A Mucinous Histochemical Study on Malignancy of Aberrant Crypt Foci (ACF) in Rat Colon

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ABSTRACT. The relationship between malignancy and number of crypts (crypt multiplicity) comprising aberrant crypt foci (ACF) was investigated, by studying changes in the mucous nature of ACF with 5 crypts or less, ACF with 6–13 crypts, adenomas and invasive adenocarcinomas induced by 1,2-dimethylhydrazine in distal colon of rats. A paradoxical Con A-staining was performed for goblet cell mucins. Of the sulfomucin-dominant ACF with 1–3 crypts, 82.6% had labile class III mucin, similar to the distal colon in the normal rats. However, in most of the goblet cell mucin produced by the ACF with 4–5 crypts with an indicated relation to colorectal carcinoma or the sialomucin (SiM)-dominant ACF with 1–3 crypts, mucin types other than class I were rarely present. The incidence of class I mucin decreased with the increase in crypt multiplicity of ACF or in the degree of histological malignancy, with the lowest incidence of 40% in adenocarcinomas. In contrast, the incidence of class II mucin increased markedly with the increase in crypt multiplicity of ACF or in the degree of histological malignancy, with the highest incidence in adenocarcinomas (95%). The ACF with 6–13 crypts had a mucous profile similar to that of adenomas. These results suggested that malignancy of ACF related to the crypt multiplicity. In the ACF with 1–3 crypts, SiM-dominant ACF had the potential to progress to malignant lesions.

KEY WORDS: aberrant crypt foci, goblet cell mucin, paradoxical concanavalin A-staining, rat colon.

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Aberrant crypt foci (ACF) are widely used as an intermediate biomarker for colon cancer to detect tumor suppressor and promoter substances. Bird [1, 2] considers ACF as preneoplastic changes of colon cancer based on their cellular, molecular and morphologic features. In addition, some ACF are themselves diagnosed as microadenoma [19], adenoma [15] or carcinoma in situ [23], indicating that ACF are preneoplastic lesions. A relative increase of sialomucins (SiMs) caused by a reduction of sulfomucins (SuMs) has been noted in colon cancer in humans and rats [4, 5, 12, 20, 32]. The change in the type of mucin is also related to the degree of histological malignancy (dysplasia) [6]. Ulex europaeus agglutinin-I (UEA-I) binding to antigenic carbohydrates has been observed in colon cancers of humans and rats [3, 7, 14, 31]. We reported that goblet cell mucin was produced by ACF, whose mucous nature is quite similar to that of adenocarcinomas, especially in ACF with more than 4 crypts [28]. Pretlow et al. [17] and Kristiansen [10] pointed out that the presence of ACF with more than 4 crypts was an important risk factor for colon cancer. Moreover, a role of ACF in colon tumorigenesis has been indicated, by the observation that showed carcinomas in lesions histologically and macroscopically diagnosed as ACF [23] and by the results of proliferating cell nuclear antigen-labeling index [22], carcinoembryonic antigen [18] and K-ras mutation molecular analysis [8, 16, 21, 24, 25, 30]. Mucin in the gastrointestinal tract of rats is classified into the 4 subtypes, namely class I, class II, stable class III and labile class III, by a modification of the concanavalin A-horseradish peroxidase method (paradoxical Con A-staining) [9]. In the colonic mucosa of normal rats, labile class III mucin and class II mucin have been noted in goblet cells, class I mucin in the cytoplasm of absorptive epithelial cells, and class II mucin in the surface coat [9, 26, 27]. Class II mucin was detected in the mucus of colon cancer induced by 1,2-dimethylhydrazine (DMH) in rats, in addition to labile class III mucin [26, 27]. We confirmed that only labile class III mucin is present in tissues of the lower distal colon of normal rats (unpublished data). The purpose of this report is to examine the goblet cell mucins of ACF with different numbers of crypts, adenomas and adenocarcinomas, based on the lesions' reactivity to paradoxical Con A-staining and to discuss the relationship between crypt multiplicity and malignancy of ACF.

