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Advances in Brief

Aberrant Crypts: Putative Preneoplastic Foci in Human Colonic Mucosa[1]

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Abstract

Aberrant crypts were identified for the first time in whole-mount preparations of normal-appearing human colonic mucosa after staining with methylene blue. The foci of aberrant crypts varied from single altered glands to plaques of greater than 30 crypts. The mean proportion of colonic mucosa altered and the number of foci with aberrant crypts per cm² of colonic mucosa were (*a*) higher in patients with colon cancer than in patients without colon cancer or predisposing conditions and (*b*) highest in our single case of Gardner's syndrome. Aberrant crypts are postulated to be the earliest identifiable potential precursors of colon cancer. Analysis of aberrant crypts may facilitate the study of the early pathological and molecular changes that precede colon cancer.

Introduction

Colorectal cancer is the second most common cause of cancer deaths in the United States (1). If the pathogenesis and risk factors associated with this disease were better understood, it might be possible to reduce significantly the incidence and/or the mortality from this disease. Recently, our laboratory (2–4); Bird (5), Bruce (6) and their associates; and Sandforth *et al.* (7) have identified lesions in the colons of animals treated with carcinogens that appear to be putative preneoplastic lesions. The purpose of this study was to determine if similar lesions can be identified in the colons of humans with colon cancer. To our knowledge this is the first description of aberrant crypts in whole mounts of grossly normal-appearing human colonic mucosa. These aberrant crypts express some enzymatic and other phenotypic alterations analogous to the alterations observed in carcinogen-treated rodents.

Materials and Methods

Grossly normal-appearing colonic mucosa was obtained from surgical resections from 22 consecutive patients with sporadic colonic carcinoma, 1 with colonic carcinoma with Gardner's syndrome, 1 with Crohn's disease, 1 with a recurrent tubulovillous polyp, 1 with diverticulitis, and 1 with clinically redundant colon (sigmoid volvulus) without pathological abnormalities. The surgical specimens were placed immediately into 0.9% saline at 4°C in the operating room. Additional tissue was provided by colonic mucosa from autopsies of patients without colonic carcinoma. The mucosa sampled from colon cancer patients was located within 15 cm of the tumor; from autopsy cases, the mucosa was collected from the right colon 15 to 30 cm distal to the ileocecal valve and from the left colon 15 to 30 cm proximal to the anus. Strips of mucosa were peeled from the submucosa and provided for us by the Tissue Conservation Core Facility of the Case Western Reserve University Cancer Center. The 22 patients with colon cancer had a mean age of 69 ± 12 (SD) years and consisted of 10 females and 12 males. Nine of the specimens were from the left colon or rectum, 1 from the transverse colon, and 12 from the right colon. The 13 autopsy patients had a mean age of 53 ± 24 years and included 6 females and 7 males; the 9 autopsy patients over the age of 50 had a mean age of 66 ± 8 years and included 4 females and 5 males.

Small segments of colonic mucosa (approximately 5 x 1.5 cm) were pinned out flat and fixed for 2 h in 2% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4) at 4°C. The fixed tissue was stained for 3 to 5 min in 0.2% methylene blue (Chroma-Gesellschaft Schmid & Co., distributed by Roboz Surgical Instrument Co., Washington, DC) in 0.1 M sodium phosphate buffer (pH 7.4) and rinsed in fresh phosphate buffer at 4°C for 30 to 60 min to allow more even distribution of the blue stain. The intact mucosal segments were placed luminal side up on microscope slides and observed with a low magnification (×4 objective lens and a × 10 or × 15 ocular lens). Aberrant crypts, as described by Bird (5), Bruce (6), and associates, were marked with permanent ink (The Davidson Marking System; Bradley Products, Inc., Bloomington, MN) with a microprobe as described by us (4) previously for aberrant crypts in rats. The marked aberrant crypts were embedded in glycol methacrylate (JB-4 embedding kit; Polysciences, Warrington, PA; molds and block holders, Bio-Rad Microscience Division, Cambridge, MA) as used by us previously for a variety of tissues including rat (3, 4, 8) and human (9) colon. Serial 2- to 4-μm sections were cut on a rotary microtome (Hacker-Bright, Fairfield, NJ) with an LKB glass knife adaptor (Mager Scientific, Inc., Dexter, MI) and stained as described previously (8) with hematoxylin, eosin, and azure II, for hexosaminidase and nonspecific esterase activity; and with the periodic acid-Schiff technique.

