学位論文の要旨

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学位論文名	Down-regulation of Serotonin Reuptake Transporter Gene Expression in Healing Colonic Mucosa in Presence of Remaining Low Grade Inflammation in Ulcerative Colitis
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論文内容の要旨

INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD), two major forms of inflammatory bowel disease (IBD), are characterized by chronic immune-mediated intestinal disorders. On the other hand, irritable bowel syndrome (IBS) is a functional disorder of the intestinal tract in the absence of organic abnormalities accompanied by alterations in bowel habits. Thus, IBS is essentially distinguished from IBD as a disease entity.

Serotonin (5-HT), a monoamine neurotransmitter, is derived from tryptophan by activation of tryptophan hydroxylase-1 (TPH-1) in the GI tract. Approximately 90% of total 5-HT is located in enterochromaffin (EC) cells in the intestine and used to regulate GI motility. The serotonin reuptake transporter (SERT) terminates 5-HT activity by removing it from interstitial space. Reduced SERT expression and function result in excess 5-HT,leading to development of diarrhea and abdominal pain, and are associated with the pathogenesis of IBS.

A recent meta-analysis showed that IBS-like symptoms occurred in approximately 35% of

IBD patients, even in those in remission, indicating the possibility of a common pathogenesis for IBS and IBD. Down-regulated colonic SERT expression in IBS patients has been reported. However, little is known regarding SERT expression in colonic mucosa of those patients during healing. In the present study, we investigated SERT expression in colonic mucosa during active and healing phases in UC patients, as well as in colitis model mice.

MATERIALS AND METHODS

Twenty-two UC patients underwent colonoscopy examinations, during which inflamed mucosa was distinguished from that undergoing healing. Healing mucosa was classified into regular and irregular vessel patterns based on narrow-band imaging (NBI) magnifying colonoscopy findings. Expressions of SERT, TPH-1, and various inflammation-related genes in biopsy samples were assessed using a PCR array system and real-time PCR. Immunohistochemistry was performed for SERT and TPH-1. Assessment of inflammatory activity was based on Matts histological grading system. The human study protocol was approved by the Ethics Committee of Shimane University and written informed consent was obtained from all subjects (No.1170).

Acute colitis was induced in 8-week-old male specific pathogen-free C57BL/6J mice by administering 2.5% dextran sodium sulfate (DSS) in drinking water for 5 days, while the controls received sterile drinking water without DSS. To evaluate the time-course changes of various gene expressions as well as colonic histology, mice were euthanized at different times (0, 7, 14, 21, 28, 56, 84 days).The present chronic colitis model was established as follows. SAMP1/Yit mice, a model of human CD, were euthanized at 30-50 weeks of age and $CD4^+$ T cells were isolated magnetically from their mesenteric lymph nodes (MLNs) by positive selection with CD4 microbeads. Isolated $CD4^+$ T cells ($5x10^5$/mouse) were then injected in an intraperitoneal manner into SCID mice (8-10 weeks old) to induce colitis. Control mice were established by adoptive transfer of $CD4^+$ T cells isolated from the MLNs of AKR mice into SCID mice. The animals were euthanized at 0 and 8 weeks after injection, then colonic tissues were obtained and subjected to real-time PCR and histological examinations. All experiments with animals in this study were approved by the Ethics Committee for Animal Experimentation of Shimane University and they were handled according to our institutional guidelines (IZ-25-135, IZ-24-152, IZ-25-135).

RESULTS AND DISCUSSION

Immunohistochemical findings showed that abundant SERT immunoreactive signaling

from epithelial cells was down-regulated in inflamed mucosa sections. Furthermore, the gene expression level of SERT in epithelial cells was significantly lower in inflamed as compared to non-inflamed healing mucosa, and SERT mRNA expression was negatively correlated with histological grade of colonic inflammation. In addition, the gene expression level of IL-8 was significantly higher in inflamed mucosa. In UC patients, we also found that the expression level of SERT was significantly decreased in inflamed as compared to non-inflamed colonic mucosa, which was negatively correlated with the expression level of pro-inflammatory cytokines as well as histological inflammatory grading. On the other hand, the expression level of TPH-1 was higher in inflamed as compared to healing mucosa, though the difference was not significant. Finally, we examined alterations of SERT expression in colonic epithelial cells in model mice with acute and chronic colitis, and found that colitis induced down-regulation of SERT expression.

Our findings obtained with NBI magnifying colonoscopy revealed two patterns of healing mucosa. The endoscopic appearance of healing mucosa with a regular vessel pattern was similar to that of uninvolved normal colonic mucosa, while histological activity and the expression level of TPH-1 were not statistically different between those. On the other hand, we found a significant decrease of SERT expression in healing mucosa with an irregular vessel pattern as compared to that with the regular vessel pattern. We also investigated the expressions of a variety of inflammation- and angiogenesis-related genes in healing mucosa with both regular and irregular vessel patterns using a PCR-array system, which confirmed increased expression levels of IL-1β, IL-8, and CXCL5 in mucosa with an irregular vessel pattern. Those results showed that expressions of various inflammation-related genes were up-regulated in mucosa with an irregular vessel pattern, whereas they were relatively low as compared to inflamed mucosa. Suppression of SERT expression in colonic tissues was also noted during the healing phase of DSS colitis in mice, which was correlated with a lower level of colonic MIP-2 expression. Together, these findings suggest that remaining low-grade inflammation may be associated with suppression of SERT expression in the colon.

CONCLUSION

We found down-regulation of SERT expression in healing colonic mucosa of UC patients. In particular, our findings showed that suppression of that expression may be dependent on remaining low grade inflammation in colonic mucosa. Additional investigations of the

regulation of SERT expression in colonic mucosa are anticipated for elucidating its relationship to the pathogenesis of IBS-like symptoms in UC patients in remission.