Subepithelial Myofibroblasts Express Cyclooxygenase-2 in Colorectal Tubular Adenomas

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ABSTRACT

Purpose: Recent data support the hypothesis that the inducible isomform of cyclooxygenase (COX-2) plays a role in the early stages of colonic carcinogenesis and that nonsteroidal anti-inflammatory drugs (NSAIDs) retard the development of colon cancer by modulating COX-2. However, the cell types responsible for producing COX-2 in colorectal adenomas remain a subject of controversy.

Experimental Design: COX-2 expression in normal colonic mucosa (n = 50), hyperplastic polyps (n = 43), sporadic adenomas (n = 67), and invasive colonic adenocarcinoma (n = 39) was studied in formalin-fixed and paraffin-embedded tissue sections from endoscopy biopsy and colonic resection specimens. Immunohistochemistry (avidin-biotin complex technique with double immunolabeling) was used to identify the phenotypes of COX-2-producing cells.

Results: In colorectal adenomas, increased expression of COX-2 was detected and localized to α smooth muscle actin (αSMA)-positive subepithelial stromal cells (myofibroblasts) in the periluminal region of the lamina propria in 63 (94%) of 67 cases. In contrast, in normal colonic mucosa and in hyperplastic polyps with intact epithelium, COX-2 expression was found only in macrophages and endothelial cells. In areas in which the surface epithelium was ulcerated in normal mucosa as well as hyperplastic or neoplastic polyps, COX-2 expression was increased in granulation tissue (and present in macrophages, endothelium, and myofibroblasts). In invasive carcinoma, COX-2 expression in myofibroblasts was limited to the adenomatous portion of the tumor and was detected in 62% of cases (n = 39). In addition, focal expression of COX-2 by malignant epithelial cells was observed in 23% of invasive adenocarcinoma.

Conclusions: These results show that increased COX-2 expression in sporadic adenoma of the colon is common and is localized specifically to subepithelial intestinal myofibroblasts. These findings further support the hypothesis that myofibroblasts are important target cells for NSAID-mediated chemoprevention of colorectal cancer.

INTRODUCTION

Attempts to reduce the incidence of colon cancer and resulting mortality are currently focused on early detection and chemoprevention. Several epidemiologic studies have shown that prolonged use of aspirin is associated with a significant reduction in the risk of developing and/or dying from cancer of the colon (1–3). Other nonsteroidal anti-inflammatory drugs (NSAIDs), have also been reported to cause a reduction in size and number of adenomas in familial adenomatous polyposis patients (4–6). In recently published double-blind clinical trial studies, daily low-dose aspirin was found to produce statistically significant reduction in the incidence of sporadic colorectal adenomas in a relatively large population of patients (n = 1121) with history of histologically documented adenomas (7), as well as in patients with history of colorectal carcinoma (8). These trials lend support to the hypothesis that the “anticancer” effects of NSAIDs derive, at least in part, from their inhibitory actions on cyclooxygenase (COX), the rate-limiting enzyme for the conversion of arachidonic acid to prostaglandins (9). The role of prostaglandins, and COX-2 in particular, in colorectal carcinogenesis has, therefore, become a major focus of research efforts to study the biology of colonic cancer and also in the concept of NSAID-related chemoprophylaxis (10). For colon cancer prevention, it is important to focus on changes in normal colonic mucosa and premalignant lesions, the adenomas, rather than the frankly malignant invasive adenocarcinoma to further the development of NSAID-based chemopreventive strategies. Increased expression of COX-2 has been shown to occur in gastrointestinal adenomatous polyps from animal models of adenomatous polyposis (11–14), familial adenomatous polyposis patients (15–17), and also in sporadic adenomas from human patients (18–22). Furthermore, the use of COX-2-selective inhibitors has resulted in significant decrease in the number and size of adenomatous polyps in both rodent models and also in familial adenomatous polyposis patients (23–25). These findings strongly suggest COX-2 may have a very significant role in promoting the development of colorectal cancer and has further encouraged the development of COX-2-based chemopreventive agents (10). However, the cellular mechanisms involved are yet to be defined. Furthermore, there are conflicting published data with regard to the cellular location of COX-2 in colorectal adenomas (11, 13–15, 19–22, 26–29). Identifying the exact cell type(s) responsible for increased COX-2 production in colorectal adenomas is essential for a thorough elucidation of the role of COX-2 in colonic carcinogenesis. Such an insight will also
help advance the development of chemoprophylactic strategies against colorectal adenocarcinoma.

