Expansion of the Epithelial Cell Proliferative Compartment and Frequency of Adenomatous Polyps in the Colon Correlate with the Strength of Family History of Colorectal Cancer

Hans Gerdes, J. Scott Gillin, Eliot Zimbalist, Carlos Urmacher, Martin Lipkin, and Sidney J. Winawer

ABSTRACT

Expansion of the proliferative compartment of epithelial cells in colonic crypts and colonic adenomas have been described as phenotypic precursors to colon cancer in individuals affected with hereditary or sporadic colon cancer. This study measured the size of the proliferative compartment in colonic crypts and the frequency of adenomas in asymptomatic members of families having sporadic colorectal cancer. The subjects were divided into 2 groups according to the frequency of colorectal cancer in their families. A shift of the compartment of proliferating epithelial cells toward the luminal surface of colonic crypts was seen in the group with a stronger family history of colorectal cancer, with significant differences in the numbers of proliferative cells in the upper and the lower crypt compartments (P < 0.05) and in the fraction of proliferative cells at the highest compartment at the luminal surface of the crypts (P < 0.05). Cell proliferation patterns in normal-appearing mucosa of the 2 groups revealed no difference in whole crypt [3H]thymidine labeling index. Colonoscopic examination of the 56 subjects revealed an overall prevalence of adenomas of 21%; when stratified by frequency of colorectal cancer in their families, 3 of 22 subjects (14%) with a weaker family history had adenomas, while 9 of 34 (26%) with a stronger family history had adenomas. Thus, parallel abnormalities of colonic epithelial cell proliferation and neoplasia were seen in individuals with a family history of colorectal cancer, both of which were more pronounced with increasing strength of family history. This observation provides further evidence of relationships among these factors in the etiology of "sporadic" colorectal cancer.

INTRODUCTION

The appearance of colorectal cancer in patients with FAP and HNPCC follows a classical autosomal dominant Mendelian pattern of inheritance. In both syndromes, the adenoma is believed to be the precursor lesion, with hundreds to thousands of colorectal adenomas occurring in FAP, and few in HNPCC. The gene for FAP has now been localized to the long arm of chromosome 5, and studies are in progress to identify the function of this gene as well as mechanisms leading to cancer (1–4).

Recent studies of sporadic colorectal cancer in the general population have also suggested that inheritance has a role in its development (5, 6). Familial clustering with approximately a 3-fold increase in colorectal cancer incidence has been observed in families having first degree relatives affected by colon cancer (7–9).

Individuals at risk for colorectal cancer by virtue of their family history have had abnormal proliferation of colonic epithelial cells (10–15), resulting in an expanded size of the normal colonic crypt zone of cell proliferation, a phenotypic abnormality preceding the development of adenomas and carcinomas. However, no studies have been carried out on relatives of individuals with "sporadic" colon cancer to specifically examine relationships between cell proliferation, the occurrence of adenomas, and the strength of the family history. A study of this type could clarify whether relatives in "sporadic" colon cancer families possess proliferative patterns similar to members of FAP and HNPCC families, whether the phenotype is inherited, and whether it parallels the presence of adenoma formation and colon cancer.

The present study investigates the expression of both colonic epithelial cell proliferation and adenomatous polyps in relatives of individuals with "sporadic" colorectal cancer. Both of these findings are potential biomarkers of an underlying tumor suppressor gene, which, like the genes responsible for FAP and HNPCC, could lead to an individual's increasing susceptibility for the development of colorectal cancer.

MATERIALS AND METHODS

Subjects with one or more relatives affected with colorectal cancer were studied. Family histories were obtained from probands to determine the presence of colorectal cancer in first, second, and third degree relatives. Verification was obtained from hospital records, death certificates, and physician reports. All subjects included had no personal history or symptoms of colorectal neoplasia or other cancer and no history of inflammatory bowel disease, and were in good general health.

All subjects had a total colonoscopy performed following a 2-day liquid diet, citrate of magnesia, and tap water enemas. All identified polyps were removed and processed for histological examination. Slides of removed polyps were reviewed by one pathologist (C. U.) without knowledge of the subject's family history, and polyps were classified as either hyperplastic, tubular, tubulovillous, villous, or other (normal mucosa, inflammation, etc.).

At the time of colonoscopy, biopsies from normal-appearing colonic mucosa were also obtained from the rectum for the study of mucosal proliferation. These biopsies were incubated with [3H]dThd according to methods described previously and were processed for microautoradiography (10). Mucosal specimens sectioned longitudinally from the base of colonic crypt to the lumen were analyzed by light microscopy. The total number of cells per crypt column was counted with each side of the crypt column being counted separately, and number and position of [3H]dThd-labeled cells were counted and scored. Measurements were performed without knowledge of the individual subject's family history.

