

Apoptosis in Normal Rectal Mucosa, Baseline Adenoma Characteristics, and Risk of Future Adenomas

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Abstract

Low apoptosis in the normal rectal mucosa has been associated with colorectal adenomas in cross-sectional studies. It is unknown whether apoptosis can predict the occurrence of new adenomas. We evaluated whether apoptosis at baseline colonoscopy, as well as patient and adenoma characteristics, could predict future occurrence of adenomas. Study subjects were participants in the Diet and Health Study III, a cross-sectional study of adenoma risk factors between August 1998 and March 2000. At baseline, subjects underwent colonoscopy and provided normal rectal mucosal biopsies to evaluate apoptosis as well as information about diet and lifestyle. The present study includes 257 subjects who returned for follow-up colonoscopy between 2000 and 2005. Apoptosis, number of adenomas, size, and atypia at baseline colonoscopy were evaluated as predictors of new adenomas. Logistic

regression was used to calculate odds ratios (OR) and 95% confidence intervals (95% CI). At baseline, low apoptosis was significantly associated with increased risk of adenomas ($P = 0.0001$). Compared with those in the lowest tertile, subjects with high apoptosis were less likely to have an adenoma at follow-up (crude OR, 0.25; 95% CI, 0.09-0.65; adjusted OR, 0.29; 95% CI, 0.08-1.06). Having three or more adenomas at baseline was associated with increased risk of new adenomas (crude OR, 2.46; 95% CI, 1.14-5.31; adjusted OR, 3.74; 95% CI, 1.01-13.83). This study suggests that lower apoptosis is associated with increased risk of future adenoma development. If confirmed in larger studies, apoptosis could potentially be used to identify patients at highest risk for developing new adenomas. (Cancer Epidemiol Biomarkers Prev 2008;17(2):306-10)

Introduction

In the United States and other developed countries, colorectal cancer is a substantial cause of morbidity and mortality. It is generally agreed that the majority of colon cancers arise from benign adenomas. Whereas most adenomas remain benign, a small proportion of adenomas undergo a multistep process that eventuates in cancer (1). Patients with adenomas are at risk to develop new adenomas (2). For that reason, individuals with prior adenomas are encouraged to undergo follow-up colonoscopy at an interval determined by the size and number of adenomas at baseline (3).

The variable intervals for follow-up colonoscopy are based on studies that have attempted to identify risk factors for the development of new adenomas (2, 4-6). Factors that have been considered include size, number, degree of atypia, family history of colorectal cancer, and adenoma location.

We have shown that a low level of apoptosis in the normal rectal mucosa was a strong predictor of adeno-

mas elsewhere in the colon in a cross-sectional study, the Diet and Health Study III (DHS3; ref. 7). Apoptosis, or programmed cell death, is an important mechanism for eliminating older or genetically damaged cells (8). Neoplasms can arise when decreased apoptosis leads to the accumulation of genetically aberrant cells (9-12). Thus, apoptosis may serve a protective role in the colorectal mucosa by preventing the development of adenomas and colorectal cancer (13-15). Observation of low apoptosis in the normal rectal mucosa supports the concept of a field effect, namely, that characteristics of the mucosa provide a substrate for the development of adenomas.

Whereas low levels of apoptosis are associated with concomitant adenomas, it is not known whether apoptosis will predict the occurrence of new adenomas. In the present study, we examined the colonoscopy findings of a subset of patients who had baseline apoptosis measurements and presented for follow-up colonoscopy to determine whether the baseline level of apoptosis and other risk factors would predict future occurrence of new adenomas.

Materials and Methods

Patient Population. We evaluated a subset of participants enrolled in the DHS3, a cross-sectional study of adenoma risk factors, who returned for follow-up colonoscopy at University of North Carolina Hospitals

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between 2000 and 2005 to determine whether baseline apoptosis would predict the development of new adenomas. The features of the patients enrolled in the initial DHS3 study have been published in detail elsewhere (16). In brief, the DHS3 study recruited patients who underwent colonoscopy for various reasons at the University of North Carolina Hospitals in Chapel Hill between August 1, 1998 and March 4, 2000. Eligible patients were 30 years of age or older, proficient in the English language, and without colitis, familial polyposis, previous colonic resection, previous colon cancer, or adenoma. In preparation for colonoscopy, patients used Golytely or Fleets Phosphosoda for bowel cleansing. Only patients who had colonoscopies achieving complete visualization of the colon were included in the study. At the time of baseline colonoscopy, six rectal pinch biopsies from normal mucosa were also obtained from patients to evaluate apoptosis and proliferation. Patients also completed a detailed lifestyle and dietary questionnaire. In the initial DHS3 study (baseline), cases were defined as patients with at least one adenoma at colonoscopy and controls were patients with no adenomas. Five hundred four subjects enrolled in DHS3 provided biopsies.

