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Original Article

2019-nCoV may create complications in colon cancer patients with ACE2 expression

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Abstract: Since December 2019, a novel coronavirus (2019-nCoV) has emerged from Wuhan, China, causing symptoms in humans. Much remains unknown about 2019-nCoV, especially the additional risks that 2019-nCoV infection may pose for colon cancer patients. Many reports show that angiotensin converting enzyme II (ACE2) is the cell receptor through which 2019-nCoV enters host cells, and this is similar to the cell entry mechanism of SARS coronavirus. Previous studies show that ACE2 is highly expressed in the gastrointestinal tract, especially in the colon. In patients with colon cancer, ACE2 expression is significantly increased in tumor tissues compared to tissues from patients with other types of cancer. One of the known regulators of endocytosis is the serine protease (TMPRSS2) and AP2-associated protein kinase 1 (AAK1), which also facilitates the passage of viruses into cells. Furthermore, the Database of Gene expression profiling interactive analysis suggests that expression levels for ACE2, TMPRSS2, and AAK1 are positively correlated in colon cells. Therefore, our findings predict that 2019-nCoV will create increased complications for patients with colon cancer.

Keywords: 2019-nCoV, colon cancer, ACE2, bioinformatics

Introduction

Since December 2019, a novel coronavirus, 2019-nCoV, has created an outbreak of severe pneumonia in Wuhan, China. This novel coronavirus quickly spread throughout entire country [1]. There are many confirmed cases of 2019-nCoV worldwide; further, the number of confirmed patients is increasing quickly [2]. The 2019-nCoV shares over 85% homology of the viral nucleic acid with the SARS-CoV, which created an outbreak in 2002-2003 [2]. Many clinical symptoms of 2019-nCoV are similar to those of SARS-CoV, including cough and fever [3]. The 2019-nCoV causes diarrhea and nausea that is much more severe than what was reported for the SARS-CoV [4]. Thus, recent studies showed that the digestive system is a likely route for 2019-nCoV infection [4]. Furthermore, 2019-nCoV spreads very rapidly and tends to infect people with low immunity, including those with diabetes and cancer.

Until now, little was known regarding the mechanism surrounding the enteric symptoms linked to 2019-nCoV. Recent reports show that cellular expression of angiotensin converting enzyme II (ACE2) is required for 2019-nCoV infection and that cells without ACE2 or other coronavirus receptors, such as dipeptidyl peptidase and aminopeptidase N, are resistant to 2019-nCoV infection [1, 5, 6]. Thus, ACE2 plays a key role in facilitating the passage of 2019-nCoV into cells [7]. Single-cell transcriptome analyses indicate that ACE2 expression is not restricted to lung AT2 cells but is indeed expressed in the gastrointestinal system [8]. A recent study demonstrated that SARS-CoV-2 uses the SARS-55 CoV receptor, ACE2, for entry and the serine protease TMPRSS2 for S protein priming [9]. A 56 TMPRSS2 inhibitor approved for clinical use blocked entry and might constitute a 57 treatment option [10]. Furthermore, enteric symptoms related to 2019-nCoV may be associated with ACE2 expression. In addition to ACE2, AP2-associated protein kinase 1 (AAK1) has also been reported to play a key role in the passage of the virus into cells [6, 8, 11].

In the present study, we establish that 2019-nCoV infects and further harms patients with

colon cancer. Using online datasets to analyze the expression of ACE2 in various organs, we found that ACE2 is highly expressed in the gastrointestinal tract. ACE2 expression was markedly enhanced in colon tumor samples compared with adjacent normal tissue samples. We also analyzed the correlation between ACE2, TMPRSS2, and AAK1 expression in colon cancer. Taken together, our study predicts that high expression of ACE2 in colon cancer patients creates an increased likelihood for 2019-nCoV infection in the digestive tract.

Materials and methods

Exploration of the expression of ACE2

In this study, the HPA database (http://www.proteinatlas.org/) was used to explore ACE2 protein and mRNA expression in different human tissues and various types of cancer. These data are available directly online.

