Predictive utility of cyclo-oxygenase-2 expression by colon and rectal cancer

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Abstract

BACKGROUND: Cyclo-oxygenase-2 (COX-2), an inducible enzyme expressed in areas of inflammation, is a target of interest for colorectal cancer therapy. Currently, the predictive significance of COX-2 in colorectal cancer remains unclear.

METHODS: Tissue microarrays were constructed using 118 colon cancer and 85 rectal cancer specimens; 44 synchronous metastatic colon cancer and 22 rectal cancer lymph nodes were also evaluated. COX-2 expression was assessed by immunohistochemistry. Univariate analysis was used to determine the predictive significance of clinicopathologic variables. Overall survival, disease-specific survival, and disease-free survival were the main outcomes examined.

RESULTS: COX-2 was found to be expressed in 93\% of colon cancers and 87\% of rectal cancers. Decreased COX-2 expression was related to decreased disease-specific survival ($P = .016$) and decreased disease-free survival ($P = .019$) in the rectal cancer cohort but not in the colon cancer cohort.

CONCLUSIONS: COX-2 expression has predictive utility for management of rectal but not colon cancer.

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In North America, colorectal cancer (CRC) has continued to be a major cause of cancer-related mortality.\textsuperscript{1} Targeted cancer therapeutics are being increasingly investigated as a means of preventing and treating CR.\textsuperscript{2} One of the targets for therapy of CRC that is currently being studied is cyclo-oxygenase-2 (COX-2). COX-2 is an inducible enzyme that catalyzes prostaglandin synthesis from arachidonic acid.\textsuperscript{3,4} This leads to the production of prostacyclin and thromboxane that are involved in the regulation of tissue homeostasis.

Unlike COX-1, which is expressed by most cells of the body, expression of COX-2 is induced in areas of inflammation.\textsuperscript{3} In epidemiologic studies, the COX inhibitor, aspirin, was originally found to decrease the risk of the development of colorectal polyps and CRC.\textsuperscript{3,5-7} Furthermore, the nonsteroidal anti-inflammatory drug (NSAID), sulindac, has been shown to reduce the number and the size of colorectal polyps in individuals diagnosed with familial adenomatous polyposis.\textsuperscript{5,9}
has led to the hypothesis that COX-2 is involved in CRC tumorigenesis and progression. Indeed, whereas COX-1 expression seems to be the similar when comparing CRC cells and normal colonic mucosa, COX-2 mRNA levels have been found to be higher in 80% of CRC cells and 40% of adenomas, when compared with normal colonic mucosa. COX-2 seems to play an important role in multiple CRC cellular functions including apoptosis, cell invasiveness, and angiogenesis.

Several studies have reported a relationship between increased COX-2 mRNA expression with larger cancer size and greater depth of cancer invasion. This has led to interest in COX-2 as a potential molecular predictor for CRC. However, currently, the predictive significance of COX-2 expression by CRC is controversial. A recent study evaluating COX-2 expression using immunohistochemistry in 76 CRC specimens has reported a correlation between COX-2 expression and reduced patient survival. Other studies were unable to identify a significant correlation between CRC patient outcomes and COX-2 expression. The objective of the present study is to evaluate COX-2 as a molecular predictor for colon and rectal cancers.

Methods

Between 1997 and 2005, there were 224 CRCs identified from a prospectively maintained database. All study subjects had undergone surgery for colon or rectal cancer, and the medical records of these individuals were retrospectively reviewed. Tissue microarray (TMA) analysis of colon and rectal cancer specimens was carried out in a manner that has been previously described. The TMAs included synchronous lymph node metastases from a subset of individuals that had regional disease. This study was approved by the Research Ethics Board of our institution. Cancers 15 cm or higher above the anus were considered colon cancer, and cancers below this level were considered rectal cancers. All individuals who underwent surgery and had adequate available archival tissue specimens for TMA construction were included in the study population. Individuals who presented with a second colonic primary cancer, or a recurrence from a previously resected colon or rectal cancer, were excluded. For survival analyses, patients who died perioperatively (defined as mortality occurring within 30 days of surgery) also were excluded from the study population. The mean follow-up time for the study population was 4 years.

All colon and rectal cancer specimens underwent histologic review by a pathologist. The TMAs were cut into 4-μm sections using a Leica microtome (Leica Microsystems Inc., Richmond Hill, Ontario, Canada), and the sections then were transferred to adhesive-coated slides for immunohistochemical staining. Immunohistochemistry studies employed the rabbit monoclonal anti-COX-2 antibody, SP21 (Lab Vision/Thermo Fisher Scientific, Kalama Zoo, MI), supplied as supernatant, and diluted to 1:100. Antigen retrieval was performed before antibody incubation in citrate buffer, pH 6, for 8 minutes in a pressure cooker. Localization was via the Envision+ avidin-biotin-based detection system (Dako Corporation, Carpinteria, CA). COX-2 expression was scored by a pathologist, blinded to all clinical data, using a scoring system based on the proportion of cancer cells expressing cytoplasmic COX-2 (Fig. 1). COX-2 expression in 76% or greater of cells was assigned a score of 3 (uniformly positive). If COX-2 was expressed by 26% to 75% of cells, a score of 2 was assigned (variably positive). A score of 1 (focally positive) was assigned if COX-2 was expressed in 5% to 25% of cells. Finally, a score of 0 was assigned for either the absence of staining or COX-2 expression by less than 5% of cancer cells. Scoring system COX-2 expression levels were given binary designations in which COX-2 staining was regarded as positive when more than 5% of the cells examined showed cytoplasmic staining. Epithelial cell COX-2 expression was evaluated in this study; stromal COX-2 expression was not assessed.

