Aberrant MUC immunohistochemical expressions in inflammatory bowel diseases

Yuichiro Hamamoto, Ph.D., M.D.[1,2], Michihiro Kawamura, Technical Associate[3], Hiroki Uchida, Bachelor of Health Sciences[3], Kojiro Takagahara, Bachelor of Health Sciences [3], Chiaki Katori, Bachelor of Health Sciences [3], Hinako Asai, Bachelor of Health Sciences [3], Hiroshi Harada, Ph.D., D.M.D.[1], Shigeki Shimizu, Ph.D., M.D.[4], Eiichi Morii, Ph.D., M.D.[2], Kyotaro Yoshida, M.D.[1,3]

1 Department of Diagnostic Pathology, Kinki central hospital, 3-1 Kurumaduka, Itami, Hyogo 664-8533, Japan.

2 Department of Pathology, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

3 Department of Clinical Laboratory, Kinki central hospital, 3-1 Kurumaduka, Itami, Hyogo 664-8533, Japan.

4 Department of Clinical Laboratory, National Hospital Organization Kinki-Chuo Chest Medical Center, 1180 Nagasonecho, Kita-ku, Sakai, Osaka, Japan

Corresponding author: Yuichiro Hamamoto

Corresponding author's e-mail: kyokui090088@gmail.com

Abstract

Ulcerative colitis (UC) and Crohn's disease (CD) are cryptogenic inflammatory bowel diseases (IBD) that are suggestive of aberrant MUC expression; however, their relationship remains unclear. Here, we examined aberrant MUC expression in intestinal samples from UC and CD patients in comparison to samples from patients with ischemic colitis (IC) and control groups. To study the expression of MUC1, MUC5AC, and MUC6 in different patient groups, we reviewed the slides stained with hematoxylin and eosin and performed immunohistochemistry. The results revealed that MUC1 was expressed more in the UC group and MUC6 in the CD group. No significant changes were observed in MUC expression in the IC group. Overall, we demonstrated changes in MUC expression in UC and CD, which can help in the diagnosis and early clinical management of UC and CD.

Keywords

Mucin; MUC; ulcerative colitis; Crohn's disease; ischemic colitis; inflammatory bowel disease; immunohistochemistry

Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) are chronic inflammatory bowel diseases (IBD) that are frequently observed in young adults. However, their pathogenesis is not completely known.[1-3] Mucins have been reported to assist in imparting mucosal defense against external pathogens.[4] Moreover, they are generally composed of core proteins (MUC and glycoprotein) and extensive O-linked glycans.[5] MUCs contain a variable number of tandem repeat sequences and demonstrate highly variable structures. They are divided into two major groups: secretory mucins and membrane-associated mucins. For instance, MUC2, MUC5AC, MUC5B, and MUC6 are classified as secretory mucins, whereas MUC1, MUC3, and MUC4 are membrane-associated mucins. Remarkably, 21 *MUC* genes have been identified.[6]

Multiple studies have demonstrated that UC pathogenesis is significantly associated with the mucin barrier.[7-13] Interestingly, MUC2 expression is often detected in the intestinal goblet cells of healthy individuals, but the expression of other MUCs has been found to be aberrant in the intestinal epithelium of patients with UC. For instance, a high incidence of MUC5AC and MUC6 expression has been reported in UC-associated non-invasive and invasive neoplasms.[14] Despite these findings, the relationship between aberrant MUC expression and UC is still indefinite and controversial.[6] Therefore, in this study, we investigated whether MUC1, MUC5AC, and MUC6, which are not normally expressed in the intestinal tract of healthy people, are useful as diagnostic markers for UC. In addition, to confirm whether the change in MUC expression was specific to UC patients, the frequency of MUC expression in patients with CD and ischemic colitis (IC) was compared.

Materials and Methods

Patients

We collected colorectal biopsy specimens of 60 patients from the pathological database (2010–2022) of Kinki Central Hospital, Itami, Japan. They were composed of samples from 38 males and 22 females (age 23–94 years, mean age 63.6 years). Fifteen patients were clinically diagnosed with UC, 15 were clinically diagnosed with CD, and 15 were clinically and histologically diagnosed with IC. The remaining 15 patients were diagnosed with colorectal cancer (CRC). Non-lesional specimens from patients with CRC were used as controls. This study was approved by the Ethical Review Board of Kinki Central Hospital (Proposal No. 416 and 432). All procedures were performed in accordance with the committee guidelines and regulations. The patient attributes are listed in Table 1.