We recently reported that the stromal cells in the lamina propria of the colonic mucosa express α smooth muscle actin (αSMA) in colonic polyps. This is different from normal colonic lamina propria, in which only pericytros stromal cells express αSMA. Thus, in colorectal polyps, there is diffuse transdifferentiation of stromal cells to myofibroblasts or alternatively, extensive proliferation of myofibroblasts (30). Studies by ourselves and confirmation by others using cell proliferation markers such as Ki67 lend support to the former (transdifferentiation; ref. 31), a scenario similar to that observed in the liver in which differentiation of perivascular stromal cells (Ito cells) to myofibroblasts is considered a differentiation into an “activated” phenotype (32). We, therefore, hypothesized that the “activated” mesenchymal cells in the lamina propria of colonic polyps may play critical roles in colorectal tumorigenesis (30). Also, studies of primary isolates from colonic biopsies of healthy patients without colonic disease or from normal parts of colorectal resection specimens showed that these intestinal myofibroblasts in an vitro cultures do express COX-2 when exposed to cytokines such as interleukin 1 (IL-1) and tumor necrosis factor α (TNFα; ref. 33).

In this study, therefore, we examined the expression and cellular localization of COX-2 protein in the colonic mucosa of a large series of normal human colon, hyperplastic polyps and sporadic adenomas, with particular emphasis on the stromal myofibroblasts. Using specific COX-2 antibodies and double immunolabeling of step tissue sections, we localized the specific differential COX-2 expression to subepithelial intestinal myofibroblasts in this large series of patients with sporadic colorectal adenomatous polyps.

MATERIALS AND METHODS

Study Materials. The study was performed on archived human paraffin-embedded tissue sections from 82 males and 68 female patients with age range of 27–92 years, according to a protocol approved by our institutional review board. The study materials consisted of randomly selected cases that included sections of tumor (polyp or carcinoma) and adjacent normal mucosa from 40 patients who had colonic resection for colorectal adenocarcinoma, as well as endoscopy biopsy and polypectomy specimens of polyps from 110 patients (67 with sporadic adenomatous polyps was scored by counting in each polyp, the number of periluminal stromal cells aggregates that stained positive for COX-2 in that region. COX-2 expression scores were then correlated with the size of the polyps. In the cases of hyperplastic polyps and 43 with hyperplastic polyps). As normal 

Table 1

<table>
<thead>
<tr>
<th>Stain</th>
<th>Catalog no./ clone</th>
<th>Source</th>
<th>Dilution</th>
<th>Antigen retrieval technique</th>
<th>Positive control tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>COX-2</td>
<td>160126*</td>
<td>Cayman</td>
<td>1:500</td>
<td>Citrate @ pH 6.0</td>
<td>18Co cell line</td>
</tr>
<tr>
<td>αSMA</td>
<td>M0851/1A4</td>
<td>DAKO</td>
<td>1:200</td>
<td>Citrate @ pH 6.0</td>
<td>Colon</td>
</tr>
<tr>
<td>Caldesmon</td>
<td>h-CD</td>
<td>DAKO</td>
<td>1:200</td>
<td>DAKO’s solution at pH 10</td>
<td>Uterus</td>
</tr>
<tr>
<td>Calponin</td>
<td>CALP</td>
<td>DAKO</td>
<td>1:400</td>
<td>DAKO’s solution at pH 10</td>
<td>Uterus</td>
</tr>
<tr>
<td>CD68</td>
<td>KP1</td>
<td>DAKO</td>
<td>1:200</td>
<td>Proteinase K</td>
<td>Tonsil</td>
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<tr>
<td>CD68</td>
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</tr>
<tr>
<td>Lysozyme</td>
<td>A0099*</td>
<td>DAKO</td>
<td>1:1200</td>
<td>Citrate @ pH 6.0</td>
<td>Tonsil</td>
</tr>
</tbody>
</table>