The significance of correlations with clinicopathologic predictive variables was determined by univariate analysis using a stepwise logistic regression analysis. Overall survival, disease-specific survival, and disease-free survival were the survival outcomes examined. Disease-free survival included stage IV patients if they underwent successful resection of all metastatic disease. Association between receptor expression and predictive variables was determined by contingency table statistics (for categorical variables) and the Mann–Whitney U test (for continuous variables). Correlational and co-expression analyses were determined by the Spearman correlation. Differential expression between paired primaries and lymph nodes were determined using the chi-square test to compare the expression scores. All tests were 2 tailed and considered significant at a P value less than .05. All statistics were performed using the SPSS statistical software package (version 13.0; SPSS Inc, Chicago, IL).

Results

The study cohort was composed of 118 (58%) colon cancer patients and 85 (42%) rectal cancer patients. There were 44 and 22 patients who had synchronous lymph node metastases available for evaluation from the colon cancer cohort and the rectal cancer cohort, respectively. There was no differential expression of COX-2 when comparing the primary tumors and their associated lymph node metastases (colon or rectal cancer). COX-2 was found to be expressed in 184 (91%) of all colon and rectal cancer specimens. Study patient characteristics are summarized in Table 1, and the correlation between COX-2 expression and clinical predictors is summarized in Table 2. Decreased COX-2 expression was related to decreased disease-specific survival (P = .016) and decreased disease-free survival (P = .019) in the rectal cancer patient cohort. The Kaplan–Meier survival curve is shown in Fig. 2. There was no significant correlation found between COX-2 expression and any of the other clinical or pathologic parameters evaluated (Table 2). Specifically, no
significant correlation was found between the COX-2 expression in the rectal cancer cohort and a history of preoperative radiation. In the colon cancer cohort, none of the patients with stage 1 disease received postoperative chemotherapy, whereas 10% (5) of stage 2% and 65% (28) of stage 3 patients did receive postoperative chemotherapy. For the rectal cancer cohort, 14% (4) of stage 1, 17% (5) of stage 2%, and 69% (20) of stage 3 patients received postoperative chemotherapy; 16% of the rectal cancer patients had received postoperative radiotherapy.

Comments

The observations made in the present study suggest that decreased COX-2 expression is directly related to decreased disease-free survival in rectal but not colon cancer patients. Early studies reporting on COX-2 expression in a smaller cohort of CRC tissue specimens (n = 76) reported by Sheehan et al\textsuperscript{12} found a correlation between COX-2 staining and decreased survival. Of the 76 tissue specimens evaluated in their study, 46% were rectal cancers and 54% were colon cancers. They reported a 5-year survival decrease from 92% to 41% in tumor specimens that expressed COX-2 ($P = .004$) with a mean follow-up of 2.7 years. Zhang and Sun\textsuperscript{14} studied 112 CRC patients (76 colon cancer cases and 30 rectal cancer cases, with the remaining 6 specimens being from unknown colorectal sites) and found that although increased expression of COX-2 was related to higher cancer stage, there was no significant difference found when survival was compared between groups that had lower compared with higher COX-2 expression. Our study cohort showed no significant difference in cancer stage when comparing the cancers that did or did not express COX-2.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Colon cancer</th>
<th>Rectal cancer</th>
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<tbody>
<tr>
<td>Age (y)</td>
<td>66.14 ± 13.68</td>
<td>62.92 ± 12.33</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>52 (44%):66 (56%)</td>
<td>48 (56%):37 (44%)</td>
</tr>
<tr>
<td>Margin status (negative:positive)</td>
<td>114 (97%):3 (3%) (1 unavailable)</td>
<td>73 (88%):10 (12%) (2 unavailable)</td>
</tr>
<tr>
<td>Lymph node invasion (positive:negative)</td>
<td>30 (25%):88 (75%)</td>
<td>7 (10%):65 (90%) (13 unavailable)</td>
</tr>
<tr>
<td>Vascular invasion (positive:negative)</td>
<td>23 (19%):95 (81%)</td>
<td>10 (13%):66 (87%) (9 unavailable)</td>
</tr>
<tr>
<td>AJCC stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>19</td>
<td>33</td>
</tr>
<tr>
<td>II</td>
<td>48</td>
<td>24</td>
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<td>III</td>
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\textit{AJCC = American Joint Commission on Cancer}
We also found a significant difference with respect to survival when comparing the 2 groups, with COX-2 expression being associated with improved survival. Although our observations are different from those reported by Sheehan et al and Zhang et al, these observations are more consistent with recent studies.