Hematoxylin and eosin staining and immunohistochemistry

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 both hematoxylin and eosin staining and immunohistochemical (IHC) staining. The latter was performed using the Roche BenchMark ULTRA IHC/ISH Staining Module (Ventana Medical Systems, AZ, USA) according to the manufacturer's instructions and a previous study.[15] The primary antibodies used in this study, dilution ratios, and positive controls are listed in Table 2.

The antibodies for MUC1 (H23), MUC5AC (MRQ-19), and MUC6 (MRQ-20) used in our analysis were connected to the tandem-repeat domain.[16,17] Immunohistochemical staining was scored by two independent pathologists (Y.H. and K.Y.). Staining was considered positive if one or more stained epithelial cells were present in the crypts. We confirmed that each specimen contained ten crypts at least. When several specimens were included in the patient group, they were analyzed together.

Statistical analysis

Pairwise comparisons using Fisher's test were performed using EZR (Easy R).[18] The test was used to examine the relationship between the groups and MUC IHC expression. The *p* value adjustment method used was Bonferroni. Statistical significance was set at $p < 0.05$.

Results

Hematoxylin and eosin staining and MUC expression using immunohistochemistry

To study the pathological characteristics of the UC biopsy tissues, hematoxylin and eosin staining was performed. The specimens of the control, CD, and IC groups were similarly studied. Representative histological images of each group are shown in Figure 1.

Subsequently, the MUC expression was assessed in the control, UC, CD, and IC group of patients using IHC; the results are presented in Table 1. MUC1 expression was

positive in 5, 13, 10, and 8 patients in the control, UC, CD, and IC groups respectively. MUC5AC expression was positive in 9, 15, 9, and 15 patients in the control, UC, CD, and IC groups, respectively. MUC6 expression was positive in 0, 1, 7, and 0 patients in the control, UC, CD, and IC groups, respectively. Compared to the control group, there were significantly more cases expressing MUC1 in the UC group ($p = 0.047 < 0.05$) and significantly more cases expressing MUC6 in the CD group ($p = 0.038 < 0.05$). No significant change was observed in the frequency of MUC expression in the IC group.

Representative immunohistochemical expressions are shown in Figure 2. The statistical results for MUC immunohistochemical expressions are summarized in Table 3. The specificity and sensitivity using the panel of MUC1 and MUC6 are shown in Table 4.

Discussion

MUC1 expression has been observed in the glandular epithelium of the breast, pancreas, fundic gland, and lungs, whereas MUC5AC expression has been detected in the gastric foveolar epithelium. Furthermore, MUC6 expression has been identified in the pyloric, cardiac, and Brunner's glands. The expressions of MUC2, MUC3, MUC12, MUC13, and MUC17 have been reported in the intestine.[19] The expressions of MUC1, MUC5AC, and MUC6 have not been concluded in UC and CD patients.[16] In this study, we analyzed the expression of MUC1, MUC5AC, and MUC6 in intestinal biopsy samples from UC, CD, and IC groups using IHC.

Interestingly, our results differ from those of a previous report,[20] according to which MUC1 is expressed more intensely in the UC group than in the non-IBD group.

Furthermore, MUC1 was found to be expressed more strongly at the site of ruptured crypt abscess in patients belonging to the severe UC group than in patients from the control group. Moreover, the expression of MUC5AC and MUC6 was not observed in the UC group. In our study, MUC1 expression was detectable regardless of crypt abscesses in the UC group. The intensity of MUC1 expression was not considered in this analysis. However, other reports have corroborated our results. For instance, MUC5AC and MUC6 expression has been reported in 63 (70.0 %) and 16 (17.8 %) out of 90 patients with UC, respectively.[21] While MUC6 expression has been frequently associated with neoplasia in UC, no neoplasia was detected in cases with MUC6 expression in our study. Moreover, Mizoshita et al. reported a connection between ectopic MUC5AC expression and ulcerative colitis.[22]

MUC1 overexpression in neo-terminal ileum biopsies was detected immunohistochemically in postoperative patients with recurrent CD.[23] Histological severity was considered in this study. Increased MUC1 gene expression was also reported in the uninflamed CD ileum and IBD colon compared to healthy controls.[24] These studies are not necessarily contradictory to our study. mRNAs and peptides of MUC5AC and MUC6, which are seldom seen in the healthy ileum mucosa, were observed in the ileal mucosa near ulcers of CD.[25] Our report is consistent with the report of MUC6 expression. Decreased MUC5AC gene expression has been reported in CD patients.[26] On the other hand, our study showed stable MUC5AC immunohistochemical expression in CD patients. This discrepancy may have been caused by differences in gene and peptide expressions.

