Increased ACE2 and TMPRSS2 expression in ulcerative colitis

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Abstract

Ulcerative colitis (UC) is a cryptogenic inflammatory bowel disease, and there is an urgent need to elucidate its pathogenesis. ACE2 and TMPRSS2, the entry molecules of SARS-CoV-2, are reportedly associated with the disease; however, no consensus has been reached yet. In this study, we examined the expression of ACE2 and TMPRSS2 in colon and rectal specimens of UC. We collected colorectal specimens from 60 patients (30 patients with UC and 30 controls from 2018 to 2021) and analyzed the proportion and intensity of ACE2 and TMPRSS2 using immunohistochemistry. The results revealed a significant increase in the proportion of ACE2 expression and the intensity of TMPRSS2 expression in patients with UC. ACE2 and TMPRSS2 expression in UC remained unaffected by the COVID-19 pandemic. We demonstrated that ACE2 and TMPRSS2 are likely involved in the pathogenesis of UC.

Introduction

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) with a high incidence rate in young adults. However, the pathogenesis is not completely understood. Diagnosis is based on clinical, endoscopic, and histological findings.

ACE2 and TMPRSS2 are believed to be involved in IBD pathogenesis. ACE2 and TMPRSS2 are the host cell membrane molecules involved in SARS-CoV-2 infection. Their expression levels vary depending on the site in the intestinal tract [1].

ACE2 regulates blood pressure via the renin-angiotensin-aldosterone pathway and is expressed in the lungs and intestines (especially in the small intestine) [1]. ACE2 possesses anti-inflammatory and anti-fibrotic effects [2] and regulates the homeostasis of intestinal amino acids, expression of antimicrobial peptides, and ecology of intestinal microbiome [3]. Toyonaga et al. [4] found that increased ACE2 expression in the colon is associated with a poor prognosis in Crohn's disease (CD), whereas Potdar AA [2], found that impaired ACE2 expression in the ileum leads to poor outcomes in CD. Thus, certain interpretations of ACE2 and CD prognoses remain difficult.

TMPRSS2 is present in the plasma and intracellular membranes; however, its physiological function is not yet understood [1].

There are limited reports on IBD and the expression of ACE2 and TMPRSS2 in

IBD, and a consensus has not yet been reached. Therefore, in this study, we investigated the relationship between UC and the expression of ACE2 and TMPRSS2 via immunohistochemistry (IHC) and clarified their involvement in the pathogenesis of UC. We also analyzed the effect of the COVID-19 pandemic on ACE2 and TMPRSS2 expression.

Materials and methods

Patients

We collected colorectal specimens from 60 patients from the pathological database (2018–2021) of the Kinki Central Hospital, Itami, Japan. They comprised samples from 31 men and 29 women (age: 22–94 years; mean age: 64.3 years). Fifteen patients were clinically diagnosed with UC in 2018 (before the COVID-19 pandemic), 15 patients were clinically diagnosed with UC between 2020 and 2021 (after the COVID-19 pandemic), 15 patients were clinically diagnosed with colorectal adenocarcinoma in 2018 (before the COVID-19 pandemic), and 15 patients were clinically diagnosed with colorectal adenocarcinoma between 2020 and 2021 (after the COVID-19 pandemic). Nonlesional specimens from patients with cancer were used as controls. This study was approved by

the Ethical Review Board of Kinki Central Hospital (Proposal No. 453). All procedures were performed in accordance with the guidelines and regulations of the committee. The patient characteristics are listed in Table 1. C-reactive protein (CRP) levels were examined at the closest time point to the date of tissue collection.

IHC

All tissue specimens were fixed in 10% formalin and neutral buffered solution, embedded in paraffin, cut into 4-μm-thick serial sections, and used for IHC. The assay was performed using the Roche BenchMark ULTRA IHC/ISH Staining Module (Ventana Medical Systems), according to the manufacturer's instructions and a previous study [5]. The primary antibodies used in this study, their dilution ratios, and positive controls are listed in Table 2.

Scoring of IHC

IHC staining was scored by two independent pathologists (Y.H. and K.Y.) using an optical microscope. We confirmed that each specimen consisted of at least 10 crypts. The specimens were limited to the colorectum, and the ileum was excluded. The target was limited to the mucosal epithelium. Positive controls for both ACE2 and TMPRSS2 were

set in the renal tubular epithelium of surgical specimens. ACE2 and TMPRSS2 of positive controls were expressed in the cytoplasm with higher expression intensities on the luminal surface.

The proportions of ACE2 and TMPRSS2 were determined at four levels: score 0 (less than 1% positive), score 1 (1% or more but less than 33% positive), score 2 (33% or more but less than 66% positive), and score 3 (66–100% positive). The intensity of the control was set at a score of 3, and complete negativity was set at a score of 0. For the intensity of both ACE2 and TMPRSS2, judgments were made in four equal steps with scores of 0, 1, 2, and 3, starting from the one with the lowest intensity.

Statistical analysis

Cochran–Armitage trend tests and Spearman's rank correlation coefficient calculations were performed using EZR (Easy R) [6]. Statistical significance was set at $P < 0.05$.

