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Original contribution

Methylation status of normal background mucosa is correlated with occurrence and development of neoplasia in the distal colon

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Summary The aim of this study is to evaluate the methylation status of normal colonic mucosa in relation to the stage of neoplasia arising from the mucosa. The methylation status of 2 age-related loci (*ESR1* and *MYOD1*) and global methylation (the mean of Alu and Sat2) in the normal colonic mucosa of 156 patients with and without colorectal neoplasia were examined. The distal colon and proximal colon were analyzed separately because neoplasia is biologically and clinically different between these sites. The methylation status was determined by MethyLight using percentage of methylated reference (PMR). In the distal colon, methylation of the age-related loci decreased as the stage of neoplasia increased (patients with no neoplasia or with adenoma ≤9 mm versus patients with advanced adenoma or with invasive cancer: *ESR1*-PMR median, 21.0 versus 15.7; $P = .015$; *MYOD1*-PMR median, 5.35 versus 3.80; $P = .0037$, respectively). Interestingly, global methylation was inversely correlated with the stage of neoplasia (59.7 versus 61.5; $P = .054$). In contrast, the proximal colon showed no significant correlations. The methylation of *MYOD1* in the normal mucosa was significantly correlated with *K-ras* mutation in neoplastic tissue arising from the mucosa. Specific epigenetic changes in normal colonic mucosa may be correlated with the occurrence and development of neoplasia in the distal colon.

1. Introduction

DNA methylation plays pivotal roles in carcinogenesis. In the context of cancer occurrence and development, 2 types of DNA methylation, CpG island methylation and global methylation, have been identified. In the case of CpG island methylation, the methylation of cytosines within promoter CpG islands is associated with the loss of protein expression by transcription repression. Therefore, gene hypermethylation is often associated with the silencing of tumor suppressor genes in cancer [1]. On the other hand, global hypomethylation, which has been shown to be associated with genomic instability and increased mutation rates, characterizes a large percentage of human cancers [2,3].

In addition, methylation can occur in DNA in the normal colonic mucosa. Therefore, there may be specific epigenetic changes in normal mucosa that make it susceptible to the occurrence and development of neoplasia. In this context,

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Shen et al [4] detected methylation of the promoter region of O^6-methylguanine-DNA methyltransferase (*MGMT*) in the normal mucosa adjacent to colorectal cancer (CRC) tissue in 22 (50%) of 44 patients whose tumors exhibited *MGMT* promoter methylation but in only 3 (6%) of 51 patients without it. Kawakami et al [5] reported that among 199 patients with CRC, age-related loci were more frequently methylated in normal mucosa of patients with CRC with microsatellite instability (MSI) or CpG island methylator phenotype (CIMP). In addition, estrogen receptor 1 (*ESR1*) in colonic mucosa was methylated more frequently in ulcerative colitis patients who had neoplasia than in those who did not [6]. Moreover, Ushijima et al [7] examined the methylation levels of healthy volunteers, patients with a single gastric cancer, and patients with multiple gastric cancers, and found that methylation levels in normal gastric mucosa was positively correlated with the risk for gastric cancers.

These previous results suggest that there may be a correlation between epigenetic changes of normal mucosa and the occurrence or development of tumors arising from it. However, although most CRCs develop from benign adenoma through an adenoma-carcinoma sequence, few reports have investigated the changes in the methylation status of normal background mucosa during the development of colorectal neoplasia, from small adenoma to advanced adenoma to invasive cancer.

In addition to the issue of cancer stage, the location of colorectal neoplasia should also be highlighted in the analysis of tumor development and methylation. There are many clinical and biologic differences between proximal and distal colon cancer [8,9]. In brief, the proportion of proximal colon cancer increases in the elderly and/or females [10]. CRCs with MSI have been frequently observed in the proximal colon, and epigenetic characteristics of CRCs also differ between the proximal and distal parts of the colon [10]. For example, CRC with CIMP-high (or CIMP+) is frequently observed in the proximal colon [10-12], and we also reported that a laterally spreading type of colorectal adenoma exhibited high frequency of CIMP-high and *K-ras* mutations especially in the proximal colon [13].

In this study, we evaluated the methylation status of normal mucosa in age-related CpG loci and global methylation. Our analyses were focused on the differences in patient and neoplastic characteristics as well as location of neoplasia, that is, proximal versus distal. We also examined correlations between the methylation status of normal mucosa and genetic and epigenetic changes of tumors.

2. Materials and methods

2.1. Patients and tissue samples

Normal colorectal mucosal samples were obtained from 88 patients, who had undergone endoscopic polypectomy, and 43 patients who had undergone surgical treatment of CRC at Okayama University Hospital (Japan) between June 2003 and May 2006. In addition, normal colorectal mucosal samples from 25 age-matched colonoscopically normal patients were obtained and used as controls. There were no cases with inflammatory bowel disease or with known history of hereditary colorectal cancer.

For patients who underwent colonoscopy or endoscopic polypectomy, normal colonic mucosal biopsy specimens were collected during polypectomy procedures from the proximal colon and distal colon of each patient. Samples of the proximal colon (defined as the cecum, ascending colon, and transverse colon) were collected from the midportion of the ascending colon, whereas those of the distal colon (defined as the descending colon, sigmoid colon, and rectum) were obtained from the sigmoid colon approximately 20 cm from the anal verge. Two samples were collected by biopsy forceps in each portion of the colon. For patients who underwent surgical resection, normal colonic mucosa specimens were scraped from the mucosal layer of resected samples more than 5 cm from the tumor margin. The samples were stored at −80°C until analysis. This study protocol was approved by the institutional review board of Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences. Informed consent was obtained from each patient.

2.2. Clinical information

The patients' clinical information, including age, sex, smoking habits, and alcohol consumption, was obtained. *Smokers* were defined as those who had a past or current smoking habit. *Alcohol drinkers* were defined as those who drank more than approximately 10 g/d. At the time of the endoscopic polyp resection, polyp location and size were determined. Polyp location was classified into 2 groups: "proximal" and "distal." Polyp size was recorded as the maximum diameter of the extirpated specimen. The location of the invasive cancer was determined according to the findings of the operation.

2.3. Pathologic findings

Histologic studies were performed on all resected polyps and cancers. Adenomas were classified as tubular, tubulovillous, villous, or serrated adenomas. They were also classified as low-grade, high-grade, or intramucosal cancer according to the grade of dysplasia. The cytologic atypia of serrated adenoma was determined to be similar to that of conventional adenoma, whose nuclei are elongated, hyperchromatic, and pseudostratified [14]. *Advanced adenomas* were defined as adenomas of 10 mm or more, with a villous component, or exhibiting high-grade dysplasia or intramucocal cancer. Hyperplastic polyps were not included in the neoplasia category.