According to Yuan[27], MUC1 is not expressed immunohistochemically in IC. This result was consistent with our results. The relationships between IC and MUC5AC

and between IC and MUC6 have not been reported. MUC expression in IC is likely to be different from that in CD and UC.

Since our analysis focused on non-dysplastic/neoplastic UC and CD in patients of Japanese ethnicity compared to IC patients, we believe that our approach is original and valuable. Our study is also helpful for accumulating evidence about MUC expression in UC and CD using monoclonal antibodies against MUC1, MUC5AC, and MUC6, which were different from other previous studies.

Mucin core proteins and glycans are diverse, as the core proteins have various repeated sequences, while the glycans exhibit heterogeneous branched structures.[19] Thus, the antigenicity of mucins may be difficult to generalize. Regardless of the antibody clone, aberrant MUC expression is a potential pathogenic alteration in UC and CD patients. Hence, if the suggestive histology for UC and CD is observed, aberrant expression of MUC can support the diagnosis of UC and CD. Additionally, the panel of MUC1 and MUC6 is helpful for the differential diagnosis of UC and CD. Especially, the combination of MUC1+/MUC6- showed a specificity of 80% and a sensitivity of 80% (Table 4). Accordingly, these findings may be useful in the early clinical management of UC and CD.

Nonetheless, this study had some limitations. For instance, the study had a limited sample size and its outcomes were not completely adjusted for disease severity and duration. We could not stratify patients according to severity because we did not find many patients with UC, CD, or IC in our hospital.

Conclusion

A change in the frequency of MUC expression in UC and CD patients was noted, which

could help in the diagnosis and early clinical management of UC and CD.

Acknowledgments

This work was financially supported by a research grant from the Kinki Central Hospital, Itami, Japan. We would like to acknowledge the cooperation of all members of the Department of Gastroenterology at Kinki Central Hospital. We would also like to thank Editage (http://www.editage.jp) for the English language editing of the manuscript.

Disclosure statement

None

Author Contributions

Yuichiro Hamamoto designed the study, analyzed the data, and drafted the manuscript and figures. Michihiro Kawamura, Hiroki Uchida, Kojiro Takagahara, Chiaki Katori, and Hinako Asai were primarily involved in the specimen processing, histological sectioning, and staining. Hiroshi Harada and Shigeki Shimizu were involved in pathological diagnosis. Eiichi Morii helped with the cross-referencing and bibliography. Kyotaro Yoshida supervised the study, analyzed the data, and carefully read the manuscript and figures. All authors reviewed and acknowledged the findings reported in the manuscript.

References

1 Ungaro R, Mehandru S, Allen PB, et al. Ulcerative colitis. *Lancet* 2017;389(10080):1756-1770.

2 Podolsky DK. Inflammatory bowel disease (1). *N Engl J Med* 1991;325(13):928-37.

3 Guan Q. A Comprehensive Review and Update on the Pathogenesis of Inflammatory Bowel Disease. *J Immunol Res* 2019;2019:7247238.

4 Olli KE, Rapp C, O'Connell L, et al. Muc5ac Expression Protects the Colonic Barrier in Experimental Colitis. *Inflamm Bowel Dis* 2020;26(9):1353-1367.

5 Strous GJ, Dekker J. Mucin-type glycoproteins. *Crit Rev Biochem Mol Biol* 1992;27(1-2):57-92.

6 Bankole E, Read E, Curtis MA, et al. The Relationship between Mucins and Ulcerative Colitis: A Systematic Review. *J Clin Med* 2021;10(9):1935.

7 Fang J, Wang H, Zhou Y, et al. Slimy partners: the mucus barrier and gut microbiome in ulcerative colitis. *Exp Mol Med* 2021;53(5):772-787.

8 Hansson GC. Mucus and mucins in diseases of the intestinal and respiratory tracts. *J Intern Med* 2019;285(5):479-490.

9 ohansson ME, Gustafsson JK, Holmén-Larsson J, et al. Bacteria penetrate the normally impenetrable inner colon mucus layer in both murine colitis models and patients with ulcerative colitis. *Gut* 2014;63(2):281-91.

10 van der Post S, Jabbar KS, Birchenough G, et al. Structural weakening of the colonic mucus barrier is an early event in ulcerative colitis pathogenesis. *Gut* 2019;68(12):2142-2151.

