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Advances in Brief

Significant Increase of Colonic Mutated Crypts in Ulcerative Colitis Correlatively with Duration of Illness[1]

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Abstract

Mild periodic acid-Schiff (mPAS) staining can discriminate non-*O*-acetylated (mPAS positive) from *O*-acetylated (mPAS negative) epithelial sialoglycoproteins in human colonic mucosa, giving three haplotypes of expression of a single polymorphic autosomal gene (*oat*). Increase in mPAS-positive crypts in heterozygotes is an indication of mutations, and wholly mPAS-positive (stem cell mutated) crypts and clusters of two or more mPAS-positive crypts in heterozygotes of ulcerative colitis ($P < 0.0001$) were found to be increased significantly, compared with controls. The observed correlation with ulcerative colitis duration ($r = 0.892$ and 0.853, respectively) supports a chronic inflammation-carcinoma sequence.

Introduction

With long-standing UC,[3] dysplastic changes occur frequently, and a dysplasia-carcinoma sequence is generally accepted (1–3). In addition to the accelerated epithelial cell turnover attributable to an increase in epithelial cell apoptosis (4–6), there is an association with p53 accumulation and high $p21^{CIP1/WAF1}$ expression in the active phase of UC, suggestive of epithelial DNA damage (7). Recently, it was demonstrated that mPAS staining can discriminate non-*O*-acetylated (mPAS positive) from *O*-acetylated (mPAS negative) epithelial sialoglycoproteins (8, 9). Thus, three patterns of staining may result from expression of a single polymorphic autosomal gene (*oat*): (*a*) uniformly mPAS positive, reflecting the homozygous recessive genotype for low or absent *O*-acetylation (oat^b/oat^b); (*b*) uniformly mPAS negative, reflecting the homozygous dominant genotype for normal or high *O*-acetylation (oat^a/oat^a); and (*c*) mPAS-negative mucosa containing scattered positive discordant crypts representing heterozygotes (oat^a/oat^b). Somatic mutation of the high acetylator allele in colonic crypt stem cells in heterozygous subjects followed by crypt colonization by the mutant progeny leads to conversion of the crypt phenotype from mPAS negative to mPAS positive. In fact, radiotherapy of heterozygotes induces a considerable increase in the mPAS-positive crypt frequency, which subsequently remains significantly elevated for 2–34 years, indicating that crypts with a mutant phenotype are stable (9, 10). Using this staining approach in the present study, we assessed mutated crypts in UC heterozygotes, as a potential indicator of DNA damage because of chronic inflammation.

Materials and Methods

UC Cases. Colectomy specimens were available from 104 patients with UC. Tissue blocks from rectosigmoid colon of cases were fixed with 10% buffered formalin (pH 7.0), embedded in paraffin. Forty cases with severe colitis (multiple ulcers and hemorrhage) were excluded. The *O*-acetylation phenotype was tested with mPAS staining in a total of 64 cases with UC. Furthermore, cases with active colitis, dysplasia or carcinoma, and cases in which tissue blocks were not sufficient for study were excluded. Finally, 14 cases who were mPAS-negative with scattered mPAS-positive crypts (heterozygotes) were obtained (age, 41.9 ± 12.7; male:female = 8:6).

Control Cases. Forty-five large bowel resection specimens from sporadic (nonhereditary) primary colorectal cancer patients were tested for mPAS staining for comparison. A total of 12 heterozygotes was chosen for study (age-matched, 41.3 ± 5.3; male:female = 9:3). The tissues studied were all obtained from the rectosigmoid colon, at a sufficient distance to guarantee noninvolvement from cancers.

mPAS-positive Crypts. The numbers of crypt profiles adjacent to the muscularis mucosa present in one central step section in each case were counted manually by a single observer (I. O.), according to the method of Campbell *et al.* (9). The total number of crypt profiles present was calculated by multiplying the number of step sections by the count in the central section. For mPAS staining, 4-μm sections were cut from paraffin-embedded blocks and step sections taken at 80-μm intervals to obtain the necessary size of sample while keeping the chances of counting the same crypt in adjacent sections at a very low level. The sections were stained with the mPAS technique (11). mPAS-positive crypts were identified and counted in longitudinal sections showing uniform staining of goblet cells from the base of the crypt to the luminal surface (complete replacement by the mutant phenotype) in >10,000 crypt profiles and expressed as numbers of positive crypts $\times 10^{-4}$. Because irregularly formed crypts were frequent in cases with UC, identification of single crypts was carefully confirmed to avoid duplication. Total numbers of crypts examined with mPAS staining were 14,275 ± 4,036 crypts in controls and 11,568 ± 1,917 crypts in UC cases.