The height-distribution patterns for labeled cells were determined as described previously (10, 16), and the location of [3H]dThd-labeled and unlabeled epithelial cells in colonic crypts of individuals in each of the 2 groups of subjects studied was analyzed by computer program (16). For the entire crypt column, and for each crypt compartment [crypt divided equally into 5 compartments: (1) base to (5) surface], the following were measured: (a) the total number of epithelial cells; (b) the number of [3H]dThd-labeled epithelial cells; and (c) the fraction of [3H]dThd-labeled epithelial cells (labeling indices). Statistical comparisons were made using the 2-tailed Student's t test for comparison of means and the χ² statistic for comparison of outcomes.

Parents, siblings, and children of a given individual were scored as first degree relatives by convention. Grandparents, aunts, and uncles were scored as second degree relatives.
second degree relatives, and cousins and great grandparents were scored as third degree relatives. In order to quantify the number of affected relatives, FICC was calculated for each individual. This index was determined by summing the value of each individual’s affected relatives. First degree relatives were assigned a value of 1; second degree relatives, 0.5; third degree relatives, 0.25; and fourth degree relatives, 0.125. Data from subjects were divided into 2 categories based on the number of relatives previously affected with colorectal cancer. Group I consisted of subjects with no more than one first degree relative and one second degree relative affected with colorectal cancer (FICC ≤1.5). Those with at least 2 first degree relatives affected with colorectal cancer or one affected first degree relative and several affected second degree relatives (FICC > 1.5) comprised Group II.

RESULTS

Family History

Group I. Twenty-two (39%) of the subjects had a FICC of ≤1.5. The mean age for this group was 44 ± 12 (SD) years with a range of 21–64 years. Half of the subjects in this group had only one affected relative, while the others had one first and one second degree relative affected with colorectal cancer, and one subject had 3 affected relatives consisting of one first degree relative, one third degree relative, and one fourth degree relative for a FICC of 1.325.

Group II. Thirty-four subjects (61%) had stronger family histories with FICC >1.5. The mean age for Group II was 48 ± 16 years with a range of 18 to 77 years of age. The difference in age between the groups was not statistically significant.

Frequency of Adenomas

Among the 56 subjects studied, 16 adenomatous polyps were removed from 12 subjects with an overall prevalence of 21%. Two subjects had 2 adenomas, 1 subject had 3, and 2 subjects had hyperplastic polyps. All the adenomas were tubular adenomas, with the exception of one 1.5-cm tubulovillous adenoma with high grade dysplasia removed from a patient in Group II. Three of 22 subjects in Group I had adenomas with a prevalence of 14% compared to 9 of 34 subjects in Group II, with 26% (Table 1). This difference was not statistically significant (P = 0.23). The mean age of those in Group I harboring colorectal adenomas was 54, compared to a mean age of 55 for those in Group II.

Colonic Mucosal Cell Proliferation

Twelve subjects from Group I and 14 from Group II had measurements of colonic epithelial cell proliferation measured using [3H]dThd labeling. The mean age of the subjects in each group was similar (Group I, 44; Group II, 42). All subjects on whom colonic epithelial cell proliferation was measured had colons that were free of adenomas. Table 2 shows the following findings: There was no statistically significant difference in the total number of colonic crypt columns assayed, epithelial cells counted, or the number of labeled cells found within the 2 groups. The total number of epithelial cells and numbers of [3H]dThd-labeled epithelial cells per crypt column were the same as were overall labeling indices. It was the distribution of proliferative cells within regions of the colonic crypts that differed between the 2 groups. Thus, there was a statistically significant difference between the groups in the number of labeled cells found in crypt compartments. Those in Group I had more labeled cells present in the lower crypt compartments (1 and 2) for those in Group I, but higher at the surface (compartments 4 and 5) for those in Group II. This difference in labeled cell distribution was statistically significant, while whole crypt labeling index was not different, as shown in Table 2. This difference in labeled cell distribution is consistent with an expansion of the zone of cell proliferation in the colon from those subjects in Group II.


dt

Table 1 Clinical characteristics of study subjects as stratified into 2 groups based on family history

<table>
<thead>
<tr>
<th>Parameter measured</th>
<th>Group I</th>
<th>Group II</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>22</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Mean age</td>
<td>44</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>F/M</td>
<td>11:11</td>
<td>21:13</td>
<td></td>
</tr>
<tr>
<td>Mean FICC</td>
<td>1.1</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>No. with adenomas</td>
<td>3 (14%)</td>
<td>9 (26%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Colonic epithelial cell proliferation in subjects with family history of colon cancer

