Altering of Gene Expression in Macroscopically Normal Colonic Mucosa from Individuals with a Family History of Sporadic Colon Cancer

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ABSTRACT

Purpose: We have shown that the expression of several genes associated with human colon cancer is altered in the morphologically normal colonic mucosa (MNCM) of APC<sub>min</sub> mice and humans with colon cancers. To determine whether these alterations also occur in the MNCM of individuals who have not developed colon cancer but are at high risk of doing so, we measured gene expression in the MNCM of individuals with a family history of colon cancer.

Methods: Expression of 16 genes in the MNCM of 12 individuals with a first-degree relative with sporadic colon cancer and 16 normal controls were measured by quantitative reverse transcription-PCR. All subjects tested had normal colonoscopic examinations. Biopsy samples of MNCM were obtained from the ascending, transverse, descending, and rectosigmoid regions of the colon (2-8 biopsy samples were obtained from each region).

Results: Relative to normal controls, the expression of several genes, including PPAR-γ, SAA1, and IL-8 were significantly altered in the macroscopically normal rectosigmoid mucosa from individuals with a family history of colon cancer.

Conclusions: Molecular abnormalities that precede the appearance of adenomatous polyp are present in the MNCM of individuals who have a family history of colon cancer. This observation raises the possibility of screening for individuals who have a family history of colon cancer. This postulate that these changes occur early in the process of colon carcinogenesis (i.e., before the development of APs or cancer), and therefore may represent the much larger set of genes that are altered in colon cancer. Expression of 13 of these 15 genes was also altered significantly in morphologically normal colonic mucosa (MNCM) before the appearance of any APs. In a previous study, we analyzed the expression of 15 genes in the APs and MNCM from APC<sub>min</sub> mice, as well as in MNCM from human colon cancer specimens (10). These 15 genes function in several pathways related to cancer development, including the APC/β-catenin pathway, the NFκB pathway, cell cycle, and inflammation and therefore may represent the much larger set of genes that are altered in colon cancer. Expression of 13 of these 15 genes was altered in the APs from APC<sub>min</sub> mice. The five genes (CXCR2, Gro-Gro-α, NIP-2, COX-2, and OPN) that were most highly up-regulated in mouse APs were also significantly up-regulated in polyp-free colon mucosa. Similarly, we found that expression of 9 of the 15 genes was also altered significantly in morphologically normal mucosa of surgical sections taken from human cancer patients, relative to colonic mucosa from individuals without cancer (10). Of the nine genes that showed altered expressions in the MNCM from patients with rectosigmoid cancers, expressions of PPAR-γ, PPAR-δ, and p21<sup>ΔN</sup> were down-regulated and expressions of IL-8, OPN, COX-2, CXCR2, MCSF-1, and CD44 were up-regulated (10).

These findings indicate that MNCM from APC<sub>min</sub> mice and human with colon cancer is not metabolically normal. We postulate that these changes occur early in the process of colon carcinogenesis (i.e., before the development of APs or cancer), and that these alterations in MNCM may indicate an increased risk of developing colon cancer.

INTRODUCTION

The adenomatous poly (AP) is a precursor lesion of colon cancer. Early detection and removal of AP has been shown to substantially reduce mortality from colon cancer (1). Currently, colonoscopy is routinely recommended for individuals ages >50 years (1). However, the inconvenience of the precolonoscopy preparation and the high cost of colonoscopy discourage compliance in the general population (2). A less taxing and less expensive method that allows early detection of APs and occult colon cancer, using biomarkers would be useful.

Early detection of AP and colon cancer may be facilitated by identifying the molecular alterations associated with early tumorigenesis. Although the expression of many genes is altered in colon cancers (3–8), it is not clear whether these alterations involve initiation and progression of the cancer, or are a consequence of genomic destabilization. This distinction must be made on precancerous colon mucosa.