Statistics. Data are given as mean ± SD values. Statistical comparison between the two groups was performed using the nonparametric Mann-Whitney *U* test. For demonstration of associations, the Pearson's correlation coefficient test was used with statistical significance defined as $P < 0.05$ and $r > 0.5$.

Results

Control Cases. All 45 noncancerous colorectum specimens removed surgically against primary colorectal cancers clearly showed one of the three phenotypes of uniformly mPAS-positive, uniformly mPAS-negative, or mPAS-negative heterozygotes with scattered positive discordant crypts (17:7:21; Fig. 1, *A–C*).

The total numbers of mPAS-positive crypts, clusters of two or more positive crypts (Fig. 1*D*), and foci of mPAS-positive crypts in rectosigmoid colons were 7.8 ± 3.4 $\times 10^{-4}$, 0.9 ± 0.8 $\times 10^{-4}$, and 6.7 ± 2.9 $\times 10^{-4}$ crypts, respectively (Table 1). Foci of mPAS-positive crypts refer to both individual crypts and clusters of two or more positive crypts, each counted as one event.

UC Cases. The total duration of illness in the 14 cases of heterozygotes were 1, 4, 5, 6, 7 (2 cases), 8, 9 (2 cases), 10 (2 cases), 11, 14,

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[3] The abbreviations used are: UC, ulcerative colitis; mPAS, mild periodic acid-Schiff.