Wu et al evaluated 139 CRCs (54 colon and 85 rectal cancers) and concluded that no correlation could be found between COX-2 expression and American Joint Committee on Cancer stage, depth of invasion, or the presence of lymph node metastases. The 118 (85%) patients whose cancers expressed COX-2 did have improved survival at 10 years of follow-up, compared with those patients whose cancers did not express COX-2, but this observation did not reach statistical significance. Furthermore, a study by Kim et al from Korea did not report a significant difference between the presence or absence of COX-2 expression by CRC cells (specific numbers of colon and rectal cancer cases not specified in the study) with respect to disease stage and the presence of lymphovascular invasion by the cancer. However, significant differences were difficult to identify in their analysis as they only had 10 (6%) patients who did not express COX-2. Finally, Chen et al reported on COX-2 expression by 85 colon cancer specimens and did not find survival differences. However, their study did find a higher level of COX-2 expression in patients with lower disease stage. They also found that staining for COX-2 correlated with fewer distant metastases. Chen et al postulated that the expression of COX-2 may play a role in early colon cancer tumorigenesis. Wasilewicz et al found that high COX-2 expression by colonic polyps was significantly associated with risk factors for malignant transformation that included larger polyp size and the presence of high-grade dysplasia. Thus, their observations suggest involvement of COX-2 in the early stages of CRC tumorigenesis and progression.

Treating cancers of the colon and rectum as distinct entities has important implications for individualizing therapy based on expression of molecular markers such as COX-2. The proximal colon differs from the distal colon and rectum in its embryologic origin, blood supply, and innervation. The proximal colon arises from the foregut, is supplied by branches of the superior mesenteric artery, and is innervated by the vagus nerve. The distal colon arises from the hindgut, is supplied by tributaries of the inferior mesenteric artery, and is innervated by fibers from S2 to S4. Frattini et al observed that K-ras mutations were more common in colon cancers, whereas a mutational pattern restricted to the APC gene was more common in rectal cancers. Microsatellite instability, which is a mechanism that underlies cancer development in the proximal colon, is rarely seen in rectal cancers. Einspahr et al reported that COX-2 mRNA expression was significantly higher in distal (including rectum and splenic flexure) adenomas than more proximal adenomas. Dimberg et al studied COX-2 protein levels using western blot analysis and found overexpression of COX-2 protein in rectal cancers compared with cancers located in other parts of the colon. It should, however, be noted that the comprehensive molecular characterization of colon and rectal cancer carried out by the Cancer Genome Atlas Network found no genetic differences between these 2 cancers. Thus, the earlier mentioned variations between colon and rectal cancers may reflect differences in the treatment regimens used in the management of these disease entities.

Because of its role in CRC tumorigenesis, COX-2 represents a potential target for anticancer drugs for the
treatment of this disease. Along with large epidemiologic studies that have demonstrated a reduction of CRC risk when taking aspirin, randomized trials have also shown a 30% reduction in adenomas in a group of 81 familial adenomatous polyposis patients when taking celecoxib.5,25,26 Clinical trials evaluating adenoma prevention using rofecoxib and celecoxib were terminated early, ending any attempt at CRC chemoprevention, when the adverse cardiovascular effects of these drugs were identified.25 There is little data regarding NSAID use for adjuvant therapy of CRC. Rofecoxib was used in addition to adjuvant chemotherapy for treatment of metastatic colon cancer, but this study was also terminated early because of a lack of improvement and the gastrointestinal toxicity that was observed in the first 10 patients entered in this study.27

The small population size is a limitation of our study, and we believe further investigation with larger cohorts is warranted in the future. Given the CRC risk reduction conferred by NSAIDs, a potential confounder of our observations is the variable utilization of aspirin and other NSAIDs by the study population before the development of their colon or rectal cancer. The design of the present study also does not address the relationship between COX-2 expression and radiosensitivity. To answer this question, a large prospective study would need to be developed in which rectal cancer patients, randomized to either receive or not receive preoperative radiation therapy, would have their COX-2 status evaluated and outcomes followed. Another limitation of our study is the short duration of patient clinical follow-up (mean follow-up was 4 years).

The present study represents the largest group of colon cancer and rectal cancer patients in whom COX-2 expression by their tumors has been reported. Our observations suggest that there is a difference in the molecular mechanisms that underlie tumorigenesis and progression for colon and rectal cancers with respect to the role of COX-2. The differences we observed in COX-2 expression, between the colon and rectal cancer study cohorts, suggests that further study of COX-2, especially for rectal cancer, is warranted and ultimately may lead to improved outcomes for individuals diagnosed with this common human malignancy.

References