The APC<sub>min</sub> mouse, which carries a mutation in the adenomatous polyposis coli (APC) gene, is the best-characterized mouse colon neoplasia model. It is analogous to the human familial adenomatous polyposis syndrome (9). Because APC<sub>min</sub> mice always develop intestinal APs within the first 6 months of life, it is reasonable to assume that molecular changes occur in their morphologically normal colonic mucosa (MNCM) before the appearance of any APs. In a previous study, we analyzed the expression of 15 genes in the APs and MNCM from APC<sub>min</sub> mice, as well as in MNCM from human colon cancer resection specimens (10). These 15 genes function in several pathways related to cancer development, including the APC/β-catenin pathway, the NFκB pathway, cell cycle, and inflammation and therefore may represent the much larger set of genes that are altered in colon cancer. Expression of 13 of these 15 genes was altered in the APs from APC<sub>min</sub> mice. The five genes (CXCR2, Gro-Gro-α, NIP-2, COX-2, and OPN) that were most highly up-regulated in mouse APs were also significantly up-regulated in polyp-free colon mucosa. Similarly, we found that expression of 9 of the 15 genes was also altered significantly in morphologically normal mucosa of surgical sections taken from human cancer patients, relative to colonic mucosa from individuals without cancer (10). Of the nine genes that showed altered expressions in the MNCM from patients with rectosigmoid cancers, expressions of PPAR-γ, PPAR-δ, and p21<sup>ΔN</sup> were down-regulated and expressions of IL-8, OPN, COX-2, CXCR2, MCSF-1, and CD44 were up-regulated (10).

These findings indicate that MNCM from APC<sub>min</sub> mice and human with colon cancer is not metabolically normal. We postulate that these changes occur early in the process of colon carcinogenesis (i.e., before the development of APs or cancer), and that these alterations in MNCM may indicate an increased risk of developing colon cancer.
Individuals with a family history of colon cancer are known to have an increased risk of developing colon cancer (11). In this study, we test our hypothesis by analyzing expression levels for 16 genes in MNCM from individuals who had normal colonoscopic examinations and who did not have a prior history of colon cancer. The study group was divided into those with and those without a family history of colon cancer. We found that expression of several genes that were altered in MNCM from colon cancer patients were also altered in the rectosigmoid mucosa from individuals with a family history of colon cancer. Our results suggest that molecular abnormalities are present in the MNCM of the individuals who are at increased risk of developing colon cancer. Because these molecular alterations are present in the absence of APs, they may represent changes contributing to the very early stages of colon carcinogenesis.

MATERIALS AND METHODS

Human Subjects. Biopsies of MNCM from the rectum and sigmoid colon were done at the time of routine colonoscopy from individuals seen at the California Pacific Medical Center who had no history of prior colon cancer and who were free of APs, colon cancer, or other colonic lesions at the time of examination. Twelve individuals with a family history of colon cancer in a first-degree relative (Table 1) and 16 individuals with no known family history of colon cancer were included in the study. Although the information of family cancer history is obtained by patients’ self-reports without confirmation from the hospital’s cancer registry, a recent study has confirmed the accuracy of self-reported family history with regard to colon cancer (12). Of the 12 individuals with a family history of colon cancer, two are mother and daughter (cases 6 and 7 in Table 1), two are sister and brother (cases 11 and 12), and the rest are not related. Study subjects ranged in age from 18 to 64 years in the group with a family history of colon cancer, and 16 to 83 years in the control group (the 16-year-old had undergone colonoscopy for chronic abdominal pain). The research protocols for obtaining normal biopsy specimens for study were approved by the California Pacific Medical Center Institutional Review Board. The appropriate procedure for obtaining informed consent was followed for all study subjects.

**Table 1** Summary of expression of PPAR-γ, IL-8, SAA1, COX-2, and PPAR-δ in rectosigmoid biopsy samples from individuals with a family history of colon cancer

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age (y)</th>
<th>Family member with cancer</th>
<th>No. biopsy samples analyzed</th>
<th>No. samples with altered expression</th>
<th>No. genes with altered expression</th>
<th>Probability that changes are due to chances</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>53</td>
<td>Mother*</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>53</td>
<td>Mother†</td>
<td>6</td>
<td>1</td>
<td>3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>43</td>
<td>Father†</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>47</td>
<td>Mother†</td>
<td>7</td>
<td>1</td>
<td>2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>52</td>
<td>Mother</td>
<td>8</td>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>52</td>
<td>Father and daughter*</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>0.26</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>18</td>
<td>Grandfather and sister*</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>35</td>
<td>Mother† and grandmother</td>
<td>8</td>
<td>8</td>
<td>2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>46</td>
<td>Father†</td>
<td>8</td>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>64</td>
<td>Sister†</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>0.26</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>36</td>
<td>Mother and grandfather</td>
<td>7</td>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>38</td>
<td>Mother and grandfather</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