The differential expression of ACE2 in tumor tissues and corresponding adjacent non-tumor tissues was analyzed using GEPIA (http://gepia.cancer-pku.cn/index.html). The correlation of ACE2 and AAK1 expression was also obtained from this database.

Tissues and IHC

Primary colon cancer tissues and corresponding adjacent non-tumor colon tissues were collected from the Biobank of Zhejiang Cancer Hospital. Prior to surgery, each patient signed a written informed consent to take part in the study. This study was approved by the Medical Ethics Committee of Zhejiang Medical College. IHC was carried out according to the Invitrogen protocol; briefly, paraffin sections were successively deparaffinized, rehydrated, and boiled to perform antigen retrieval. Then, ACE2 antibody (21115-1-p, PTG, PROTEINTECH GROUP, INC; 1:1000) was added to each section for 2 h at room temperature. After paraffin sections were washed gently three times with PBS, 400 μL of histochemical polymer enhancer was added for 20 min at room temperature. After paraffin sections were washed gently again three times with PBS, anti-mouse antibody (1:5000) was added to each section for 30 min at room temperature. Finally, 400 μL of DAB was added sequentially to each section; sections were counterstained with hematoxylin (P0203, Beyotime), dehydrated and dried with gradient alcohol, clarified with xylene, and sealed with neutral gum.

Evaluation of immunohistochemical staining

The expression of ACE2 detected by immunohistochemistry was evaluated by measuring staining intensity and the proportion of positively-stained cells. According to the staining intensity, there were four levels including negative (0 point), weak positive (1 point), intermediate positive (2 points), and strong positive (3 points). The proportion of positive cells was scored as follows: < 5% = 0 point, 5%-25% = 1 point, 26%-50% = 2 points, 51%-75% = 3 points, and > 75% = 4 points. If the sum of two scores was < 5 points, it was considered low expression; 0 point indicated not expressed; and ≥ 5 points indicated high expression.

Database of immune cell expression (DICE)

DICE (https://dice-database.org/landing) was used to identify the expression quantitative trait loci for a total of 12,254 unique genes, which represent 61% of all protein-coding genes expressed in different cell types. Strikingly, a large fraction (41%) of these genes showed a strong cis-association with genotype only in a single cell type. It also showed that biological sex is associated with major differences in immune cell gene expression in a highly cell-specific manner. In this study, the expression levels of ACE2 are available directly online.

Clinical data

We summarized clinical data for immune cell expression in patients with cancer. All clinical data were directly obtained from the clinic lab at Zhejiang Cancer Hospital.

Results

Expression of ACE2 in different organs

Recent studies have demonstrated that ACE2 is the cellular receptor for 2019-nCoV, and that expression of this receptor is necessary for the virus to enter a cell. The expression level of ACE2 in various organs was analyzed using online datasets, and the results indicated that ACE2 was highly expressed in the digestive tract, including the stomach and the colon (**Figure 1A**). We also analyzed the Human