Results

Aberrant crypts were identified in grossly normal human colonic mucosa stained with methylene blue (Fig. 1). These foci of aberrant crypts were identified in samples from 9 of 9 left colons, 0 of 1 transverse colon, and 4 of 12 right colons when the grossly normal mucosa was collected from patients with sporadic colonic carcinoma. A total of 358.0 cm² of mucosa were evaluated from these patients, an average of 16.3 ± 7.1 cm²/patient. For comparison, the grossly normal colonic mucosa was evaluated from 5 additional patients with colonic resections for other conditions (Table 1) that included one patient with Gardner's syndrome (Fig. 2). Since we were able to obtain normal colonic mucosa from resections of only two patients without predisposing conditions for colon cancer during this time period, we used colonic mucosa from autopsy patients who never had colon cancer for additional comparisons. Similar segments of left and right colon were evaluated from the autopsies of 13 patients without colon cancer. No aberrant crypts were observed in the mucosa from the surgically resected descending colon of a patient with a recurrent tubulovillous polyp, from the surgically resected left colon from a patient with clinically redundant colon, or in 253 cm² from the right colons of 13 autopsies of patients without colon cancer.

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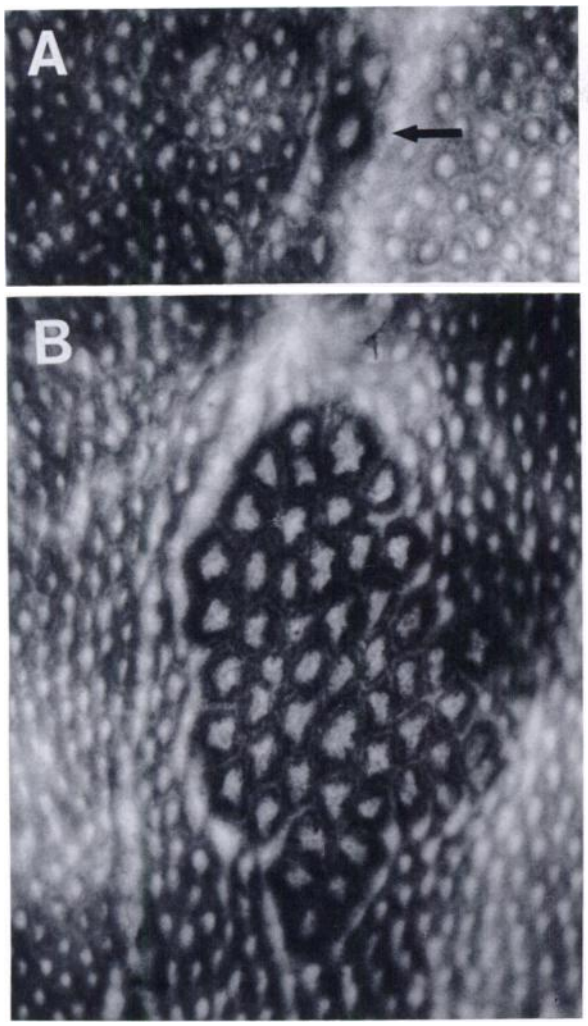

Fig. 1. Whole-mount segments, stained with methylene blue, of grossly normal human colonic mucosa from patients with colon cancer. The microscope light through the central portion of each crypt reveals a circular lumen. The lumina of the aberrant crypts are generally more elliptical and sometimes irregular in shape. *A, arrow,* single aberrant crypt. *B,* larger focus or plaque of aberrant crypts. The elevation of the plaque above the mucosal surface is apparent from the fact that it is in a different focal plane from that of the surrounding normal mucosa. × 29.