No. individuals with altered gene expression

| 5 | 4 | 2 | 2 |

**Table 1** Summary of expression of PPAR-γ, IL-8, SAA1, COX-2, and PPAR-δ in rectosigmoid biopsy samples from individuals with a family history of colon cancer

NOTE. Number of biopsy samples that exhibited expression levels below (for PPAR-γ and PPAR-δ) or above (for IL-8, SAA1, and COX-2) the cutoff point (P < 0.01). Individuals with all the biopsy samples exhibit expression levels within the normal range are indicated with a (−) sign. All the grandparents with colon cancers in this study are maternal. Ages of the rest of the family members when colon cancer was diagnosed are not available. None of the 12 patients in the family history group reported other type of cancers in the family except the father of the patient for case 10 had lung cancer in his seventies.

*Colon cancer was diagnosed before age of 50 years.
†Colon cancer was diagnosed before age of 60 years.
‡Colon cancer was diagnosed after age of 60 years.

Extraction and Preparation of RNA and cDNA. Biopsy samples obtained from the segment of colon between the cecum and the hepatic flexure were classified as ascending colon samples; those from the segment of colon between the hepatic flexure and the splenic flexure as transverse colon samples; those from the segment of colon below the splenic flexure as descending colon; those from the winding segment of colon below the descending colon were classified as rectosigmoid colon samples (~5-25 cm from rectum). The number of biopsy samples obtained from each patient varied. Two to eight biopsy samples were obtained from each colon segment, except that only one sample was obtained from the transverse and the descending colon segments in one subject of the family history group. A total of 39 ascending colon, 37 transverse colon, 45 descending colon, and 77 rectosigmoid specimens were obtained from the 12 individuals with a family history of colon cancer; and a total of 53 ascending colon, 48 transverse colon, 49 descending colon, and 104 rectosigmoid specimens were obtained from the 16 individuals with no family history of colon cancer. All biopsy samples were snap-frozen on dry ice and taken immediately to the laboratory for RNA preparation and reverse transcription as described (10).

**Analysis of Gene Expression.** The expression levels of oncogene c-myc, CD44 antigen (CD44), cyclooxygenase 1 and 2 (COX-1 and COX-2), cyclin D1, cyclin-dependent kinase inhibitor (p21[cdc42]), interleukin 8 (IL-8), interleukin 8 receptor (CXCR2), osteopontin (OPN), melanoma growth stimulatory activity (Gro/MSGA), Gro3 oncogene (Gro3), macrophage colony stimulating factor 1 (MCSF-1), peroxisome proliferative activated receptor α, δ, and γ (PPAR-α, PPAR-δ, and PPAR-γ), and bone morphogenetic protein 4 (BMP-4) were determined by reverse transcription-polymerase chain reaction (RT-PCR) as described (10).
Alteration of Gene Expression and MNCM

and serum amyloid A1 (SAA1) were analyzed by quantitative reverse transcription-PCR. Except for SAA1, the same 15 genes had been analyzed in our previous studies of APC<sup>min</sup> mice and colon cancer patients (10). Quantitative reverse transcription-PCR were carried out as described in our previous study (10). In brief, the cycle numbers (C<sub>T</sub>) were recorded when the accumulated PCR products crossed an arbitrary threshold. To normalize this value, ΔC<sub>T</sub> was determined as the difference between the C<sub>T</sub> for each gene tested and the C<sub>T</sub> for β-actin. The average ΔC<sub>T</sub> for each gene in the control group was calculated. The ΔΔC<sub>T</sub> was determined as the difference between the ΔC<sub>T</sub> for each individual sample and the average ΔC<sub>T</sub> for this gene obtained from the control samples. These ΔΔC<sub>T</sub> values were then used to calculate relative gene expression values as described (Applied Biosystems, Foster City, CA, User Bulletin 2, December 11, 1997). All PCR were done in duplicate when cDNA samples were available. The results were also verified using histidyl-tRNA synthetase as internal control (10). Relative gene expression values yielded similar results using either β-actin or his-tRNA synthetase as reference. Statistical analyses reported here were obtained using β-actin as normalization controls.

**Statistical Analysis.** Gene expression patterns were compared between individuals with a family history of colon cancer and the control group subjects who had no family history of colon cancer. Rather than testing expression of each gene separately and adjusting for multiple comparisons by methods that reduce statistical power, we tested the expression patterns of all genes by multivariate ANOVA with Wilks’ lambda criterion. This test is a multivariate analogue of the F test for univariate ANOVA, which tests the equality of means. This type of analysis takes into account correlations among gene expression levels and controls the false-positive rate by providing a single test of whether the expression patterns, based on all the genes in the subset, differ between groups.

