Cyclooxygenase-2 Expression is Associated with Increased Size in Human Sporadic Colorectal Adenomas

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Abstract. Background: Cyclooxygenase-2 (COX-2) has been implicated in colorectal carcinogenesis but its role is not completely defined. Materials and Methods: The expression of COX-2 was evaluated in 68 paraffin-embedded sporadic colorectal adenomas by immunohistochemistry. Associations between COX-2 expression and the clinicopathological characteristics of the adenomas were studied by contingency tables and the Chi-square test. Results: Cytoplasmic staining for COX-2 protein was present in epithelial cells of 62 out of the 68 adenomas. COX-2 expression was not associated with age or gender. Furthermore, no significant correlations were found between the expression of the protein and histology (tubular vs tubulovillous), localization (proximal vs distal) or morphology (sessil vs pedunculated) of the adenomas. Both stromal and epithelial COX-2 expressions were higher in larger (>4 mm) compared with smaller (≤4 mm) adenomas (p=0.037 and p=0.024). Conclusion: These data support the hypothesis that the expression of COX-2 may occur as a general phenomenon in colorectal adenomas. A size-dependent increase of COX-2 expression might be involved in colorectal carcinogenesis.

Colorectal cancer (CRC) is the second leading cause of cancer mortality in the United States (1). A multistep process involving many different oncogenes has been associated with the progression of colorectal dysplasia from normal mucosa up to the occurrence of carcinoma: APC, DCC, p53, ras, etc. (2-7). However, the events involved in CRC carcinogenesis require further studies, with much effort oriented to the understanding of the molecular pathways involved in this disease.

In recent years, increasing evidence has been reported on the role of cyclooxygenases (COX) in promoting colorectal carcinogenesis. Several epidemiological studies have demonstrated a decreased risk of CRC in people taking aspirin or other non-steroidal anti-inflammatory drugs (NSAIDs) (8, 9), and many researchers have shown that NSAIDs can inhibit chemically-induced carcinogenesis in rodents, as well as cause regression of human colorectal adenomas (10-14). The putative mechanism by which NSAIDs prevent colorectal carcinogenesis involves the inhibition of COX, the enzyme which metabolizes arachidonic acid to eicosanoids, including prostaglandins (PG) (15,16). Two forms of COX have been identified: COX-1 and COX-2. While COX-1 is constitutively expressed in most cells and tissues, COX-2 is induced by a variety of agents including cytokines, hormones, growth factors and inflammation mediators (17-22).

Furthermore, COX-2 is elevated in colorectal adenomas (CRAs) and CRC as compared with normal mucosa and promotes tumor cell growth, angiogenesis, tumor invasion and metastasis (17, 19, 23, 24). Interestingly, the lack of COX-2 in mice results in decreased tumor growth of colorectal tumors (25).

In this study, we evaluated the expression of COX-2 in paraffin-embedded archival CRAs and determined the correlations between the expression of COX-2 and the characteristics of CRAs.

Materials and Methods

Formalin-fixed, paraffin-embedded 5-μm tissue sections of 68 sporadic CRAs, collected between March 2002 and April 2003, were immunostained using the biotin-streptavidin-peroxidase method (YLEM). Sections were subjected to routine deparaffinization and rehydratation. The slides were then immersed in 10 mM sodium...
citrate buffer (pH 6.0), boiled for 10 min on a hot plate, and then allowed to cool for 20 min. The sections were then incubated with biotin-labelled secondary antibody (1:30) and streptavidin-peroxidase (1:30) for 20 min each. The slides were stained for 5 min with freshly prepared 0.05% 3,3'-diaminobenzidine tetrahydrochloride and then counterstained with hematoxylin, dehydrated and mounted in Diatex.

The specimens immunostained for COX-2 were evaluated independently by two investigators (FT and MDB). Negative controls were obtained by omitting the primary antibody. All controls gave satisfactory results. Staining was categorized into four semi-quantitative classes (-, +, ++, +++), based on the amount of stained tumor cells (0%, 0-<10%, 10-<50%, 50-100%, respectively). Negative or equivocable results were repeated two times. Discordant cases were discussed and concordance was then achieved. The primary antibody was also tested on 22 colorectal carcinomas.

Data on patient (age, sex) and baseline adenomas characteristics (size, location, histology, degree of dysplasia) were obtained from the endoscopy and pathology reports of the NCI and reviewed by the study pathologists (FT, MDM, GB). The size of the adenomas was assessed by the pathologists. The cut-off size (≤4 mm and >4 mm) was retrospectively derived. Statistical analyses were performed by contingency tables and χ²-test.

Results

Sixty-eight sporadic CRAs were analyzed. The median age of the patients was 66 (range: 32-87); 43 (63%) patients were males, 25 (37%) females. Neither age nor sex were associated with the expression of COX-2. No pure villous adenomas were observed. Figure 1 shows some examples of COX-2 staining.

There were no significant associations between COX-2 expression and localization (proximal vs distal) and morphology (sessil vs pedunculated) of the adenomas. A slight trend was observed towards an increase of epithelial COX-2 expression in tubulovillous adenomas, although it did not reach statistical significance (p = 0.067) (Table I).

Both stromal and epithelial COX-2 expression were significantly higher in larger adenomas (>4 mm) compared to smaller adenomas (≤ 4 mm) (p = 0.024 and p = 0.037, respectively) (Table I).

Discussion

There is evidence that CRC can progress from normal tissue to carcinoma through CRA, which accumulates multiple genetic alterations. Thus, CRA is a very interesting model to study the progression of the CRC transformation (2, 26). We studied, by immunohistochemistry, the expression of COX-2 in epithelial and stromal cells of CRAs with different clinicopathological characteristics and we found that COX-2 is overexpressed in large CRAs.

In recent years, a few studies have already been reported dealing with the expression of COX-2 in CRAs, though only two included a number of specimens greater than the present study (27-29). However, a major
limitation of these studies was that the results were limited to mRNA expression and did not assess the active protein. Only two studies (30, 31), with many fewer specimens that the present one, detected the expression of the COX-2 protein by immunohistochemistry in CRAs. One of these studies investigated the expression of COX-2 in 35 adenomas and showed that, with an increase in the size of the adenoma, there was an increase in the expression of COX-2 protein (31). In addition, many studies found adenoma size to be related to mRNA COX-2 expression (29, 32, 33). A relationship between adenoma localization and COX-2 expression has been described by some authors (32-34). In contrast, other studies have reported that neither localization (32, 34) nor size (28) are associated with COX-2 expression.

In the present study, we showed that both epithelial and stromal COX-2 expression are associated with adenoma size. This data may indicate that the alteration of COX-2 expression occurs as a general phenomenon. It could be a sort of “environmental shift” within the adenoma mucosa that confers a selective growth advantage for adenomas expressing higher levels of COX-2. In addition, a trend of COX-2 overexpression was observed in tubulovillous adenomas compared with the tubular ones, but it did not reach statistical significance.

A large body of literature supports the promise that COX-2 inhibitors might be useful in the prevention of intestinal polyposis and colon cancer. Our data support the hypothesis that the inhibition of COX-2, in particular within large adenomas, might play a critical role in blocking colorectal carcinogenesis.

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### References


