The American Journal of Surgery (2014) 207, 712-716

The American Journal of Surgery®

North Pacific Surgical Association

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

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KEYWORDS:
Cyclo-oxygenase-2;
Colon cancer;
Rectal cancer;
Colorectal cancer;
Immunohistochemistry

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.

In North America, colorectal cancer (CRC) has continued to be a major cause of cancer-related mortality.[1] Targeted cancer therapeutics are being increasingly investigated as a means of preventing and treating CR.[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.[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.[3] In epidemiologic studies, the COX inhibitor, aspirin, was originally found to decrease the risk of the development of colorectal polyps and CRC.[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.[8,9] This

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Manuscript received October 28, 2013; revised manuscript December 16, 2013

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http://dx.doi.org/10.1016/j.amjsurg.2013.12.019

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.[10] COX-2 seems to play an important role in multiple CRC cellular functions including apoptosis, cell invasiveness, and angiogenesis.[3]

Several studies have reported a relationship between increased COX-2 mRNA expression with larger cancer size and greater depth of cancer invasion.[11] 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.[12] 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.[13] 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 TMA blocks 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, Kalamazoo, 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