If there was evidence that expression patterns differed between groups, we used univariate t tests to determine which genes were contributing to the global difference. All multivariate ANOVA tests were based on the Wilks’ lambda criterion and were carried out on log (base 2) of the expression levels, because ANOVA tests were based on the Wilks’ lambda criterion and genes were contributing to the global difference. All multivariate analyses of the expression values of all the biopsy samples from the control group was used to calculate the cutoff point for either up-regulation or down-regulation of each gene. A table of tolerance bounds for a normal distribution was used to define cutoff points so that a fraction of the distribution of no more than P would lie above the cutoff point for up-regulated genes or below the cutoff point for down regulated genes. Each cutoff point was defined by cutoff point = mean + k (SD), where the mean and SD are based on values from the control group. Values of k are found in the table and depend on the P and the number of normal samples (13). Assuming a Gaussian distribution of expression levels of each gene, one would expect <1% of the biopsies from a normal population to have an expression level exceeding the 99% tolerance limit (P = 0.01).

To calculate the probability that the number of observed samples outside the upper 99th percentile was due to chance in each case, we used the binomial distribution method with μ = 0.01 and n = the number of samples for each case multiplied by the number of genes tested. For example, for case 1 (Table 1) we had two samples; both showed abnormal expression for PPAR-γ and SAA1, one of two for PPAR-6, and neither was abnormal for IL-8 and COX-2. Thus, for this case, 5 of 10 tested were beyond the upper 0.01 boundary. The probability that this happened by chance is 2.4 × 10<sup>-8</sup>. The general formula is given by: Pr{X ≥ k|P, n} = ∑<sub>i=0</sub><sup>n</sup>(0.01)^i(0.99)^{n-i}, where k is the number beyond the 99th percentile and n is the number of samples (5 is the number of genes tested).

**RESULTS**

**Altered Gene Expression in the Rectosigmoid Mucosa of Individuals with a Family History of Colon Cancer.** Twelve individuals (10 women and 2 men) comprised the group with a family history of colon cancer; 16 individuals (9 women and 7 men) served as the control group. We analyzed a total of 92 ascending colon biopsy samples, 85 transverse colon samples, 94 descending colon biopsy samples, and 181 rectosigmoid biopsy samples for levels of expression of 16 genes. Expressions of these genes are known to be altered in the late stages of human colon cancers. We have also shown that some of these genes are altered in the MNCM of individuals with a family history of sporadic colon cancer patients (10).

Multivariate analysis of the expression values of all 16 genes indicated a significant difference in the biopsy samples from the rectosigmoid region (P = 0.01) between those with and those without a family history of sporadic colon cancer. Gene expression in biopsy samples from the descending, ascending and transverse colon did not vary significantly between these two groups of individuals (P = 0.06, 0.22, and 0.52, respectively). Most of the differences in rectosigmoid biopsy samples were contributed by just five of these genes (Table 2): PPAR-γ, SAA1, IL-8, COX-2, and PPAR-β. Similar to the alterations of gene expression in the MNCM of cancer patients (10), we found that the expression levels of SAA1, IL-8, and COX-2 were up-regulated and those of PPAR-γ and PPAR-β were down-regulated in the MNCM of individuals with a family history of sporadic colon cancer.
The mean (±SD) age in the family history group was younger (45 ± 12 years) than that of the control group (56 ± 16 years), presumably because of heightened awareness of the need for early colonoscopy in the group with a family history of colon cancer. In addition, there is a sex difference between these two groups (10 women and 2 men in the family history group versus 9 women and 7 men in the control group). However, we found that sex did not affect the level of gene expression (P = 0.67). Moreover, there was no correlation between age and the expression levels of SAA1, IL-8, COX2, and PPAR-γ (all P > 0.05) except for PPAR-δ (P < 0.01). Nevertheless, abnormal expression (down-regulation) of PPAR-δ increases with age. Thus, comparison between younger family history group and older controls would be biased toward finding fewer, rather than more, abnormal expressions in the family history group. In other words, we may underestimate the incidence of altered expression of PPAR-δ in the family history group.