Data Collection. The present study reviewed medical records at University of North Carolina Hospitals to identify previous DHS3 participants who returned for follow-up colonoscopy. We searched the University of North Carolina Hospitals computerized medical records system (WEBCIS) and identified patients from the DHS3 study who returned to University of North Carolina Hospitals for follow-up colonoscopy between January 2000 and July 1, 2005. Of the 504 patients initially enrolled in the DHS3 study, 275 returned for follow-up colonoscopy. We excluded 18 patients from the study because of unsatisfactory preparation of the colon at follow-up colonoscopy, leaving 257 men and women who returned for follow-up colonoscopy. This population included both patients who had adenomas at baseline and who returned for surveillance and patients who were free of adenomas at baseline but who returned for clinically indicated colonoscopy.

For each patient with follow-up colonoscopy, one investigator (A.A.) recorded the number of adenomas found and the characteristics of each adenoma including size, location, histologic type, and degree of atypia. An experienced gastroenterologist (R.S.S.) reviewed a 10% sample of records to assess reliability. The reliability was 100%.

Measurement of Apoptosis. Baseline apoptosis data were available on 191 subjects. The methods for evaluating apoptosis have previously been described for the DHS3 study (7). Briefly, each biopsy specimen was sectioned into five sections spaced 50 μ m apart to avoid double counting of crypts. Apoptosis was assessed on H&E-stained sections of biopsies of normal rectal mucosa obtained at baseline colonoscopy using standard morphologic criteria. Cells were considered apoptotic by the presence of cell shrinkage, chromatin condensation, and nuclear fragmentation. Apoptosis was confirmed on a subset of specimens in DHS3 by the terminal deoxyribonucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) method (17). For TUNEL, cells were considered apoptotic if they had nuclear brown staining of condensed chromatin or pyknotic dots of

condensed chromatin. Cells were not scored as apoptotic if the nucleus did not meet these criteria. There was a positive correlation between the two apoptosis methods (Spearman correlation coefficient = 0.73, $P = 0.01$). Using well-defined criteria for identifying scorable crypts and apoptotic cells, an experienced technician who was blinded to adenoma status scored all sections. A sample of slides was resubmitted for scoring in a blinded fashion to the same scorer as well as an independent scorer to evaluate reproducibility. The level of agreement for intrarater scoring was 99% whereas inter-reader scoring reproducibility was 94%. The number of apoptotic cells for each crypt was combined to calculate a mean apoptosis score per crypt. A total of 8 to 12 longitudinal crypts were scored per biopsy on H&E- and TUNEL-stained slides.

Data Analysis. Summary statistics were generated both overall and by case status in the form of means and SEs for continuous variables or frequencies and percentages for categorical variables. Cases and controls were compared via *t* tests for continuous variables and Fisher's exact test for categorical variables. Means and SEs of apoptosis were generated by categories for case (adenoma versus no adenoma), age (under 45, 50-59, >60 years), race (self-reported race, White, Black), sex (male/female), body mass index (BMI; normal, 18-24.9; overweight, 25-29.9; obese, >30), nonsteroidal anti-inflammatory drug (NSAID) usage (<15 or >15 times/mo), smoking (never, former, current), and family history of colorectal cancer (no/yes), and those categories were compared by *t* tests and ANOVA. Tertile cutoff points for apoptosis were also generated using the original DHS3 apoptosis data in controls. Logistic regression models were used to examine the association between occurrence of new adenomas and baseline demographic/lifestyle characteristics. Logistic regression was conducted among those with adenomas at baseline only to examine the relationship between characteristic of baseline adenoma and whether or not a subject had new adenomas at follow-up. The location of adenoma was classified as proximal (cecum, ascending colon, hepatic flexure, and transverse colon) or distal (splenic flexure, descending colon, sigmoid, rectosigmoid, and rectum). Both unadjusted and adjusted models were run. For adjusted models, all covariables of interest were entered into the model.