11 ang J, Wang H, Zhou Y, et al. Slimy partners: the mucus barrier and gut microbiome in ulcerative colitis. *Exp Mol Med* 2021;53(5):772-787.

12 Johansson ME, Gustafsson JK, Holmén-Larsson J, et al. Bacteria penetrate the normally impenetrable inner colon mucus layer in both murine colitis models and patients with ulcerative colitis. *Gut* 2014;63(2):281-91.

13 Olli KE, Rapp C, O'Connell L, et al. Muc5ac Expression Protects the Colonic Barrier in Experimental Colitis. *Inflamm Bowel Dis* 2020;26(9):1353-1367.

14 Tatsumi N, Kushima R, Vieth M, et al. Cytokeratin 7/20 and mucin core protein expression in ulcerative colitis-associated colorectal neoplasms. *Virchows Arch* 2006;448(6):756-62.

15 Hamamoto Y, Kukita Y, Kitamura M, et al. Bcl-2-negative IGH-BCL2 translocation-

negative follicular lymphoma of the thyroid differs genetically and epigenetically from Bcl-2-positive IGH-BCL2 translocation-positive follicular lymphoma. *Histopathology* 2021;79(4):521-532.

16 Dhanisha SS, Guruvayoorappan C, Drishya S, et al. Mucins: Structural diversity, biosynthesis, its role in pathogenesis and as possible therapeutic targets. *Crit Rev Oncol Hematol* 2018 Feb;122:98-122.

17 Mazor Y, Keydar I, Benhar I. Humanization and epitope mapping of the H23 anti-MUC1 monoclonal antibody reveals a dual epitope specificity. *Mol Immunol* 2005 Jan;42(1):55-69.

18 Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant* 2013;48(3):452-8.

19 Hansson GC. Mucins and the Microbiome. *Annu Rev Biochem* 2020;89:769-793.

20 Longman RJ, Poulsom R, Corfield AP, et al. Alterations in the composition of the supramucosal defense barrier in relation to disease severity of ulcerative colitis. *J Histochem Cytochem* 2006;54(12):1335-48.

21 Borralho P, Vieira A, Freitas J, et al. Aberrant gastric apomucin expression in ulcerative colitis and associated neoplasia. *J Crohns Colitis* 2007;1(1):35-40.

22 Mizoshita T, Tanida S, Tsukamoto H, et al. Colon Mucosa Exhibits Loss of Ectopic MUC5AC Expression in Patients with Ulcerative Colitis Treated with Oral Tacrolimus. *ISRN Gastroenterol* 2013;2013:304894.

23 Hashash JG, Beatty PL, Critelli K, et al. Altered Expression of the Epithelial Mucin MUC1 Accompanies Endoscopic Recurrence of Postoperative Crohn's Disease. *J Clin Gastroenterol* 2021;55(2):127-133.

24 Vancamelbeke M, Vanuytsel T, Farré R, et al. Genetic and Transcriptomic Bases of Intestinal Epithelial Barrier Dysfunction in Inflammatory Bowel Disease. *Inflamm Bowel Dis* 2017;23(10):1718-1729.

25 Buisine MP, Desreumaux P, Leteurtre E, et al. Mucin gene expression in intestinal epithelial cells in Crohn's disease. *Gut* 2001;49(4):544-51.

26 Niv Y. Mucin Genes Expression in the Intestine of Crohn's Disease Patients: a Systematic Review and Meta-analysis. *J Gastrointestin Liver Dis* 2016;25(3):351-7.

27 Yuan M. Mucin core peptide expression in malignant and non-malignant colorectal tissues. *Zhonghua Zhong Liu Za Zhi* 1992;14(4):267-9.

Figure 1

Representative hematoxylin and eosin staining of terminal ileum or colorectum samples from control, ulcerative colitis (UC), Crohn's disease (CD), and ischemic colitis (IC) group. In case number 6 of control, there was no significant change. In case number 17 of UC, crypt distortion and crypt abscess were seen. In case number 32 of CD, irregular branching crypt was seen. In case number 49 of IC, goblet cell depletion and ghost-like appearance were seen. (Original magnification 200×, scale bar = 50 μm).

Figure 2

Representative immunohistochemical staining MUC1, MUC5AC, and MUC6 of terminal ileum or colorectum samples from control, ulcerative colitis (UC), Crohn's disease (CD), and ischemic colitis (IC) group. Case number 6 of control, case number 17 of UC, case number 32 of CD, and case number 49 of IC are shown. (original magnification 400×, scale bar = 20 μm). The staining was seen in cytoplasm of the crypt.