* Only the catalog number was provided by the manufacturer, DAKO, (Carpinteria, CA).
cell pellets and processed into formalin-fixed paraffin-embedded cell blocks for COX-2 immunohistochemistry. Another group of treated cells (with appropriate controls) were lysed and analyzed by Western blot for COX-2 expression. Western blot analyses were performed as described previously (33) with the following modifications: 345 μg of total protein was fractionated in 10% SDS-PAGE gels prepared using a single-well preparative comb (11.5 cm) flanked by conventional wells containing molecular mass markers. Proteins were then transferred to Immobilon-P (Millipore, Bedford, Mass.) membranes. After containing molecular mass markers. Proteins were then transferred following modifications: 345 μg of total protein was fractionated in 10% SDS-PAGE gels prepared using a single-well preparative comb (11.5 cm) flanked by conventional wells containing molecular mass markers. Proteins were then transferred to Immobilon-P (Millipore, Bedford, Mass.) membranes. After transfer, membranes were cut longitudinally into strips of 0.5-cm width (15 μg of total protein per lane) and blocked in 5% fat-free dry milk in Tris-buffered saline for 1 h at room temperature. Individual membrane strips were then incubated overnight at 4°C with the appropriate primary antibody (a panel of 8 commercially available anti-COX-2 antibodies). Each antibody was tested in 1:10,000 and 1:20,000 dilutions in 5% bovine serum albumin TBS-T (TBS containing 0.1% Tween 20). To equalize for background signals due to the secondary antibodies, a premix of horseradish peroxidase-conjugated, antigoat, antirabbit secondary antibodies were used to detect the primary antibodies. After three washes with TBS-T, membrane strips were aligned to the original configuration with adhesive tape and chemiluminescent detection was done with a chemiluminescence detection kit (Amersham Biosciences, Piscataway, NJ) according to the supplier’s recommendations. The results from each antibody dilution were qualitatively similar.

RESULTS

Study Materials. The colorectal polyps range from 1.5 mm to 1.1 cm in greatest dimension. Three of the benign (hyperplastic) polyps had mucosal ulceration with no additional distinguishing features. All of the premalignant (adenomatous) polyps (n = 67) were devoid of areas with high-grade dysplasia or intramucosal carcinoma. The frankly malignant neoplasms (invasive adenocarcinoma, n = 40) ranged from 1.2 cm to 13 cm. in greatest dimension and consist of 8 American Joint Committee on Cancer (AJCC) stage I tumors, 17 stage II, 9 stage III, and 6 stage IV tumors.

Specificity of COX-2 Antibodies. Of the eight commercially available COX-2 antibodies that we tested, six of them showed spurious bands (nonspecific background staining) with and without treatment with IL-1α and/or TNFα (Fig. 1; Table 2). The COX-2 antibody that we used was selected from the panel based on its specificity in Western blot assays performed with 18Co, an intestinal myofibroblast primary isolate that is known to produce COX-2 after treatment with IL-1 and/or TNFα. In Western blots, the selected antibody showed a single band with correct size (Mr, 72,000) and an absence of spurious bands in both untreated and treated cells. Details of the antibodies are listed in Table 2.