One focus of aberrant crypts was observed in 253.3 cm^2 from the left colons of 13 autopsies of patients without colon cancer, and a very small focus (2.8×10^{-4} cm^2) of aberrant crypts was observed in the left colon of one patient with both diverticulitis and an inflammatory polyp.

All crypts identified as aberrant were at least 3 times larger in diameter than normal crypts, and most had lumina that were oval or slit-shaped rather than circular (Figs. 1 to 3). The lumina of some aberrant crypts were dilated and smooth while others were serrated or had invaginations. The foci of aberrant crypts varied from single altered glands (Fig. 1*A*) to large plaques (Figs. 1*B* and 2*C*) of greater than 30 crypts, had a mean size of 1.4 ± 2.0 mm^2 (range, 0.001 to 8.1 mm^2), and appeared to be slightly elevated above the mucosal surface when viewed microscopically. Aberrant crypts were manyfold more frequent in our colon cancer patients than in our autopsy control patients and more frequent in the left colons than in the right colons resected for colonic carcinoma (Table 1). The mean percentage of mucosa altered in the colons of cancer patients (0.24 ± 0.50%) was much greater than that in patients without colon cancer or predisposing conditions including those at least 50 years old (Table 1). The frequency of aberrant crypts in the single patient with Crohn's disease was very similar to that observed in patients with colon cancer. In Gardner's syndrome, foci of aberrant crypts were more frequent and occupied a larger area (greater proportion) of mucosa as compared to our sporadic colon cancer patients. The lumina of most crypts not involved in aberrant foci in our single patient with Gardner's syndrome were abnormal in shape; a larger number of patients is needed to confirm this observation.

Methacrylate-embedded sections of some aberrant crypts were characterized histologically (Fig. 3) and histochemically. The nuclei of the aberrant crypts varied from normal to dysplastic. Two-thirds of the aberrant crypts from human colons had increased histochemically demonstrable hexosaminidase activity; many also had increased α-naphthyl butyrate esterase activity and periodic acid-Schiff-staining material.

Discussion

The present study is the first to demonstrate the presence of putative preneoplastic lesions in the grossly normal-appearing colonic mucosa of patients with colon cancer that resemble

Table 1 *Foci of aberrant crypts (AC) in human colonic mucosa stained with methylene blue*

Source of tissue	No. of patients	Total tissue (cm^2)	Specimens with AC	Foci of AC/cm^2	% of mucosa altered
Left colon with cancer	9	134.0	9	0.33 ± 0.17[a]	0.50 ± 0.71[a]
Less than 50 yr old	1	9.0	1	0.67	2.33
50 yr old or greater	8	125.0	8	0.29 ± 0.13	0.27 ± 0.18
Transverse colon with cancer, 75 yr old	1	12.2	0		
Right colon with cancer, 50 yr old or greater	12	211.8	4	0.023 ± 0.037	0.063 ± 0.142
Total: colon with cancer	22	358.0	13	0.15 ± 0.19	0.24 ± 0.50
Gardner's syndrome with rectal cancer, 68 yr old	1	14.9	1	2.10	2.86
Crohn's disease: right colon, 89 yr old	1	20.4	1	0.15	0.54
Recurrent polyp, left colon, 53 yr old	1	19.5	0		
Left colon without cancer,[b] less than 50 yr old	2	29.2	1	0.073 ± 0.103	$2.0 \times 10^{-5} \pm 2.9 \times 10^{-5}$
Left colon from autopsy	13	253.3	1	0.004 ± 0.014	$1.3 \times 10^{-5} \pm 4.8 \times 10^{-5}$
Less than 50 yr old	4	75.9	0		
50 yr old or greater	9	177.4	1	0.005 ± 0.016	$1.9 \times 10^{-5} \pm 5.8 \times 10^{-5}$
Right colon from autopsy	13	253.0	0		
Less than 50 yr old	4	63.5	0		
50 yr old or greater	9	189.5	0		
Total: colon from autopsy	26	506.3	1	0.002 ± 0.01	$0.7 \times 10^{-5} \pm 3.4 \times 10^{-5}$

[a] Mean ± SD.
[b] One with diverticulitis and one clinically redundant without pathological abnormalities.