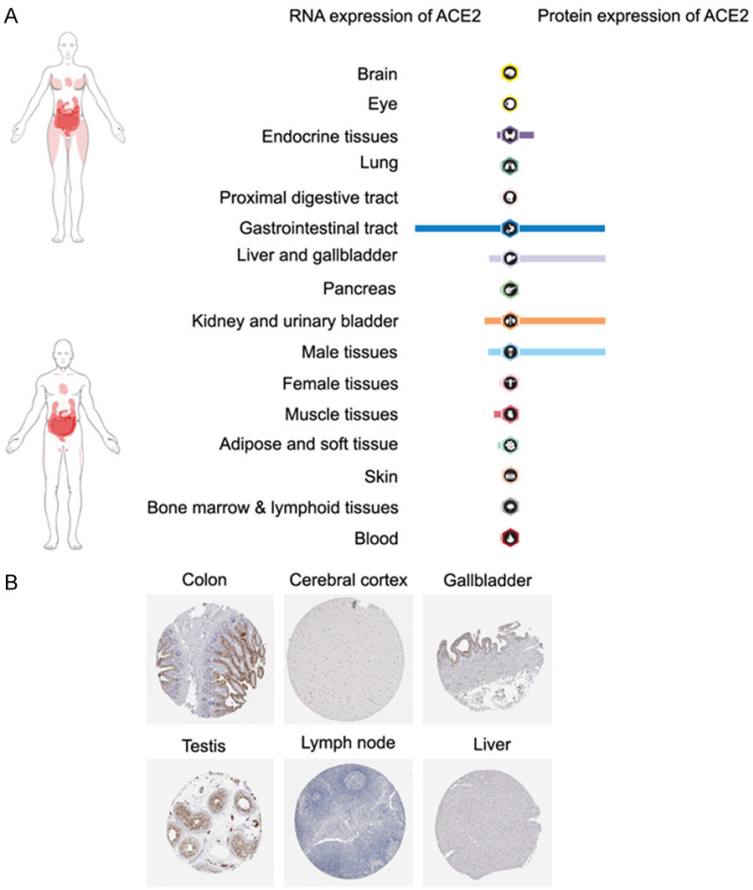

Figure 1. The level of ACE2 in various human tissues. A. The Human Protein Atlas database was used to analyze the expression of ACE2 in 16 different human tissues, including the gastrointestinal tract. B. Representative IHC staining of ACE2 in colon, cerebral cortex, gallbladder, testis, and lymph node.

Protein Atlas (HPA) database and investigated ACE2 protein expression level in various cell types. As shown in the database, immunohistochemistry (IHC) analysis indicated that ACE2 protein expression is significantly higher in the colon compared to other tissues (**Figure 1B**). These results suggest that cells of the digestive tract are likely targets for 2019-nCoV infection.

The level of ACE2 in various cancers

To further determine the expression level of ACE2 in various cancers, HPA analysis indicated that ACE2 expression is significantly higher in patients with colon cancer, gastric cancer, and renal cancer (**Figure 2A**). Therefore, we analyzed ACE2 expression in tumor tissues compared to normal tissues using the GEPIA database and found that expression of ACE2 mRNA is higher in colon cancer tissues compared to normal tissues (**Figure 2B** and **2C**). These results suggest that colon cancer tissues are more susceptible to 2019-nCoV infection than normal tissues.

High expression of ACE2 in colon cancer tissues

IHC was used to verify the expression of ACE2 in primary colon tumor samples compared with normal tissue samples. We analyzed ACE2 expression levels in 60 colon tumor tissues. For each tumor sample, an adjacent non-cancerous colon tissue sample was also analyzed. The results showed that ACE2 was abundantly and uniformly expressed in tumor samples, but its expression was significantly down-regulated in all adjacent non-cancerous tissues (**Figure 3**). ACE2 was highly expressed in 55 cases of colorectal cancer (91.67%) and was low in 5 cases of colorectal cancer (8.33%). In matched normal intestinal tissues, the expression was high in 3 case (5%) and low in 57 cases (95%). Nonparametric test showed that the expression of ACE2 in human colorectal cancer tissue was significantly higher than that in normal intestinal tissue ($P < 0.001$) (**Table 1**).

Positive correlation between ACE2, TMPRSS2 and AAK1 in colon cells

A recent study reported that 2019-nCoV also relies on AAK1 to enter cells. TMPRSS2 is also a likely target for the 2019-nCoV. Therefore, we next analyzed the correlation between ACE2, TMPRSS2, and AAK1 expression in the colon. A GEPIA database analysis showed a positive correlation between ACE2, TMPRSS2, and AAK1 expression in the colon (**Figure 4**). Taken together, these findings indicate that ACE2 is highly expressed in patients with colon cancer. Thus, high levels of ACE2 expression in tumor tissues of colon cancer patients reveal not only a poten-