**Comparison with Cutoff Points for “Normal” Gene Expression.** Relative gene expression levels in the rectosigmoid samples varied among individuals, much more so in samples obtained from the individuals with a family history of colon cancer than the corresponding values from the controls (Table 2). We therefore use the expression level of each gene in the control group to define the “normal” expression level for each gene by calculating a cutoff point (P = 0.01) for each gene. Figure 1 shows the distribution of the log$_2$ expression values for genes, PPAR-γ, IL-8, SAA1, and COX-2 and their cutoff points. As expected, <1% of the biopsy samples from the control group had expression of these genes above or below the cutoff lines (P = 0.01, Fig. 1). However, 21%, 12%, and 8% of the biopsy samples from the family history group had expression of SAA1, IL-8, and COX-2, respectively, above the cutoff points and 12% of them had expression of PPAR-γ below the cutoff point (Table 3).

We next analyzed each individual in the family history group (Table 1). For the five most commonly altered genes, 9 of the 12 individuals with a family history of colon cancer had at least one biopsy samples with expression levels below or above the cutoff point. Two individuals (cases 1 and 2) had altered expression of three of these genes in apparently normal rectosigmoid mucosa. In contrast, only one of the 16 individuals in the control group had altered expression of one of these five genes (Table 1). The cutoff is set so that 1% of expressions could be false positives. However, the numbers of biopsy samples obtained from each individual are different. To make an adjustment for the number of specimens, we also calculated, for each case, the probability that the number of observed samples outside the upper 99th percentile was due to chance. This calculation was based on the binomial distribution. As shown in Table 1, the observed altered gene expression in 7 of the 12 individuals of the family history group is unlikely due to chances (P < 0.01). In these seven cases, expressions of at least two of the five genes were altered. In addition, among the 16 genes analyzed, PPAR-γ and SAA1 are the most frequently altered genes that occurred in 5 of the 12 individuals with a family history of colon cancer (Table 1).

**Table 2** Gene expression levels in normal rectosigmoid biopsy samples from individuals with family history of colorectal cancer compared with controls

<table>
<thead>
<tr>
<th>Genes</th>
<th>Controls (n = 104)</th>
<th>Patients with family history (n = 77)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>PPAR-γ</td>
<td>0.44-1.65</td>
<td>1.07 ± 0.41</td>
</tr>
<tr>
<td>SAA1</td>
<td>0.17-22</td>
<td>2.16 ± 3.67</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.14-13</td>
<td>1.71 ± 1.94</td>
</tr>
<tr>
<td>COX-2</td>
<td>0.17-18</td>
<td>1.82 ± 2.75</td>
</tr>
<tr>
<td>PPAR-δ</td>
<td>0.39-2.66</td>
<td>1.11 ± 0.48</td>
</tr>
<tr>
<td>CD44</td>
<td>0.35-1.13</td>
<td>1.14 ± 0.64</td>
</tr>
<tr>
<td>c-Myc</td>
<td>0.24-3.66</td>
<td>1.21 ± 0.75</td>
</tr>
<tr>
<td>MCSF-1</td>
<td>0.38-22</td>
<td>1.81 ± 2.59</td>
</tr>
<tr>
<td>Gro-α</td>
<td>0.01-51</td>
<td>2.61 ± 5.48</td>
</tr>
<tr>
<td>Gro-γ</td>
<td>0.16-35</td>
<td>2.18 ± 4.29</td>
</tr>
<tr>
<td>P21</td>
<td>0.51-2.15</td>
<td>1.10 ± 0.62</td>
</tr>
<tr>
<td>PPAR-α</td>
<td>0.31-2.38</td>
<td>1.09 ± 0.55</td>
</tr>
<tr>
<td>CXCR2</td>
<td>0.22-1.13</td>
<td>1.45 ± 1.78</td>
</tr>
<tr>
<td>OPN</td>
<td>0.19-13</td>
<td>1.66 ± 2.05</td>
</tr>
<tr>
<td>CyclinD</td>
<td>0.34-3.48</td>
<td>1.28 ± 0.85</td>
</tr>
<tr>
<td>COX-1</td>
<td>0.27-5.97</td>
<td>1.21 ± 0.85</td>
</tr>
</tbody>
</table>