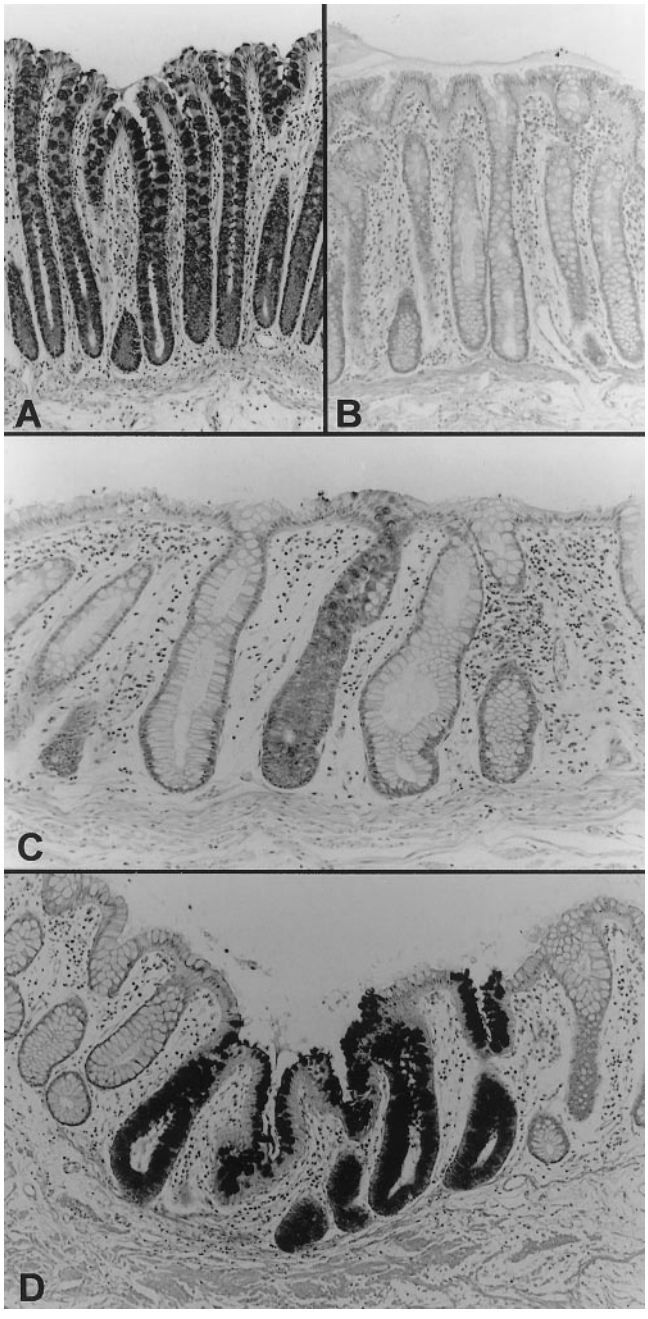

Fig. 1. mPAS staining. *A*, uniformly mPAS-positive mucosa in a control (×128); *B*, uniformly mPAS-negative mucosa in a control (×128); *C*, uniformly mPAS-negative mucosa with scattered positive discordant crypt (heterozygote) in a control (×320); *D*, cluster of mPAS-positive crypts in a UC heterozygote (×267).

and 17 years. None of the cases had either carcinoma or dysplastic lesions.

Significant increases of total numbers of mPAS-positive crypts, clusters of two or more mPAS-positive crypts, and foci of mPAS-positive crypts were found in UC cases, compared with the controls (Table 1).

There were significantly positive correlations between total numbers of mPAS-positive crypts, clusters of mPAS-positive crypts, and foci of mPAS-positive crypts and the duration of UC illness ($n = 26$ cases; $r = 0.892$, 0.853, and 0.731; $P < 0.0001$, respectively; Fig. 2, *A–C*). On the other hand, no significant correlations were found between the above described, each category, and patients' ages ($n = 26$ cases; $r = 0.071$, 0.074, and 0.275; $P = 0.7323$, 0.7203, and 0.1743, respectively).

Discussion

Concerning mPAS positivity in the present study, the uniformly mPAS-positive mucosa:uniformly mPAS-negative:uniformly mPAS-negative with scattered positive discordant crypts (heterozygotes) ratios were 17:7:21 in controls and 22:13:29 in UC cases, in line with those (34:10:56 cases; predicted percentage, 34:17:49) in Japanese cases in a previous report (12), indicating consistency with the staining method. The frequency of mPAS-positive crypts in 10^4 crypts in controls was 7.8 ± 3.4, also relatively similar to the value in another earlier report (Ref. 10; 7.5, 0–53.4, mean and range), suggesting our assessment method to also be accurate.

In UC cases, heterogeneity of mPAS positivity was observed in the present study, as described by Jass *et al.* (13). In particular, slightly mPAS-positive goblet cells were seen frequently in hyperplastic colonic mucosa. To avoid confusion with this kind of crypt, only definitely and wholly involved crypts with strong mPAS positivity were counted. Furthermore, definitely mPAS-positive discordant crypts tended to appear as clusters in UC cases. Because of this situation, mPAS-positive crypts were counted carefully to avoid duplication.

Thus, the present study provided clear evidence that mPAS-positive crypts in heterozygotes are increased significantly with a positive correlation to the duration of illness, indicating accumulation of mutations because of chronic inflammation in UC. This increase of mPAS-positive crypts in long-standing UC cases appears to be greater than that to 31.7×10^{-4} crypts, found in irradiated colonic mucosa (4500–4800 cGy internal; Ref. 10). Furthermore, significant increase of clusters composed of two or more mPAS-positive crypts, along with the duration of illness was revealed in UC cases, suggesting development from single mutated crypts by growth, although simultaneous common mutation occurring in several crypts because of the common DNA damage cannot be precluded.

In the present study, no significant correlations were found between any categories of mPAS-positive crypts and patients' ages. mPAS-positive crypts were 7.5 (range, 0–53.4) $\times 10^{-4}$ in rather elderly cases (age, 73; range 49–94) and 10 (range, 1.7–44) $\times 10^{-4}$ in left-sided colorectal cancer cases (age, 70; range, 51–83) in previous reports (10, 14), both being not significantly different from the values in our relatively young control cases. Furthermore, significant increase of clusters of two or more mPAS-positive crypts has hitherto not been reported in the background mucosa in colorectal cancer cases

Table 1 *Frequency of mPAS-positive crypts in colonic mucosa of controls (noncancerous colonic mucosa) and ulcerative colitis cases*

	No. of cases	Age (years)	Total duration of illness (years)	Frequency of mPAS-positive crypts ($\times 10^{-4}$)	Clusters of mPAS-positive crypts ($\times 10^{-4}$)[a]	Foci of mPAS-positive crypts ($\times 10^{-4}$)[b]
Controls	12	41.3 ± 5.3	0	7.8 ± 3.4	0.9 ± 0.8	6.7 ± 2.9
UC	14	41.9 ± 12.7	1–17	37.7 ± 16.5	7.9 ± 3.6	12.5 ± 4.0
				$P < 0.0001$	$P < 0.0001$	$P = 0.0008$

[a] Clusters of two or more mPAS-positive crypts.
[b] Both individual crypts and clusters of two or more positive crypts, each counted as one event; *P* compared with controls.