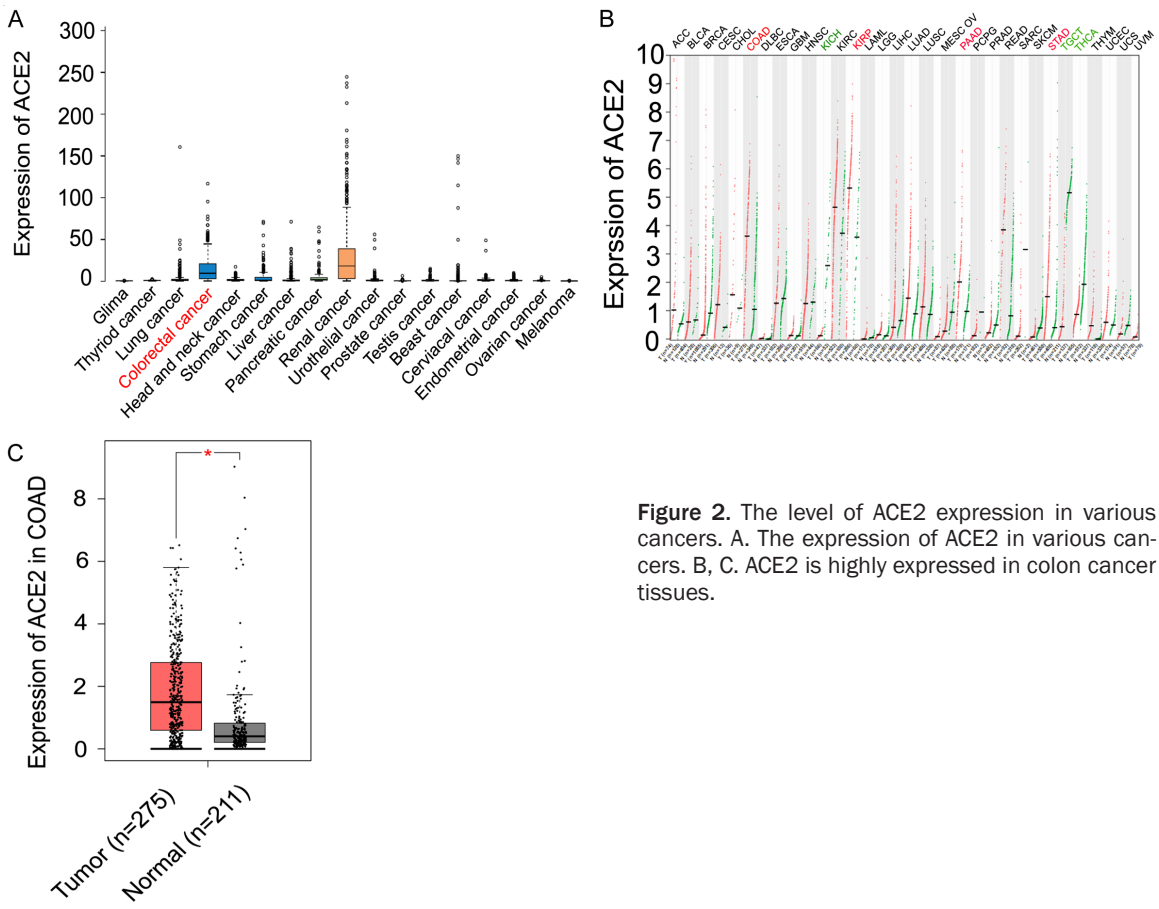

Figure 2. The level of ACE2 expression in various cancers. A. The expression of ACE2 in various cancers. B, C. ACE2 is highly expressed in colon cancer tissues.

tial mechanism of infection, but also a possible mechanism for direct damage of renal tubules and testes in which ACE2, TMPRSS2, and AAK1 function as host cell receptors for 2019-nCoV.

Discussion

In December 2019 and January 2020, a novel coronavirus-infected pneumonia outbreak in Wuhan, Hubei province, China, which spread rapidly and has posed a major threat to global public health [12-14]. Despite the public health significance of this outbreak, little was known until now about the additional risks that 2019-nCoV infection may pose for cancer patients.