Table 2 List of COX-2 antibodies tested

<table>
<thead>
<tr>
<th>Antibody index no.</th>
<th>Source (manufacturer)</th>
<th>Type</th>
<th>Catalog no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cayman Chemicals</td>
<td>(Murine) Rabbit polyclonal</td>
<td>160106</td>
</tr>
<tr>
<td>2</td>
<td>Cayman Chemicals</td>
<td>(Human) Rabbit polyclonal</td>
<td>160107</td>
</tr>
<tr>
<td>3</td>
<td>Cayman Chemicals</td>
<td>(Human) Mouse monoclonal</td>
<td>160112</td>
</tr>
<tr>
<td>4</td>
<td>Cayman Chemicals</td>
<td>(Murine) Rabbit polyclonal-affinity-purified</td>
<td>160126</td>
</tr>
<tr>
<td>5</td>
<td>Santa Cruz</td>
<td>Goat polyclonal-affinity-purified (C-20)</td>
<td>sc-1745</td>
</tr>
<tr>
<td>6</td>
<td>Santa Cruz</td>
<td>Goat polyclonal-affinity-purified (N-20)</td>
<td>sc-1746</td>
</tr>
<tr>
<td>7</td>
<td>Santa Cruz</td>
<td>Rabbit polyclonal (H-62)</td>
<td>sc-7951</td>
</tr>
<tr>
<td>8</td>
<td>BD Transduction Laboratories</td>
<td>Mouse monoclonal</td>
<td>610203</td>
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ical antibody absorption tests using blocking peptide with cell blocks as well as tissue controls (Fig. 2C and D). Strong COX-2 expression in ganglion cells in the myenteric plexus of sections of normal colon was eliminated when the tissue sections were preincubated with COX-2 peptide.

Expression of COX-2 in Normal Colonic Mucosa. In the normal colonic mucosa, COX-2 expression was detected in macrophages and endothelial cells in the lamina propria of the normal colonic mucosa in all resection specimens (n = 40; Fig. 3A). No COX-2 expression was seen in the epithelium or in the (noninflammatory) stromal cells of the lamina propria. Similar results were also obtained in the 10 biopsies from patients with normal colons on endoscopy as well as in the normal mucosa fragments that were present in the endoscopic specimens obtained from patients with colonic polyps.

Expression of COX-2 in Hyperplastic Polyps. In all of the hyperplastic polyps (obtained from 43 patients), COX-2 expression was similar to that in the normal mucosa. In areas with intact surface epithelium, COX-2 expression was observed only in macrophages and endothelial cells in all of the hyperplastic polyps (Fig. 3B). At foci of mucosal ulceration in hyperplastic polyps, COX-2 expression was markedly increased in the granulation tissue at the ulcer base at which COX-2 was expressed by endothelial cells, macrophages, and now also by myofibroblasts, all of which were present in granulation tissue (Fig. 3C). No positive COX-2 immunoreactivity was found in the epithelium of any of the hyperplastic polyps that we studied.

Expression of COX-2 in Neoplastic Polyps. In tubular adenomas, COX-2 expression was seen in endothelial cells and macrophages, as was observed in the normal colonic mucosa and hyperplastic polyps. In addition, marked COX-2 expression was seen in the lamina propria in a group of stromal cells located just beneath the surface epithelium (Fig. 3D). This periluminal subepithelial expression of COX-2 in adenomatous polyps was present in 63 of 67 patients whose sporadic adenomas had intact surface epithelium. In the remaining four patients with adenomas, the surface of the adenoma was significantly eroded such that the subepithelial stromal cells beneath the surface epithelium were absent and could not be assessed for COX-2 expression. Double immunolabeling of step-tissue sections of adenomas with intact surface epithelium (from the remaining 63 patients) showed these COX-2 positive subepithelial stromal cells to be myofibroblasts (αSMA-positive cells) and not macrophages, as evidenced by their negative immunoreactivity with all of the macrophage-specific markers (CD68 KP1, CD68 PGM, Lysozyme) and positive reaction with αSMA antibody (Fig. 4A–D).
In contrast, no such periluminal increased expression of COX-2 by subepithelial myofibroblasts was seen in hyperplastic polyps or in normal colonic mucosa sections with an intact surface epithelium. Also, no COX-2 epithelial staining was detected in the adenomatous polyps. Thus, the only difference in COX-2 staining pattern seen, when normal colonic mucosa was compared with hyperplastic polyps with tubular adenoma, was the expression of COX-2 by periluminal myofibroblasts in the adenomatous polyps. Furthermore, these COX-2-expressing subepithelial stromal cells stained positive for αSMA (the universal marker for myofibroblasts) but negative with other classic smooth-muscle-specific markers such as calponin and caldesmon. Thus, the COX-2 expressing myofibroblasts are devoid of antibody reaction to markers specific for macrophages or smooth muscle except αSMA. Also, we found no correlation between the expression of COX-2 and the size of the adenomatous polyps nor the degree of the dysplasia therein.