The results of the two pathologists were evaluated using Spearman's rank correlation coefficient, and significant positive correlations were found for ACE2 proportion (Spearman's rank correlation coefficient = 0.594, P = 0.00000056), ACE2 intensity (Spearman's rank correlation coefficient = 0.589, P = 0.00000076), TMPRSS2 proportion (Spearman's rank correlation coefficient = 0.725, P = 0.00009369654), and

TMPRSS2 intensity (Spearman's rank correlation coefficient = 0.393, P = 0.00189). A final decision was made after discussing whether the two judgments differed.

Results

The proportion and intensity of ACE2 and TMPRSS2 were assessed in the control pre and post COVID-19 pandemic and the UC pre and post COVID-19 pandemic groups using IHC (Table 1). Representative IHC images of ACE2 and TMPRSS2 are shown in Fig. 1.

The following results were obtained from the Cochran–Armitage test: (i) the COVID-19 pandemic had no significant effect on the ACE2 proportion (P = 0.374), ACE2 intensity (P = 0.238), TMPRSS2 proportion (P was not available), or TMPRSS2 intensity (P was not available) in the UC group; (ii) the TMPRSS2 intensity of the control group significantly increased after the COVID-19 pandemic (P = 0.0309), but there was no significant effect of the COVID-19 pandemic on the ACE2 proportion (P = 0.309), ACE2 intensity (P = 0.108), and TMPRSS2 proportion (P = 0.0871) in the control group; (iii) without considering the effect of the COVID-19 pandemic, the proportion of patients with UC significantly increased with an increase in ACE2 proportion (P = 0.0271) (Fig. 2); (iv) the proportion of patients with UC significantly increased with an increase in

TMPRSS2 intensity (P = 0.00488) (Fig. 3); and (v) with regard to ACE2 intensity (P = 0.575) and TMPRSS2 proportion (P = 0.0948), there was no significant association with the proportion of patients with UC.

Furthermore, the correlation between the ACE2 proportion and CRP was examined in 28 patients in the UC group, excluding two patients with deficient CRP values, but no significant correlation was found (Spearman's rank correlation coefficient = 0.0557, P = 0.778).

Discussion

In this study, we used IHC to examine the expression of ACE2 and TMPRSS2, which are SARS-CoV-2 entry molecules, in colon and rectal specimens from patients with UC. We examined whether the expression of these molecules was affected by the COVID-19 pandemic.

The results of this study did not reveal any effects of the COVID-19 pandemic on ACE2 or TMPRSS2 expression in the UC group. In patients with IBD, increased risks of infection, severe disease, or death from SARS-CoV-2 have not been observed [7], which is consistent with the findings of this study.

Although this study found an increase in ACE2 expression in the UC group, it

could not be determined whether the increase in ACE2 expression was protective against or promoted inflammation. In other words, it could not be determined whether the increase in ACE2 levels was a cause or consequence of inflammation. A previous report showed significantly higher ACE2 staining intensity in the cytoplasm of the UC group than that in the control group, although these results were not consistent with those of our study [8]. In this study, no significant correlation was found between the ACE2 proportion and CRP values, suggesting disease activity; thus, the ACE2 proportion may be a universal marker independent of disease activity.

An increase in the intensity of TMPRSS2 expression was observed in the UC group compared with that in the control group. In contrast, an increase in TMPRSS2 intensity was observed in the control group after the COVID-19 pandemic; however, we do not believe that this affected the interpretation of the results.

A previous study using microarray data showed no significant difference in gene expression of *ACE2* and *TMPRSS2* in colon mucosal biopsies between the control and UC groups [9]. Discrepancies may exist between the gene and protein expression levels of ACE2 and TMPRSS2.

This study has some limitations. First, no distinction was made regarding the area of the bowel (e.g., ascending or transverse colon) in the specimens collected, and the

effect of different areas on the results remains unknown. The intestinal expression of ACE2 and TMPRSS2 in IBD depends on the site of the disease; both TMPRSS2 and ACE2 are reported to be highly expressed in the ileum and weakly expressed in the colon [10]. Second, this study was based on a limited number of cases from a single institution, and further accumulation of cases is required.

Conclusion

ACE2 and TMPRSS2 expression in UC was not affected by the COVID-19 pandemic. There was a significant increase in the proportion of ACE2 expression and intensity of TMPRSS2 expression in patients with UC. ACE2 and TMPRSS2 are likely to be involved in the pathogenesis of UC.

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Conflict of interest

The authors declare no conflict of interest.

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Figure legend

Fig. 1. Representative immunohistochemical expression of ACE2 and TMPRSS2. ACE2 proportion, ACE2 intensity, TMPRSS2 proportion, and TMPRSS2 intensity were scored as 3, 3, 3, and 3, respectively, in patient 45. ACE2-positive cells were observed in the crypt epithelium. Especially, strong positive staining on the luminal side was observed (A: Original magnification 100×, scale bar = 200 μm, B: Original magnification 400×, scale bar = 50 μm,). TMPRSS2-positive cells were also observed in the crypt epithelium. The positive staining on cytoplasm was observed (C: Original magnification 100×, scale bar = 200 μm; D: Original magnification 400×, scale bar = 50 μm,).

Fig. 2. Statistical analysis. ACE2 proportion score was significantly increased in patients with UC ($P = 0.0271$).

Fig. 3. Statistical analysis. TMPRSS2 intensity score was significantly increased in patients with UC ($P = 0.00488$).

Conflict of interest

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