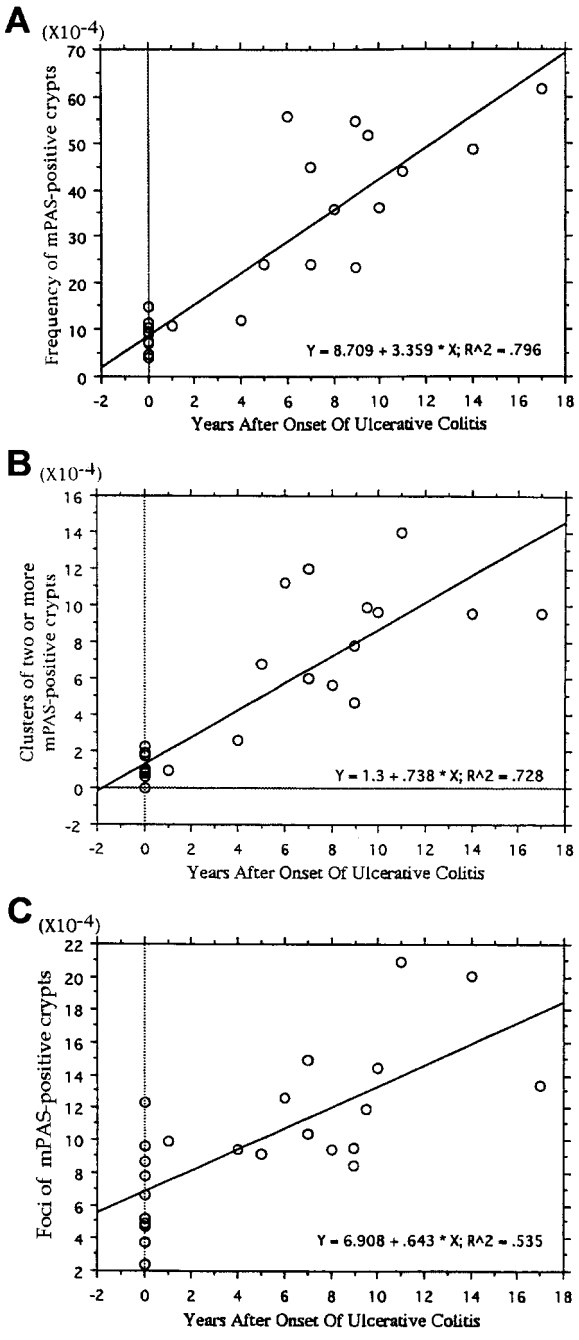

Fig. 2. *A*, correlation between the frequency of discordant mPAS-positive crypts with the duration of UC illness ($P < 0.0001$); *B*, correlation between values for clusters of two or more discordant mPAS-positive crypts with the duration of UC illness ($P < 0.0001$); *C*, correlation between values for foci of mPAS-positive crypts with the duration of UC illness ($P < 0.0001$).

or postirradiation state. Therefore, the potential role of age can be considered to be negligible in the present results.

It has been shown recently that genomic instability (15), chromosomal alterations (16), and *p53* gene mutations (17–19) occur in dysplastic lesions with a background of UC. In particular, genetic alterations of p53 may be evident even in nondysplastic regenerative mucosa with p53 protein overexpression in long-standing UC cases (19). Furthermore, it is considered that oxidative stress, including generation of peroxides, may play an important role via DNA damage in inflammation-associated tumorigenesis (20).

In conclusion, the present study demonstrated a significant correlative increase of wholly mPAS-positive (mutated) crypts in heterozygotes with the duration of UC, indicating accumulation of genetic mutations because of DNA damage with chronic inflammation. This is in line with the postulated chronic inflammation-carcinoma sequence.

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