**NOTE.** Results of analysis of 104 biopsy samples from 16 individuals without family history and 77 biopsy samples from 12 individuals with family history of colon cancer in a first-degree relative. Samples were analyzed for gene expression as described in MATERIALS AND METHODS. The numbers in the table represent the expression level relative to the average ΔΔC_T of the control group. If there is no variation among individuals, the normal gene expression level in the control group should equal to 1. Multivariate analysis using the Wilks’ lambda criterion was carried out on log$_2$ expression values of the 16 genes to determine the significance of the difference between the two groups. Genes are listed from smallest to largest P values.
We also observed that the difference in gene expression between the two groups of individuals increased along the length of the colon for PPAR-γ (P = 0.001 for trend) and SAA1 (P < 0.001), but not for IL-8 (P = 0.20), COX2 (P = 0.58), nor PPAR-δ (P = 0.54). These results suggest that there is an increasing abnormality along the colon going from the ascending to the rectal portion between the two groups of individuals that can be detected despite reduced numbers of samples toward the ascending portion in this study.

**DISCUSSION**

Approximately 5% to 10% of colorectal cancers occur among patients with one of the two autosomal dominant hereditary forms of colon (familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer) or who have inflammatory bowel disease (11). Of the remaining colon cancers, ~20% are associated with a family history of colon cancer, which is associated with a 2-fold increased risk of developing colon cancer (1). Although linkage to chromosomes 15q13-14 and 9q22.2-31.2 has been reported in a subset of patients with familial colorectal cancer (14), the genetic basis for most of these cases is not known. In this study, we have shown substantial alterations in the expression of PPAR-γ, IL-8, and SAA1 in the rectosigmoid MNCM from individuals with a family history of sporadic colon cancer, although these individuals had no detectable colon abnormalities. Our previous study showed that, in addition to PPAR-γ, IL-8, and SAA1, expressions of PPAR-δ, p21, OPN, COX-2, CXCR2, MCSF-1, and CD44 were also altered significantly in the MNCM of colon cancer patients when compared with normal controls without colon cancer, polyps, or family history. These observations suggest that altered expression of genes related to cancer development in the MNCM may be a sequential event and may occur earlier than the appearance of gross morphologic abnormalities. For example, altered expression of PPAR-γ, SAA1, and IL-8 may occur in MNCM of individuals who have not developed colon cancer, but are at high risk of doing so, whereas altered expressions of other genes, such as PPAR-δ, p21, OPN, COX-2, CXCR2, MCSF-1, and CD44, may occur later in MNCM of individuals who have already developed a colon cancer (10).

![Gene expression in rectosigmoid biopsy samples from normal individuals and individuals with a family history of colon cancer. Control group (crosses) or family history group (circles) in each column are different rectosigmoid biopsy samples. log2 expression level of each gene is used (y-axis). The average expression level of each gene in the control group is set at 1; thus, the log2 of the average expression level in the control group of each gene equals 0 on the y-axis. Ninety-nine percent tolerance limits of each gene (dashed lines).](clincancerres.aacrjournals.org/article/11/6/1404/206696)
Genetic and epigenetic changes have been reported in macroscopically normal tissues for several neoplasms (15). For example, allelic loss has been shown in normal breast terminal ductal lobular units adjacent to primary breast cancers (16). Such allelic loss is associated with an increased risk of local recurrence (17). In addition, normal-appearing colonic mucosal cells from individuals with a prior colon cancer are more resistant to bile acid–induced apoptosis than mucosal cells from individuals with no prior colon cancer. (18, 19). Because apoptosis is important in colonic epithelium to eliminate cells with unrepaired DNA damage (20), reduction in apoptosis could result in the retention of DNA-damaged cells and increase the risk of carcinogenic mutations.

PPAR-γ is down-regulated in several carcinomas. Ligands of PPAR-γ inhibit cell growth and induce cell differentiation (21), and loss-of-function mutations in PPAR-γ have been reported in human colon cancer (22). Thus, our observation of down-regulation in PPAR-γ expression in MNCM may represent an early event that promotes colonic epithelial cell growth and inhibits cellular differentiation. In addition, PPAR-γ also negatively regulates inflammatory response (23). Inflammation favors tumorigenesis by stimulating angiogenesis and cell proliferation (24). Similarly, IL-8 and the acute-phase protein SAA1 modulate the inflammatory process (25, 26). Up-regulation of proinflammatory cytokines and acute phase proteins has been reported in the colon mucosa of individuals with inflammatory bowel disease (27, 28), who are at very high risk of developing colon cancer (29). Epidemiologic observations also suggest that chronic inflammation predisposes to colorectal cancer (30, 31). Thus, the observation of down-regulation of PPAR-γ and up-regulation of IL-8 and SAA1 in the normal mucosa of individuals with a family history of sporadic colon cancer and individuals with inflammatory bowel disease may indicate the involvement of common pathways leading to colon carcinogenesis in these two groups.