<table>
<thead>
<tr>
<th>Parameter measured</th>
<th>Group I</th>
<th>Group II</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. subjects studied</td>
<td>12</td>
<td>14</td>
<td>NS *</td>
</tr>
<tr>
<td>Mean age of subjects assayed</td>
<td>44</td>
<td>42</td>
<td>NS</td>
</tr>
<tr>
<td>Columns assayed</td>
<td>616</td>
<td>586</td>
<td></td>
</tr>
<tr>
<td>Cells counted</td>
<td>27,960</td>
<td>25,993</td>
<td></td>
</tr>
<tr>
<td>Labeled cells counted</td>
<td>4,809</td>
<td>4,555</td>
<td></td>
</tr>
<tr>
<td>No. of epithelial cells/crypt column</td>
<td>45.2 ± 1.60</td>
<td>43.94 ± 2.46</td>
<td>NS</td>
</tr>
<tr>
<td>No. of labeled epithelial cells/column</td>
<td>7.69 ± 0.86</td>
<td>7.72 ± 0.72</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 3 Measurements of individual crypt compartment epithelial cell proliferation

<table>
<thead>
<tr>
<th>Parameter measured</th>
<th>Group I</th>
<th>Group II</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compartment 1 (base)</td>
<td>1211/5324 (0.227)</td>
<td>902/4939 (0.182)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Compartment 2</td>
<td>1638/5615 (0.291)</td>
<td>1435/5215 (0.275)</td>
<td>0.02</td>
</tr>
<tr>
<td>Compartment 3</td>
<td>1197/5581 (0.214)</td>
<td>1170/5212 (0.224)</td>
<td>0.209</td>
</tr>
<tr>
<td>Compartment 4</td>
<td>595/5615 (0.105)</td>
<td>717/5215 (0.137)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Compartment 5 (lumen)</td>
<td>164/5825 (0.028)</td>
<td>331/5412 (0.061)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

DISCUSSION

Our understanding of the adenoma-adenocarcinoma sequence during the development of cancer of the colon from the normal mucosa has been significantly clarified or enhanced by recent molecular genetic research. This has led to a proposed model of colon cancer tumorigenesis as a multistep process beginning with normal mucosa, progressing through a stage of abnormal cell proliferation, and then to successive stages of adenoma formation with progressively advanced...
histology leading to cancer (17). Genetic alterations that occur in this model have been reported (18).

Abnormal colonic cell proliferation and adenoma formation have previously been reported in well-delineated inherited syndromes of FAP and HNPCC, as well as in "sporadic" colorectal cancer (10–16). A recent report has further identified an expansion of the compartment of proliferating cells as a predictive indicator for the evolution of future adenomas (20). In some studies, the frequency of adenoma formation has been shown to correlate with the strength of the family history, e.g., with 1 versus 2 first degree relatives having colorectal cancer (21–25).

The present study demonstrated parallel abnormalities of 2 stages of the colonic tumorigenesis model that appear to correlate with increasing strength of family history among first degree relatives of patients with sporadic colorectal cancer. There was both an increased frequency of abnormal cell proliferation (P < 0.001) and a trend to increased frequency of adenoma formation in individuals having a stronger family history of colorectal cancer (Group II), compared with those having a weaker family history of colorectal cancer (Group I). The data thus support a relationship between the biomarker of expanded size of the proliferative compartment and adenoma formation within the colonic mucosa, in this model of tumorigenesis. The parallel occurrence of the 2 phenotypic abnormalities correlating with increasing strength of family history suggests further evidence of an inherited predisposition in the "sporadic" colorectal cancer group.

Epidemiological studies have shown that there are aggregations of colorectal cancer in affected families that are, on the average, 3 times the expected rate, thereby suggesting a role of inherited factors in the disease (7–9). Endoscopic studies also have demonstrated a greater prevalence of colorectal adenomas in individuals with one or more first degree relatives affected with colorectal cancer (21, 22, 24). Thus, these studies suggest that the extent of family history has a role in risk for colorectal adenoma and cancer development. Similarly, studies of large pedigrees having an aggregation of close relatives affected with colorectal cancer demonstrated not only a high prevalence of colorectal adenomas and cancer in family members, but also phenotypic segregation in a pattern best fitting a model for autosomal dominant inheritance of a gene putatively associated with transmission of the disease (5).

The findings in the present study strengthen the possibility that colorectal cancer development is partly controlled by an inherited susceptibility gene, the expression of which follows the model noted above, with increased colonic epithelial cell proliferation and adenoma formation as intermediate steps. The risk of developing colorectal adenomas and cancer may follow a continuum from average risk with no family history of colorectal cancer, to increased risk depending on the strength of the family history. This increased risk appears greatest among affected first degree relatives, but affected second and third degree relatives also could reflect the expression of the segregation of a susceptibility gene in a given family. A FICC that includes first degree, second degree, and third degree relatives could have utility in quantitating the colon cancer risk of individuals based on family history. It would be of interest to test the possible value of the FICC within the framework of screening trials. Further studies between the relationship of inherited and acquired genetic alterations and changes in colonic epithelial proliferation may help clarify the mechanisms of carcinogenesis and the clinical expression of these genetic alterations.

REFERENCES


Expansion of the Epithelial Cell Proliferative Compartment and Frequency of Adenomatous Polyps in the Colon Correlate with the Strength of Family History of Colorectal Cancer


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/53/2/279

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/53/2/279.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.