall susceptibility of cancer patients to SARS-CoV-2.
Figure 1 Differences in the expression of candidate genes between colorectal cancer tissues and normal tissues. (A) The mRNA expression of ACE2 determined from RNA-Seq. (B) The protein expression of ACE2 determined by proteomics. (C) Representative immunohistochemical images of ACE2 expression in colorectal tumour tissues from the HPA database. (D) Forest plots of TMPRSS2 mRNA expression in six RNA-Seq databases. (E) Representative immunohistochemical images of TMPRSS2 in colorectal tumour tissues from the HPA database. (F) Forest plots of FURIN mRNA expression in six RNA-Seq databases. (G) Representative immunohistochemical images of FURIN in colorectal tumour tissues from the HPA database. (H) Heat map of TMPRSS2 and FURIN mRNA expression from the TCGA database. (I–L) The box plot of TMPRSS2 and FURIN expression includes data from colorectal adenoma tissues from the GEO databases. (M) The tSNE plot displays the major cell clusters for the colon tissues from the GEO database (GSE125970). (N) The tSNE plot shows different cell types in the colon. (O) The tSNE plots show the expression of ACE2, TMPRSS2, and FURIN in the colon. (P) The tSNE plot displays the major cell clusters for the rectal tissues from the GEO database (GSE125970). (Q) The tSNE plot shows different cell types in the rectum. (R) The tSNE plots show the expression of ACE2, TMPRSS2, and FURIN in the rectum.
activity of the anti-cancer immune response (Figure 3C). These results suggested that higher mRNA expressions of ACE2 and TMPRSS2 influenced the TME in colorectal cancer.

Furthermore, the low mRNA expression of ACE2 was significantly associated with a poor survival time of patients, but no significant association was observed between the overall survival time and the levels of TMPRSS2 or FURIN (Figure 3D). The overall survival time of patients in the higher level score group was longer than patients in the lower level score group for the combination of ACE2 and FURIN but not for the combination of other
FIGURE 3  The association between the expression of candidate genes and the immune response, prognosis, and somatic mutation patterns in colorectal cancer tissues. (A and B) The correlation of the expression of three genes with immune score (A) and stromal score calculated by the ESTIMATE algorithm (B). (C) The association of the expression of three genes with the status of anti-cancer immunity by the TIP algorithm. (D) Kaplan–Meier survival analysis of ACE2 (left), FURIN (middle), and TMPRSS2 (right) in colorectal cancer patients without details on whether they suffered from COVID-19. (E) Patients were divided into low- and high-expression groups according to the median expression of selected genes. Kaplan–Meier survival analysis of ACE2 and FURIN (left), ACE2 and TMPRSS2 (middle), and all three genes (right). Patients were divided into high- and low-score groups based on the median risk score under different combinations of selected genes. (F) The relationship between survival status, risk score rank (upper), and survival time (bottom). (G) Somatic mutation frequency of the three genes. (H) Boxplot of ACE2 expression in association with the status of ACE2 mutations. (I) Box plot of tumour mutational burden (TMB) in association with the status of ACE2 mutations. (J) Linear correlation between ACE2 mRNA expression and TMB.

groups (Figure 3E,F). These data suggested that ACE2 may have dual functions in accelerating SARS-CoV-2 infection and prognosis in colorectal cancer.

We further explored the association of genetic variants in ACE2 and its co-factors with colorectal cancer susceptibility (Figures S9 and S10). However, no significant association was detected between candidate genetic variants and the susceptibility of colorectal cancer after false discovery rate correction (Table S3). Haplotypes with possible risk and the effect of each haplotype are shown in Table S4. The haplotype GCGGGGTTGA in TMPRSS2 significantly decreased colorectal cancer risk compared to the most common haplotype GCGGGGGGGA (OR = .63, p = .031).

To identify somatic mutation patterns in ACE2 and its co-factors, we extracted mutational signatures of these genes in colorectal cancer. Missense mutations in ACE2 (73.33%), FURIN (75.00%), and TMPRSS2 (83.33%) were common in colorectal cancer tissues (Figure 3G and Table S5). Notably, we observed a significant increase in the mutation frequency of ACE2 in the early-onset colorectal cancer group compared to the late-onset group (Table S6). We also detected a decreasing trend in ACE2 expression in patients with ACE2 mutations (Figure 3H). Tumour mutational burden (TMB) is an emerging biomarker for the immunotherapy response of cancers. Therefore, we analysed the association of TMB with ACE2 mutations and expression. TMB was higher in individuals with ACE2 mutations compared to patients without ACE2 mutations (Figure 3I) and was negatively associated with ACE2 mRNA expression (Figure 3J).

In conclusion, we identified that ACE2 was upregulated and positively correlated with TMPRSS2 expression in colorectal cancer tissues. SARS-CoV-2 infection was significantly higher in ACE2-positive colon cells. The mRNA expressions of ACE2 and TMPRSS2 were associated with
the immune infiltration level of colorectal cancer. Our results suggest that ACE2 and its co-factors participate in the mechanisms underlying the association of colorectal cancer with COVID-19 (Figure S11).

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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