There was a marked variation and overlap in the COX-2 expression scores with respect to the size of the adenomatous polyps. The scores ranged from 1 to 8 for polyps that were less than 2 mm in diameter, 1 to 20 for polyps that were 2 to 2.9 mm; 1 to 17 for 3 to 3.9-mm polyps, 1 to 19 for 4 to 4.9-mm polyps; 4 to 21 for 5 to 5.9-mm polyps, and 2 to 45 for polyps that were ≥6 mm in diameter. There was also marked variation in COX-2 expression from patient to patient even in polyps that were of the same size. For instance, seven polyps of the same size (2 mm) from different patients had COX-2 scores of 1,1,1,3,7,8, and 20. The factors responsible for this variation are subjects of additional studies in our laboratory.

**Expression of COX-2 in Invasive Adenocarcinoma.** In 62% (24 of 39) of invasive adenocarcinoma cases, COX-2 expression in αSMA-positive subepithelial stromal cells was observed in the periluminal adenomatous portion of the tumor. Thus COX-2 expression in stromal cells of carcinomas was similar to, but less frequent, than that seen in adenomas with intact surface epithelium (62% versus 94–100%; Fig. 5A). In one of the 40 tissue blocks selected for this study, the tumor was not of sufficient size for the multiple-step sections required for this study. In the invasive region of the cancer, positive COX-2 immunoreactivity was present in endothelial cells, in stromal

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*Fig. 3* COX-2 expression in normal colonic mucosa and benign (hyperplastic) polyps and premalignant (adenomatous) polyps. In A, COX-2 is observed in endothelial cells (thick arrows) and macrophages (thin arrows) in normal colonic tissue (×200); B, COX-2 expression in hyperplastic polyps with intact surface epithelium is limited to endothelium and macrophages (thin arrows; ×200); C, in hyperplastic polyps with ulcerated surface epithelium and underlying granulation tissue, COX-2 expression is increased and present in endothelium (arrowheads), macrophages (thin arrows), and myofibroblasts (thick arrows; ×x400); D, marked up-regulation of periluminal COX-2 expression in the stroma of adenomatous polyp (×40).
cells, and, focally in a few malignant epithelial cells in 9 of 40 cases (23%) in which COX-2 expression was limited to a few single cells or nests of cells in relatively few high-power fields. Depending on the availability of tumor tissue on the immunostained slides, 52 to 285 fields were counted in each case, and COX-2-positive epithelial cells were found in 1 to 29 fields. (Fig. 5B). The nine cases of frankly invasive adenocarcinoma with COX-2-positive malignant epithelial cells consisted of 1 of 8 AJCC stage I tumors, 4 of 17 stage II tumors, 4 of 9 stage III tumors and none of 6 stage IV tumors. The focal expression of COX-2 in malignant epithelial cells did not show any correlation with grade, stage, or histomorphologic features of the tumors. These results of immunohistochemical expression of COX-2 in colorectal polyps and carcinoma are summarized in Table 3.

DISCUSSION

In this study, we found that in the normal mucosa as well as in hyperplastic polyps with intact epithelial lining, COX-2 was expressed by macrophages and endothelial cells. In contrast, in adenomatous polyps, we observed additional marked expression of COX-2 in the αSMA subepithelial myofibroblasts just beneath the surface (luminal) epithelial lining. These findings suggest intestinal subepithelial myofibroblasts are the main source of up-regulation of COX-2 in adenomatous polyps. Similar expression of COX-2 by subepithelial myofibroblasts was also detected in the adenomatous portion of sporadic adenocarcinoma. We also found epithelial COX-2 expression in only a small subset (23%) of invasive cancer. Similar to previous reports (37–39), we found no correlation between COX-2 expression in malignant epithelial cells and the grade, stage, or histomorphologic features of the tumors. This is in contrast to the findings of Zhang and Sun (40), who reported a more frequent expression of COX-2 in tumors with better histologic differentiation than in poorly differentiated tumors, and to the findings of Fujita et al. (41) and Sheehan et al. (42), who reported correlation between higher COX-2 expression with larger tumor size and more advanced Duke’s stage. These con-
Flicting findings may be due to factors such as different antibodies used in those studies (43) and differences in the genetic features and molecular biology of the tumors.

