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.

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論文審査の結果の要旨

過敏性腸症候群 irritable bowel syndrome (IBS) は腸の過敏運動を主因とする腸疾患であり，内視鏡検査では器質的異常が無いと考えられている。また, serotonin (5-HT: 5-hydroxytryptamine) は消化管機能を亢進する生理活性アミンであり，生体内では 90% が消化管に存在する。そして，腸管での 5-HT は，tryptophan の生合成を担う tryptophan hydroxylase-1 (THP-1) を発現する enterochromaffin 細胞による 5-HT の産生と，serotonin reuptake transporter (SERT) を発現する腸管上皮細胞による 5-HT の取り込みとのバランスで制御されている。一方，炎症性腸疾患である潰瘍性大腸炎 ulcerative colitis (UC) の寛解期に IBS 様症状が生じることが報告されているが，その機序は解明されていない。本研究では，UC患者の粘膜組織とマウス腸炎モデルを用いて，腸炎の急性期や寛解期・回復期における5-HT 関連遺伝子や炎症性サイトカイン遺伝子の発現の変化を検討することにより機序の解明を試みた。UC患者の炎症部位と非炎症部位でのSERTとTHP-1のタンパク質の発現を免疫組織染色で，mRNA 発現をreal-time PCRとPCR assay systemで検討したところ，SERTの発現は炎症の grade や IL-8 と逆相関していた。THP-1の発現は増加する傾向にあったが，炎症との相関を認めなかった。また，NBI (narrow band imaging)併用拡大内視鏡による非炎症部位の血管パターンの評価では，治癒した regular パターンの部位と比較し，炎症からの治癒過程と考えられる irregular パターンの部位では，炎症性サイトカインのmRNA発現が高まり，SERT mRNAの発現は低下していた。一方，2.5% dextran sodium sulfate を飲水させるマウス腸炎モデルでは，急性期だけでなく慢性回復期においても腸組織でのSERT mRNAの発現が低下し，MIP-2 mRNAの発現は増加していた。さらに，SAMP1/Yitマウスの CD4陽性T細胞をSCIDマウスに移入する慢性腸炎モデルにおいても同様な結果が得られた。以上の結果は，UCの寛解期に生じるIBS様症状は，寛解期でも持続している軽度の炎症による腸上皮細胞でのSERTの発現低下が原因であることを示唆しており，UC寛解期のIBS様症状の病態を解明した意義のある研究と考えられる。