Our observation of altered expression of genes associated with cancer and inflammation in normal colonic mucosa in some individuals with a family history of colon cancer is consistent with the recent report of association of elevated serum C-reactive protein concentration before the development of colon cancer (32). These findings suggest that inflammation is a risk factor for the development of colon cancer in average-risk individuals (32). However, C-reactive protein is a nonspecific marker of inflammation that may indicate inflammation in tissues other than colon. In our study, we have analyzed the tissue where

### Table 3
Number of biopsy samples (n) with gene expression above/below the cutoff point in normal individuals and individuals with a family history of colon cancer

<table>
<thead>
<tr>
<th>Genes</th>
<th>Biopsy samples from normal controls (n = 104), n (%)</th>
<th>Biopsy samples from individuals with family history (n = 77), n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPAR-γ</td>
<td>0</td>
<td>9 (12%)*</td>
</tr>
<tr>
<td>SAA1</td>
<td>0</td>
<td>16 (21%)†</td>
</tr>
<tr>
<td>IL-8</td>
<td>0</td>
<td>9 (12%)†</td>
</tr>
<tr>
<td>COX-2</td>
<td>1 (1%)†</td>
<td>6 (8%)‡</td>
</tr>
<tr>
<td>PPAR-δ</td>
<td>0</td>
<td>2 (3%)*</td>
</tr>
<tr>
<td>Grx-γ</td>
<td>1 (1%)†</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>PPAR-α</td>
<td>0</td>
<td>2 (3%)*</td>
</tr>
<tr>
<td>Gro-α</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M-CSF-1</td>
<td>1 (1%)†</td>
<td>0</td>
</tr>
<tr>
<td>OPN</td>
<td>1 (1%)†</td>
<td>0</td>
</tr>
<tr>
<td>P21</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CD44</td>
<td>1 (1%)†</td>
<td>0</td>
</tr>
<tr>
<td>CXCR2</td>
<td>1 (1%)†</td>
<td>0</td>
</tr>
<tr>
<td>c-Myc</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CycD1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>COX-1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*With gene expression level below the cutoff point.
†Number of patients with alterations are listed in Table 1.
‡With gene expression level above the cutoff point.

Genetic and epigenetic changes have been reported in macroscopically normal tissues for several neoplasms (15). For example, allelic loss has been shown in normal breast terminal ductal lobular units adjacent to primary breast cancers (16). Such allelic loss is associated with an increased risk of local recurrence (17). In addition, normal-appearing colonic mucosal cells from individuals with a prior colon cancer are more resistant to bile acid–induced apoptosis than mucosal cells from individuals with no prior colon cancer. (18, 19). Because apoptosis is important in colonic epithelium to eliminate cells with unrepaired DNA damage (20), reduction in apoptosis could result in the retention of DNA-damaged cells and increase the risk of carcinogenic mutations.

**A Rectosigmoid region**

**Case 3** (n=5)

- COX-2
- IL-8
- SAA1
- PPAR-γ

**Case 4** (n=7)

- COX-2
- IL-8
- SAA1
- PPAR-γ

**Case 8** (n=8)

- COX-2
- IL-8
- SAA1
- PPAR-γ

**B Complete colon**

**Case 3**

- COX-2
- IL-8
- SAA1
- PPAR-γ

**Case 4**

- COX-2
- IL-8
- SAA1
- PPAR-γ

**Case 8**

- COX-2
- IL-8
- SAA1
- PPAR-γ

![Fig. 2](clincancerres.aacrjournals.org) Distribution of alterations of gene expression in normal colon mucosa of individuals with a family history of colon cancer. A, altered gene expression in rectosigmoid biopsy samples from three individuals (cases 3, 4, and 8). Number of biopsy samples from each individual (n). Biopsy sample (box). B, altered gene expression in ascending (Asc), transverse (Trans), descending (Des), and rectosigmoid (S/R) colon. Indicated colon segments (box). Names of the genes analyzed (left of each row). Presence of altered gene expression in the corresponding samples/colon segments analyzed (shaded box).
colon cancer arises and would be more specific in assessing the risk of developing colon cancer.