Although COX-2 expression is frequently observed in the epithelial portion of colorectal adenocarcinomas (11, 15, 18, 42, 44–46), epidemiologic and animal studies have demonstrated that the chemopreventive effect of COX inhibitors occurs at very early stages of colorectal carcinogenesis, at times before the appearance of adenocarcinoma (9). Given these data, it is important to identify the sites of COX-2 expression in normal mucosa and in precancerous colorectal adenomatous polyps.

Although a few studies have observed epithelial COX-2 expression in adenomatous tissue (11, 15, 19), the majority find that COX-2 is located primarily (20), if not exclusively (13, 14, 21, 22, 28, 29, 47–49), in the stromal compartment in human and rodent tissues.

The stromal cell types responsible for the increased COX-2 expression in colorectal tubular adenomas have been a point of controversy. Most studies, including ours, have localized the bulk of stromal COX-2 expression to subepithelial cells on the luminal aspect of adenomatous polyps (13, 14, 21, 22, 28, 29, 47–49). In normal colonic mucosa, cells likely to be located in this area include endothelial cells, macrophages, leukocytes, fibroblasts, and subepithelial myofibroblasts (30). In normal colonic mucosa and in hyperplastic polyps with intact surface epithelial lining, macrophages and endothelial cell-derived COX-2 can be seen (Fig. 3, and Arao et al. (20)). However, in the adenomatous polyps, we observed significant COX-2 expression in subepithelial myofibroblasts as well. A number of previous studies have concluded that macrophages are the principal source of COX-2 expression in colorectal adenomatous polyps (21, 22, 28). This conclusion was based upon colocalization of the macrophage marker CD68 and COX-2 in step sections and in double labeling experiments. In the histologic sections in those studies as in ours, however, there remained significant numbers of COX-2 expressing cells that do not coexpress CD68. In the present study, with the use of both step-sections and double immunolabeling techniques, we now show that the subepithelial COX-2-positive and CD68-negative cells in adenomatous polyps are αSMA-positive myofibroblasts (Figs. 4). We find no evidence that these COX-2 expressing αSMA-positive stromal cells are different from those in normal colonic mucosa in terms of possible hematopoietic origin or histiocytic phenotype, because they did not stain with macrophage markers (CD68 KP1, CD68 PGM, and Lysozyme). These findings concur with those reported in tumors from IL-10−/−, Min, and azothymehane-treated mice by Shattuck-Brandt et al. (14). Using immunohistochemistry and in situ hybridization performed on tissue step sections, they showed the periluminal COX-2 expressing cells to be myofibroblasts (by virtue of their positive staining with αSMA). In contrast, Sonoshita et al. (29), in their study of human familial adenomatous polyposis and its Apc mutant knockout mouse model, showed the periluminal COX-2 expressing cells in familial polyposis to occur mostly in αSMA-negative fibroblasts. This different observation may be due to differences in the pathologic and molecular changes associated with the different models of colorectal carcinogenesis. The important point, however, is that these two studies, like

Table 3: Immunohistochemical expression of COX-2 in tissue sections

<table>
<thead>
<tr>
<th>Lesions</th>
<th>Epithelium</th>
<th>Endothelium</th>
<th>Macrophages</th>
<th>Myofibroblasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal mucosa (n = 50)</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Hyperplastic polyps (n = 43)</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Tubular adenoma (n = 67)</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>94*</td>
</tr>
<tr>
<td>Invasive cancer (n = 39)</td>
<td>23</td>
<td>100</td>
<td>100</td>
<td>62</td>
</tr>
</tbody>
</table>