MATERIALS AND METHODS

Induction of ACF, adenomas and adenocarcinomas in the rat colon: ACF with 5 crypts or less were induced in 6-week-old F344:DuCrj male rats supplied by Charles River Japan (Kanagawa, Japan) by the administration of 20 mg/10 m/kg of DMH (Tokyo Kasei Kogyo Co., Ltd.) into the abdominal cavity twice at a one-week interval. ACF observed in the distal colon of 5 rats at 35 weeks after the initial DMH administration were used in the study. ACF with 6–13 crypts, adenomas and adenocarcinomas were induced in 25 five-week-old F344:DuCrj male rats by the subcutaneous administration of 50 mg/kg of DMH once a week, for a total of 10 times. The lesions observed in the distal colon of rats at 15 weeks after the initial DMH administration were used in the study. The distal colon (the rectal half of the colon, excluding the plicate part of proximal

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colon and a 2-cm portion at the anal end of rectum) was used
[28].

**Histological technique**: The proliferative lesions were stained with 0.2% methylene blue as previously described [28], and the number of crypts was confirmed by stereoscopic microscopy. Nodular lesions in which it was difficult to confirm the crypt number were regarded as colon cancer. ACF with 5 crypts or less were embedded in paraffin, parallel to the mucosal surface, and 20–30 consecutive cross-sections, 3–5 µm in thickness each, were made from upper to basal levels of crypts. For proliferative lesions with 6 crypts or more, approximately 30 consecutive sections were made from upper to basal levels of crypts in parallel to the mucosal surface (except for nodular lesions that were cut vertically to the mucosal surface).

**Histological analysis**: Every tenth section was stained with hematoxylin-eosin (H-E) and high-iron diamine Alcian blue (HID-AB). Goblet cells were observed in these sections. The ACF score [28] was calculated from the results of HID-AB staining for ACF with 5 crypts or less. Twenty-three SuM-dominant and 22 SiM-dominant ACF with 1–3 crypts and 11 SuM-dominant and 20 SiM-dominant ACF with 4–5 crypts were obtained. Among the proliferative lesions with 6 crypts or more, only those with a relatively large number of goblet cells were used. The lesions were classified according to nuclear arrangement, appearance of tubular structures, and invasiveness of proliferation. There were 68 ACF with 6–13 crypts including those that showed regularly arranged nuclei in one part and disarranged nuclei with stratification tendency in the others, 20 adenomas that exhibited invagination of epithelial cells into ductal lumen in addition to nuclei with stratification tendency, and 20 adenocarcinomas with clearly stratified nuclei and invasive proliferation to submucosal tissue of dysplastic tubuli. Borderline lesions were not used, to avoid confusion between adenomas and adenocarcinomas. The goblet cell mucin of these lesions was classified as class I (mucus that lacked characteristics of other classes of mucin), class II, stable class III and labile class III by the reactivity to paradoxical Con A-staining. In paradoxical Con A-staining, lesions that could be stained were regarded as positive and the incidence was compared. For the evaluation of the mucous nature of ACF accompanying the increases in crypt number (crypt multiplicity), all ACF with 4–5 crypts were used regardless of their ACF scores.

**Statistical analysis**: A statistical analysis of the incidences of class I, class II and labile class III mucin was performed using the χ²-test with Yates’ correction.

**RESULTS**

In the goblet cell mucin of ACF with 5 crypts or less, class I, class II and labile class III mucin were seen. These ACF were classified in detail by ACF score and crypt number, and the mucous nature of their goblet cells was evaluated by paradoxical Con A-staining (Fig. 1). Of the SuM-dominant ACF with 1–3 crypts, 82.6% contained labile class III mucin, like normal colon, whereas class I and class II mucin were present in 21.7% each of the ACF. In the SuM-dominant ACF with 4–5 crypts and SiM-dominant ACF with 1–3 or 4–5 crypts, however, labile class III mucin
was detected significantly less often (p<0.001) than in SuM-dominant ACF with 1–3 crypts. Most of these ACF had only class I mucin (Figs. 3, 4), and goblet cells with other mucous types were only found in one each of the SuM-dominant ACF with 4–5 crypts and SiM-dominant ACF with 1–3 or 4–5 crypts.