We do not know which cell type is responsible for the observed altered gene expression. There are many cell types involved in the colorectal mucosa, including several types of mucosal epithelial cells, stromal cells, and blood-borne cells. Studies from our group and others have shown that the up-regulation of COX-2 protein in MNCM is localized primarily to the infiltrating macrophages and secondarily to the epithelial cells in aberrant crypt foci in the MNCM of APCmin mice (10, 33). From our previous studies of MNCM of APCmin mice, detection of the gene products that are up-regulated or down-regulated in MNCM by in situ hybridization was found to be technically difficult, perhaps because the secreted proteins, such as IL-8 and SAA1, are evanescent in tissue sections (10). Due to the limited amount of the biopsy samples and technical difficulties, we were unable to perform immunohistochemical staining to show the cell types contributing to the altered gene expression. If the absolute RNA quantities are sufficient, RNA in situ hybridization may be a better method to determine the cellular locations of alterations. Alternatively, laser microdissection followed by reverse transcription-PCR may be able to define the cell types involved. Regardless of the cell types responsible for the altered gene expression, our results show that relative to normal individuals without family history of colon cancer, altered gene expression is present in normal colon mucosa of some individuals with a family history of colon cancer, and these individuals are known to have an increased risk of developing colon cancer (11).

Among patients with altered gene expression in the rectosigmoid biopsy samples, some showed alterations in all biopsy samples (i.e., expression of SAA1 in cases 4 and 12), whereas others showed altered expression in some biopsy samples only (i.e., PPAR-γ in cases 2 and 3; Table 1). Because most samples were assayed with multiple genes in duplications to ensure the quality of cDNA, such heterogeneity is unlikely due to technical variation. We speculate that this heterogeneity might reflect the frequency and/or the distribution of “hotspots” in these individuals. It is possible that the individuals with altered gene expression in all rectosigmoid biopsy samples may have widespread molecular abnormalities in their rectosigmoid mucosa, whereas those with altered expression in some of the biopsy samples have discrete hotspots. Thus, individuals in the former group may have a global predisposition to development of colon polyps or cancer, whereas those in the latter group may have local predisposition. Whether the risks in developing colon cancer or polyps differ between these two groups is unknown. In addition, altered expression of different combination of genes were observed in the rectosigmoid biopsy samples of individuals in the family history group. This observation suggests that different molecular pathways may be involved in the early stages of colon carcinogenesis. Whether altered gene expression in certain molecular pathways is associated with higher risk of polyps or cancer also remains to be determined.

Consistent with the reports of more aberrant crypt foci (the preneoplastic colonic lesions) in the distal colon than in the proximal colon of the sporadic colon cancer patients and the carcinogen treated mice (34, 35), we found that most of the alterations in gene expression were found in the distal colon of the individuals from the family history group. We speculate that the distal colon mucosa of the susceptible individuals may be exposed to higher concentration of exogenous substances present in the stool than mucosa in other colon regions after most of the water is reabsorbed at the end of the large intestine, and such exposure may lead to higher rate of altered gene expression at this region.

We have shown that family history of colon cancer, but not age or sex, is the factor responsible for the observed differences in gene expression in the rectosigmoid mucosa of the two groups. The available information did not indicate any specific difference in diet or medication between these two groups of patients. However, we cannot eliminate the possibility that diet or medication affect gene expression without further study. Not all individuals with a family history of colon cancer will develop cancer or APCs of the colon (1). Consistent with this clinical observation, our analysis also showed that not all the individuals with a family history of colon cancer have altered gene expression in MNCM. Because the genes analyzed in this study are involved in the development of colon cancer, we hypothesize that individuals with altered gene expression in the MNCM may be more susceptible to developing polyps or cancer than those without altered gene expression. To test this hypothesis, a prospective study with a larger number of study subjects will be needed. If such an association is confirmed, it may be possible to identify individuals at increased risk of developing colon cancer by using gene expression analysis of rectosigmoid biopsy samples. Theoretically, it is easier to identify individuals with global alterations in the MNCM than individuals with local alterations by analysis of random MNCM samples. However, if an appropriate panel of genes was selected for analysis using multiple samples, it may have enough predictive power to identify such patients.

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REFERENCES


Alteration of Gene Expression in Macroscopically Normal Colonic Mucosa from Individuals with a Family History of Sporadic Colon Cancer

Chun-Yi Hao, Dan H. Moore, Patrick Wong, et al.