* In three polyps from three patients, there was surface erosion and, therefore, COX-2 expression in the subepithelial region could not be assessed in these cases.
ours, localized COX-2 expression to stromal cells (myofibroblasts and fibroblasts) rather than to macrophages. Inflammatory myofibroblasts are thought to be a source of soluble factors (eicosanoids and cytokines) that modulate epithelial growth, differentiation, and repair and that play vital roles in mucosal immunopathology, inflammatory bowel disease, and neoplasia (50, 51). We recently reported (30) diffuse activation (and expansion) of \( \alpha \)-SMA-positive stromal cells in adenomatous colonic mucosa, a finding that suggests these transdifferentiated mesenchymal cells may play critical roles in colonic tumorigenesis. Also, studies of cultures of primary intestinal myofibroblast isolates in our laboratories showed that these cells do express COX-2 when exposed to cytokines such as IL-1 and TNF\( \alpha \) in \textit{in vitro} cultures (33, 35, 52). Presently, we demonstrate that these activated myofibroblasts (stromal cells) also express high levels of COX-2 in the periluminal region of adenomatous mucosa, a feature not seen in normal mucosa or hyperplastic polyps. Although transdifferentiation of fibroblasts to myofibroblasts in the stroma of colorectal adenomas is diffuse, COX-2 expression is limited to a small proportion (<5\%) of the activated noninflammatory \( \alpha \)-SMA-positive stromal cells located in the periluminal region. This focal periluminal expression suggests the presence of local factors that induce stromal COX-2 expression. Because transepithelial permeability is compromised in colorectal polyps (53), it is possible that luminal contents such as bile acids (54) or microflora-derived Toll receptor ligands (55) are the inducing agents. It is likewise possible that increased secretion of transforming growth factor \( \beta \), or another soluble factor from the neoplastic epithelial cells, induces stromal COX-2 expression (46).

There is considerable circumstantial evidence that COX-2 involvement is an early event in colorectal carcinogenesis (56) and probably influences the process by modulating prostaglandin \( \text{E}_2 \)-mediated paracrine effect on epithelial cell proliferation (57–60), apoptosis (61, 62), intercellular adhesion (58, 61), and matrix adhesion (61). In the normal colonic mucosa, proliferation of epithelial cells is most marked at the bases of the crypts. In contrast, proliferation of epithelial cells in adenomas is greatest in the periluminal region, in which COX-2 is also expressed in the subjacent \( \alpha \)-SMA\(^+ \) stromal cells (myofibroblasts; ref. 31). A similar spatial relationship has also been reported between COX-2 expression and angiogenesis, with the highest concentration of microvessels occurring in the vicinity of COX-2-expressing periluminal stromal cells (63). COX-2-dependent prostaglandin \( \text{E}_2 \) has also been shown to up-regulate production of proangiogenic factor vascular endothelial growth factor in adenomas of Apc\(^{\Delta 716} \) mice (64). The chemopreventive activity of NSAIDs, therefore, may result from direct inhibition of the COX-prostaglandin pathway in the subepithelial myofibroblasts with subsequent reduction of its paracrine influence on the epithelial cells and/or vascular endothelial cells. In summary, we localized the specific differential expression of COX-2 in colorectal tubular adenomas to subepithelial myofibroblasts in the periluminal region. In contrast, in intact (nonulcerated) normal colonic mucosa and in hyperplastic polyps, COX-2 expression was expressed only by endothelial cells and macrophages. These results conclusively show subepithelial myofibroblasts to be a specific source of increased COX-2 expression in colorectal adenomas and support the notion that the role of COX-2 begins early in the adenomatous phase of colorectal carcinogenesis. Thus, these findings provide rationale for in-depth additional studies of the role of intestinal stromal cells (subepithelial myofibroblasts) in colorectal tumorigenesis and their importance as target cells in NSAID-based chemoprevention of colorectal adenocarcinoma.

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COX-2 Expression in Colorectal Adenomas


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