Several studies have demonstrated that ACE2 acts as the entry receptor for 2019-nCov and that ACE2 expression is required for infection [4, 15, 16]. This infection mechanism is similar to that of SARS-CoV and MERS-CoV. The receptor-binding motif of 2019-nCov directly interacts with ACE2, and this is consistent with the reported capacity for human cell infection for 2019-nCoV [17]. Furthermore, traditional Chinese medicine approaches were used to decrease ACE2 expression as strategies for anti-2019-nCoV therapy [15]. Since ACE2 is highly expressed in renal tubular epithelial cells and in Leydig cells of the testis, patients infected with 2019-nCoV show abnormal renal function [18].

A recent study indicated that TMPRSS2 plays a key role in spread of several clinically relevant viruses, such as coronaviruses and influenza A viruses [19]. There are indications that some protection against 2019-nCoV infection was afforded by neutralizing antibody responses raised against SARS-S [20]. A recent study also revealed that disruption of AAK1 expression may prevent passage of the virus into cells. The AAK1 inhibitors, sunitinib and erlotinib, have been shown to inhibit viral infection of cells [21].

In the present study, we analyzed online datasets and found out that ACE2, which acts as the

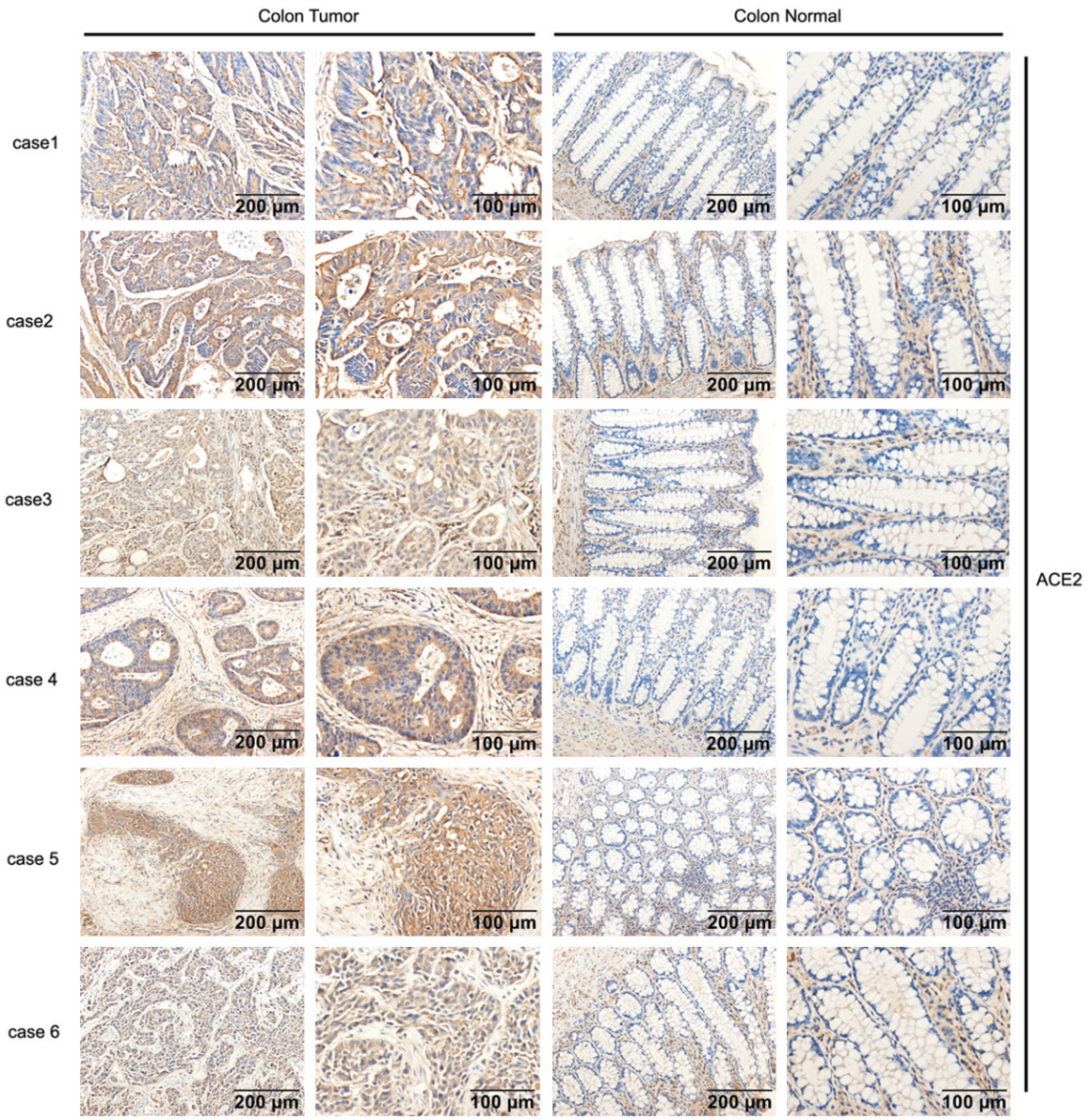

Figure 3. Expression of ACE2 protein in 60 pairs of colon tumor. Immunohistochemistry to examine the expression of ACE2 in colon tumor and the corresponding nontumor-adjacent colon tissues.

Table 1. Summary of characteristics of colon patients

Patients	Number (pairs)	Age (P25, P75)	Male (%)	Tumor		Normal	
				High	Low	High	Low
Colon cancer	60	57.5 (48.75, 65)	74.1%	91.67%	8.33%	5%	95%

major receptor for 2019-nCoV infection, is expressed at high levels in stomach and colon tissues. The elevated expression of ACE2 in colon cancer tissues compared with other tissues was verified by IHC. Expression of TMPRSS2 and AAK1, which have been predicted as other receptors for 2019-nCov, is positively correlated with expression of ACE2. Therefore, our findings suggest that 2019-nCoV poses additional risks to patients with colon cancer. Further analysis of colon cancer patients is required.

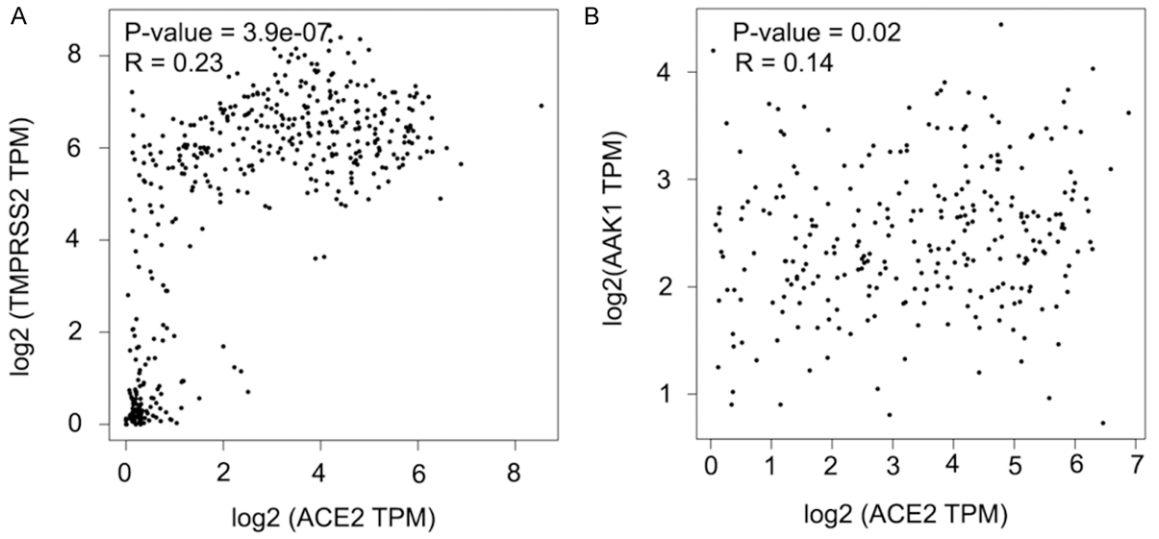

Figure 4. Correlation of ACE2, TMPRSS2, and AAK1. According to GEPIA database, ACE2 has a positive correlation with TMPRSS2 and AAK1 ($P < 0.05$).

Disclosure of conflict of interest

None.

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