Results

The baseline characteristics at of DHS3 participants who returned for follow-up are presented in Table 1. The number of subjects with baseline adenomas (case) who returned for follow-up ($n = 129$) was similar to those without baseline adenomas (control) who returned for follow-up ($n = 128$). Of the patients who had an adenoma at baseline colonoscopy, 41% had at least one new adenoma at follow-up. In contrast, only 15% of individuals who were free of adenoma at baseline had at least one new adenoma at follow-up. Among subjects who returned for follow-up colonoscopy (48.4%), the mean age was 58.4 years, 48% were males, and 28% had a family history of colorectal cancer. Among those that did not return for follow-up, the mean age was

Table 1. Baseline characteristics of DHS3 study participants who returned for follow-up

Characteristic	Overall (n = 257)*
Baseline case/control status	
Adenoma, n (%)	129 (50)
No adenoma, n (%)	128 (50)
Mean age (SE), y	58.4 (0.7)
Race	
White, n (%)	198 (80)
Black, n (%)	51 (20)
Sex	
Male, n (%)	123 (48)
Female, n (%)	134 (52)
BMI (kg/m ²)	
Normal (18-24.9), n (%)	92 (36)
Overweight (25-29.9), n (%)	82 (32)
Obese (>30), n (%)	80 (32)
Mean NSAID use/mo (SE)	8.1 (0.8)
Smoking status, n (%)	
Never	107 (43)
Former	102 (41)
Current	38 (15)
Family history of colorectal cancer, n (%)	69 (28)
Yes	69 (28)
No	178 (72)
Mean daily calories (SE), kcal	1,525 (36)
Mean apoptosis (SE)	2.70 (0.05)

*All subjects who returned for follow-up colonoscopy.

54.6 years, 38% were males, and 22% reported a family history of colorectal cancer. There were no significant differences in the baseline demographic characteristics of subjects who did or did not return for follow-up (data not shown).

We examined whether the time interval from initial colonoscopy was related to adenoma status at follow-up. Overall, those that had an adenoma at baseline had a shorter time to follow-up. Among individuals that had adenomas at baseline, the time to follow-up was longer for participants that had new adenomas at follow-up compared with those that did not (1,389 versus 1,253 days), but the results were not statistically significant. Among controls without adenomas at baseline, there was no statistically significant difference in the time to follow-up for those that had new adenoma at follow-up compared with those that did not (1,611-1,516 days).

Table 2 presents mean (SE) apoptosis scores by patient characteristics at baseline. The mean apoptosis score was significantly lower in subjects with adenoma compared with those with no adenoma at baseline. The mean apoptosis score was lower in males than in females but there was no significant difference for any of the other patient characteristics.

We examined whether apoptosis and other risk factors such as baseline adenoma status, age, and NSAID usage predicted the risk of future adenomas among subjects who returned for follow-up colonoscopy (Table 3). Compared with subjects without adenomas at baseline, those with adenomas at baseline were at significantly increased risk of having new adenomas at follow-up [odds ratio (OR), 2.95; 95% confidence interval (95% CI), 1.19-7.33]. NSAID use significantly predicted reduced risk of new adenoma occurrence. The results for apoptosis were borderline significant (crude OR, 0.25; 95% CI, 0.09-0.65; adjusted OR, 0.29; 95% CI, 0.08-1.06) after adjustment for age, race, sex, BMI, baseline case

control status, and other variables listed in the footnote for Table 1.

Among individuals with adenomas at baseline colonoscopy, certain characteristics of baseline adenomas were associated with development of future adenomas (Table 4). The number of adenomas at baseline positively predicted the occurrence of new adenomas (adjusted OR, 1.67; 95% CI, 1.11, 2.52). Compared with those with one or two adenomas, having three or more adenomas at baseline was associated with the likelihood of developing new adenomas at follow-up (adjusted OR, 3.74; 95% CI, 1.01-13.83). There was no statistically significant association between the occurrence of new adenomas and presence of advanced adenoma at baseline or baseline adenoma location. Forty-eight percent (48%) of patients with proximal adenomas (cecum, ascending colon, hepatic flexure, transverse colon) at baseline had occurrence of new lesions compared with 34% of those with distal baseline adenomas (splenic flexure, descending colon, sigmoid rectosigmoid, and rectum), but this difference was not statistically significant.