In ACF with 6 crypts or more, adenomas and adenocarcinomas, class I, class II or labile class III mucin was present in all lesions, as in the ACF with 5 crypts or less (Fig. 5). Different classes of mucin were co-existed in the same lesions in most cases (Fig. 6). The incidence of class I mucin tended to decrease with the number of crypts composing the ACF or the degree of malignancy of the lesions. The tendency was significant (p<0.01 or 0.001) in the ACF with 6 crypts or more, adenomas and adenocarcinomas when compared to the ACF with 4–5 crypts and in the adenocarcinomas compared to the ACF with 6–13 crypts. The incidences of class II mucin (64.7–95%) in the ACF with 6 crypts or more, adenomas and adenocarcinomas were significantly higher than that in the ACF with 4–5 crypts. Moreover, compared to those in the ACF with 6–13 crypts and adenomas, the incidence of class II mucin in the adenocarcinomas was significantly high (p<0.01 or 0.05). The incidence of labile class III mucin tended to increase with the increase in crypt multiplicity and malignancy. The ten-
tendency was significant (p<0.05 or 0.001) in the adenomas and adenocarcinomas when compared to the ACF with 4–5 crypts, and in the adenocarcinomas (p<0.01) compared to the ACF with 6–13 crypts. However, in all mucinous types, no clear difference was seen between the ACF with 6–13 crypts and adenomas.

DISCUSSION

ACF have been considered putative preneoplastic lesions or precursors of colon cancer. In the distal colon after carcinogen treatment, temporary decreases in the total number of ACF and incidence of ACF with 1–3 crypts were reported by McLellan et al. [13]. The incidence of labile class III mucin similar to the distal colon of normal rats was high in the SuM-dominant ACF with 3 crypts or less among those with 5 crypts or less. In ACF with 3 crypts or less, the incidence of SiM-dominant ACF and that of UEA-I lectin-bound crypts decreased with time after the initial DMH administration [28]. These findings implied that some of the SuM-dominant ACF with 3 crypts or less are normalized with time. In ACF with 4–5 crypts [10, 17] or in SiM-dominant ACF [28], which show similarities to colon cancer, the lesions with mucous types other than class I mucin were few. Our observation in normal fetal rats with paradoxical Con A-staining revealed that class I mucin is the type expressed in 18-day fetal rats, while class II mucin was detected in goblet cells of the small intestine from adult rats (unpublished data). Therefore, the fact that only class I mucin was detected in the goblet cells of ACF indicates that the mucous production of ACF was poorly differentiated. This is interesting in regard to the mechanism of ACF development. In DMH-induced colon cancer of rats, the presence of class II mucin has been confirmed [26, 27]. In the present study also, most of the adenocarcinomas examined had class II mucin. By identifying the changes in mucin from ACF with 4–5 crypts to adenocarcinomas, we confirmed that the incidence of class I mucin decreased with the increase in malignancy of the lesions as evaluated by the crypt multiplicity in ACF and degree of malignancy in neoplastic lesions. In contrast, the incidence of class II mucin in colon cancer was clearly increased. The changes in mucin with progression of malignancy are consistent with those reported by Filipe [6] in early malignancy using SiM as a marker. Since class II mucin is present in goblet cells of the small intestine of humans and rats, mucus in the colon indicates small intestinal type production. The increase of class II mucin in ACF with 6–13 crypts seems to be resulted from the production of small intestinal-type mucin by ACF, in the process of progression to larger lesions. Yamachika et al. [29] reported that goblet cell-type mucin produced in human colon cancer changed into the small intestinal-type, from the pattern of sialosyl-Tn antigen seen in typical SuM of colon and normal small intestine. SiM-dominant mucous production seen in distal colon cancer can be considered an oncofetal characteristic [11] and is reported to be associated with the degree of dysplasia [6]. In goblet cells of the distal lower colon of the 18-day fetal rats, class I mucin was present along with class II mucin (unpublished data). These findings indicate that class I mucin potentially changes into class II mucin in the distal colon.

Labile class III mucin increased with degree of crypt multiplicity in ACF and malignancy in neoplastic lesions. However, this change was not considered to be of biological significance because this type of mucin is also found in distal colon of normal rats.

The nature of mucin in ACF with 6–13 crypts was similar to that in adenomas. ACF with 6–13 crypts are a borderline lesion to adenomas and include neoplastic lesions called “microadenomas” [19]. These findings suggested that malignancy of ACF related to crypt multiplicity. In the ACF with 1–3 crypts, the SiM-dominant ACF had the potential to progress to malignant lesions.

REFERENCES