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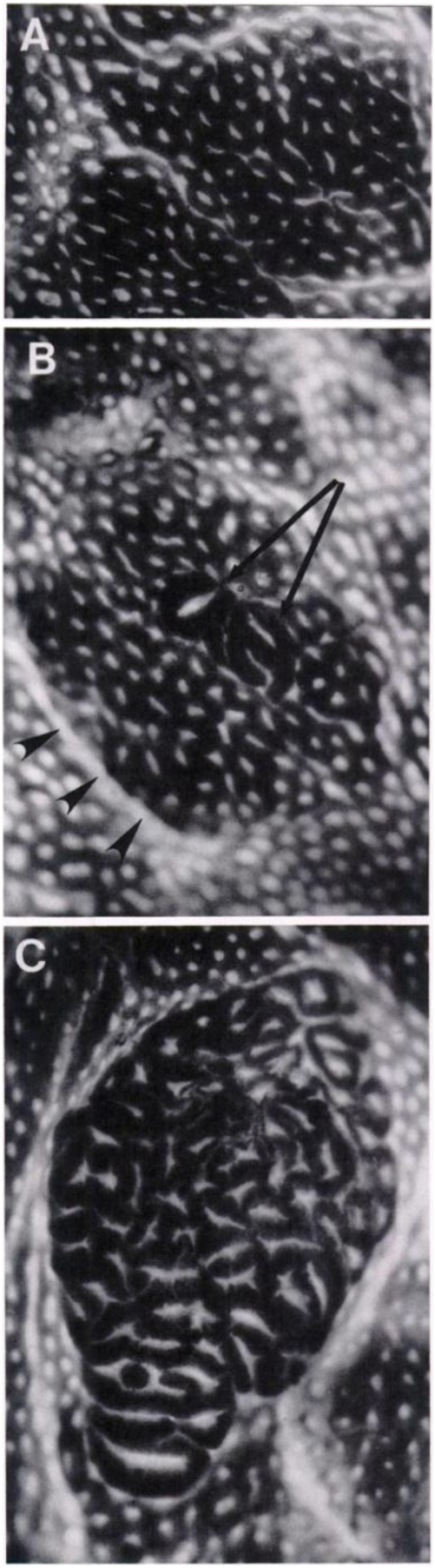

Fig. 2. Whole-mount segments of grossly normal human colonic mucosa from a patient with Gardner's syndrome. *A*, field of relatively "normal" mucosa revealing small uniform crypts. This mucosa from a single patient with Gardner's syndrome differed from samples of mucosa from all other patients in that the lumina are elliptical rather than circular. *B*, central cluster of aberrant crypts with elliptical lumina and thickened epithelium (*arrows*) surrounded by normal-sized crypts with elliptical lumina (*arrowheads*). This large focus is surrounded by more normal crypts with circular lumina. *C*, very large plaque of aberrant crypts from the same patient. × 25.

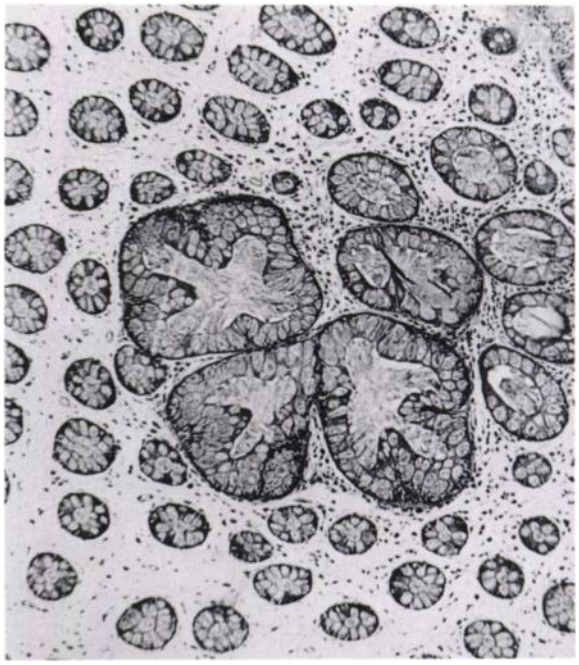

Fig. 3. Cross-section of a focus of aberrant crypts surrounded by normal crypts from a colon of a patient with sporadic colon cancer. Section is embedded in methacrylate, cut at 3 μm, and stained with hematoxylin, eosin, and azure II. × 64.