Discussion

The present study evaluated level of apoptosis as a predictor of occurrence of new adenomas among subjects who underwent an initial baseline colonoscopy and returned for follow-up. We also explored baseline patient and adenoma characteristics in relation to new adenoma development. We observed an inverse association between apoptosis in the normal rectal mucosa and occurrence of new adenomas. Subjects in the highest

Table 2. Apoptosis and baseline patient characteristics

Variable	Mean apoptosis (SE)	P*
Case status		
Adenoma	2.43 (0.06)	0.0001
No adenoma	3.00 (0.06)	
Age (y)		
<45	2.88 (0.10)	0.11
50-59	2.69 (0.08)	
≥60	2.62 (0.07)	
Race		
White	2.72 (0.06)	0.71
Black	2.68 (0.10)	
Sex		
Male	2.59 (0.07)	0.03
Female	2.79 (0.06)	
BMI (kg/m ²)		
Normal (18-24.9)	2.78 (0.08)	0.10
Overweight (25-29.9)	2.55 (0.08)	
Obese (>30)	2.74 (0.08)	
NSAIDs used >15 times/mo		
No	2.74 (0.06)	0.18
Yes	2.58 (0.10)	
Smoking status		
Never	2.71 (0.07)	0.60
Former	2.66 (0.08)	
Current	2.81 (0.12)	
Family history of colorectal cancer		
No	2.68 (0.06)	0.38
Yes	2.78 (0.10)	

*P values were generated via ANOVA.

†Family history of colorectal cancer includes first-degree relatives.

Table 3. Baseline characteristics and occurrence of new adenomas

Baseline measure	Crude OR (95% CI)	Adjusted OR* (95% CI)
Case status		
No adenoma	1.0 (reference)	1.0 (reference)
Adenoma	4.00 (2.19-7.29)	2.95 (1.19-7.33)
Age (5-y increment)	1.10 (0.96-1.25)	1.08 (0.87-1.34)
Race		
White	1.0 (reference)	1.0 (reference)
Black	0.74 (0.36-1.52)	0.39 (0.14-1.03)
Sex		
Female	1.0 (reference)	1.0 (reference)
Male	1.54 (0.89-2.66)	1.98 (0.82-4.74)
BMI (kg/m ²)		
Normal (18-24.9)	1.0 (reference)	1.0 (reference)
Overweight (25-29.9)	1.97 (1.01-3.85)	1.48 (0.56-3.94)
Obese (>30)	1.45 (0.73-2.90)	1.74 (0.62-4.86)
Used NSAIDs >15 times/mo		
No	1.0 (reference)	1.0 (reference)
Yes	0.58 (0.28-1.21)	0.28 (0.10-0.80)
Smoking		
Never	1.0 (reference)	1.0 (reference)
Former	1.12 (0.62-2.04)	1.30 (0.54-3.13)
Current	0.69 (0.28-1.66)	0.69 (0.19-2.59)
Family history of colorectal cancer, <i>n</i> (%)		
No	1.0 (reference)	1.0 (reference)
Yes	0.97 (0.52-1.81)	0.70 (0.26-1.90)
Daily calories (increment of 100)	0.98 (0.93-1.03)	0.99 (0.91-1.06)
Apoptosis [†]		
Tertile 1 (1.29-2.46)	1.0 (reference)	1.0 (reference)
Tertile 2 (2.48-3.00)	0.99 (0.48-2.01)	1.75 (0.70-4.41)
Tertile 3 (3.04-6.90)	0.25 (0.09-0.65)	0.29 (0.08-1.06)

*OR adjusted for age, race, sex, BMI, NSAIDs, smoking, family history of colorectal cancer, calories, apoptosis, bowel prep, and baseline case/control status.

[†]Apoptosis data were available for 102 cases and 89 controls (the number of patients in apoptosis tertiles was for tertile 1, case/control = 61/18; tertile 2, case/control = 29/30; tertile 3, case/control = 27/39; 27 cases were missing and 39 controls were missing).

tertile of apoptosis were less likely to have adenoma recurrence. Previously, we have shown in this population that low apoptosis predicted increased risk of adenomas in a cross-sectional study (7, 16). Thus, the findings from the current prospective analysis support our previous observations and show that apoptosis may be a valuable risk biomarker.

Studies in animal models and humans also implicate deregulation of apoptosis in the neoplastic process (18-21). Low levels of apoptosis in the colonic epithelium may provide the fertile ground that allows for the unchecked growth of aberrant cells in the colon (7, 15). Studies have shown that individuals who lack an adenoma at baseline are unlikely to develop another adenoma soon afterwards, and the recommended follow-up interval for colonoscopy is prolonged for these patients (3). However, there is still a risk for future development of new adenomas even among those that are free of adenoma at baseline. The present study suggests that apoptosis in the normal rectal mucosa may identify those patients who may be at higher risk and who would benefit from earlier follow-up colonoscopy. Unfortunately, the techniques for apoptosis measurement are currently too tedious to be of clinical utility. If the measurement could be automated, analysis of apoptosis in the rectal biopsies could serve as a risk biomarker to determine the interval

for the next colonoscopy. Larger longitudinal studies are needed to confirm these findings. In addition, apoptosis in the normal rectal mucosa could serve as one of a panel of potential biomarkers to evaluate effectiveness of a chemopreventive agent. If the agent had a beneficial effect for apoptosis, it might be evaluated using other end points. Reports from chemopreventive studies of colorectal cancer support the potential utility of apoptosis as a biomarker. One study assessed the efficacy of celecoxib in familial adenomatous polyposis patients and observed that increased apoptosis was significantly associated with polyp regression and high apoptosis significantly correlated with better response to celecoxib (22). Others have also reported similar findings (14, 23-25). Because we have shown that levels of apoptosis at baseline predict future adenomas, an intervention that raised apoptosis at baseline could be a good candidate agent for adenoma prevention.

The present study showed that baseline NSAID use was strongly associated with reduced risk of developing new adenomas. This observation is consistent with reports from other studies (26, 27). Aspirin and NSAIDs have been associated with reduced risk of colorectal adenomas (7, 28, 29). A proposed mechanism for the chemoprotective effect of aspirin and NSAIDs is through inhibition of prostaglandin synthesis. Although induction of apoptosis has also been proposed as a mechanism for the protective effect of NSAIDs on colorectal adenomas and cancer (30, 31), we found that the association between adenomas and NSAIDs in average-risk individuals was independent of apoptosis (7).

Certain risk factors, such as presence of adenomas at baseline, and characteristics of adenomas, such as size and number of adenomas, may predict the development of new adenomas (2, 4-6, 32). We found that the number of adenomas at baseline colonoscopy was associated with elevated risk of new adenoma occurrence. Our results are

Table 4. Baseline adenoma characteristics and occurrence of new adenomas

	Crude OR (95% CI)	Adjusted OR* (95% CI)
Indication for earlier endoscopy [†]		
No	1.0 (reference)	1.0 (reference)
Yes	2.39 (1.17-4.92)	2.03 (0.69-6.00)
Advanced adenoma at baseline		
No	1.0 (reference)	1.0 (reference)
Yes	1.61 (0.77-3.38)	1.09 (0.32-3.70)
No. adenomas at baseline		
1-2	1.0 (reference)	1.0 (reference)
≥3	2.46 (1.14-5.31)	3.74 (1.01-13.83)
Location [‡]		
Distal	1.0 (reference)	1.0 (reference)
Proximal	1.78 (0.72-4.41)	3.95 (0.85-18.33)
>1	2.79 (1.06-7.31)	1.54 (0.37-6.39)

*OR adjusted for age, race, sex, BMI, NSAIDs, smoking, family history of colorectal cancer, calories, apoptosis, bowel prep, and baseline adenoma status.

[†]Indication for earlier endoscopy was defined as having adenoma at least 1 cm in diameter or villous histology or severe atypia or (43 patients), 3 or more adenomas (21 patients). Advanced adenoma was defined as having an adenoma at least 1 cm in diameter, histology of villoglandular or villous or severe atypia.

[‡]Adenoma distal location included splenic flexure, descending colon, sigmoid, rectosigmoid, and rectum. Proximal location included cecum, ascending colon, hepatic flexure, and transverse colon.

compatible with prior reports in the literature (2, 5). However, adenoma size and location were not significant predictors of new adenoma occurrence, possibly due to small sample size. Previous studies have shown inconsistent results for the association between these two variables and occurrence of new adenomas (2-5).

Our study makes a unique contribution to the literature by suggesting apoptosis as a predictor of occurrence of new adenomas. Additional advantages of this study are inclusion only of patients with adequate prep and complete visualization of the colon to the cecum at baseline and follow-up colonoscopy. We minimized inter- and intra-observer bias by having the same pathologist to evaluate all pathologic lesions at baseline and follow-up using predefined criteria. We also had one technician to evaluate apoptosis in the study. Limitations of this study include small sample size and incomplete follow-up. Because not all subjects in the initial study returned for follow-up, our study may be biased. However, the baseline demographic characteristics of those who returned for follow-up were similar to those who did not return for follow-up. Likewise, the proportions of subjects with and without adenomas at baseline who returned for follow-up were comparable.

In summary, our study provides new evidence that lower levels of apoptosis at baseline are associated with the risk of developing new adenomas. Apoptosis could potentially be used to identify patients at highest risk for development of future adenomas especially among those who are free of adenomas during an initial colonoscopy.

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