those described previously in the colons of rodents treated with carcinogens (4–7). The ability to identify multiple early potential precursors of colon cancer may facilitate the study of the pathological and molecular changes that take place in these earliest identifiable lesions as they progress to cancer in both familial polyposis and sporadic colon cancer. It is interesting that many of the same phenotypic markers that are altered in rat aberrant crypts (10) are also demonstrable in human aberrant crypts, although the direction of change is not necessarily the same. In contrast to the marked depletion of hexosaminidase activity in all aberrant crypts from carcinogen-treated rats observed to date [30 described by us earlier (4) and over 20 since then], histochemically demonstrable hexosaminidase activity was slightly elevated in some or not changed in other human aberrant crypts. Increased hexosaminidase activity in extracts of human colonic carcinomas has been demonstrated previously by our laboratory (11). The other histochemical phenotypic markers tested to date also demonstrate less marked changes in human aberrant crypts as compared with those observed in rat colons.

The increased frequency and amount of mucosa altered in Gardner's syndrome, a hereditary condition that predisposes to colon cancer, help support the hypothesis that aberrant crypts are putative precursors of colon cancer. The similarity between the mucosa from our single case of Crohn's disease and mucosa from sporadic cancer patients was unexpected. This may reflect the slightly increased incidence of cancer of the gastrointestinal tract that is observed in patients with Crohn's disease (12). Although the sample is small, it is interesting that the sporadic cancer patient with the most frequent aberrant crypts (0.667

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aberrant crypts/cm^2) and the most area of mucosa altered (2.33%) was the youngest patient (42 years old).

Histological sections of aberrant crypts resemble the minute (microscopic) adenomatous polyps that have been studied extensively in familial polyposis (13–15) but have been observed only rarely in the mucosa of other patients (16, 17). Oohara *et al.* (17) identified microscopic adenomas in 8 of 17 (47%) patients with colon cancer by examining microscopic sections (an average of 65 sections/patient) from the entire length of resected colons compared with 13 of 22 (59%) patients with aberrant crypts in this study. The observation of manyfold more aberrant crypts in the mucosa from colon cancer patients compared to the mucosa from autopsy patients without colon cancer suggests that an increased frequency of these very early lesions predisposes to colon cancer, *i.e.*, suggests the hypothesis that some of these lesions may be putative precursors of colon cancer. We should emphasize the fact that, with the available tissue, we are unable to assess rigorously the potential effect of autolysis on our quantification of foci in colonic mucosa obtained from autopsies. We need a much larger study of grossly normal mucosa from colonic resections from patients with colon cancer as well as from those without conditions that predispose to colon cancer. Since the identification of aberrant crypts with methylene blue in whole-mount segments of grossly normal human colonic mucosa provides a rapid method to screen large areas of colonic mucosa for the presence of these lesions, it should now be possible to test this hypothesis.

The finding of early pathological lesions in humans similar to those observed in rodents after a brief treatment with colon carcinogens (4–7) lends further support to the validity of these animal systems as models for human colon cancer. Others (18, 19) have discussed the similarity between the tumors observed in rodents treated with 1,2-dimethylhydrazine or its derivative, azoxymethane, and those observed in humans. The occurrence of these early lesions in the colonic mucosa of cancer patients and their rare occurrence in humans without colon cancer suggest that these lesions are not merely a curiosity observed in laboratory rodents after the administration of a high dose of carcinogen. The availability of an animal model with early putative precursors of colon cancer that can be rapidly scored may complement the work of Lipkin *et al.* (20) in the study of compounds and nutrients that might interrupt the progression of these early lesions to colon cancer. The quantification of these lesions may also have value in epidemiological studies of human colons.

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