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Expression of cyclooxygenase-2 in colorectal cancer and its clinical significance

Bin Xiong, Tao-Jiao Sun, Wei-Dong Hu, Fu-Lin Cheng, Min Mao, Yun-Feng Zhou

Bin Xiong, Tao-Jiao Sun, Wei-Dong Hu, Fu-Lin Cheng, Min Mao, Yun-Feng Zhou, Department of Oncology, Affiliated Zhongnan Hospital of Wuhan University, Wuhan 430071, Hubei Province, China
Supported by Hubei Province Natural Science Foundation, No. 2000J054
Correspondence to: Dr. Yun-Feng Zhou, Affiliated Zhongnan Hospital of Wuhan University, Wuhan 430071, Hubei Province, China. binxiong88@yahoo.com
Telephone: +86-27-67813358
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Abstract

AIM: To clarify the clinicopathologic significance of COX-2 expression in human colorectal cancer.

METHODS: A total of 128 surgically resected colorectal cancer specimens were immunohistochemically analyzed with the use of anti-COX-2, anti-VEGF and anti-MMP-2 antibodies. The relationship between the cyclooxygenase-2 expression in primary lesions of colorectal cancer and clinicopathologic parameters was evaluated by chi-square test.

RESULTS: Among 128 cases of colorectal cancer, 87 (67.9%) were positive for cyclooxygenase-2. The expression of cyclooxygenase-2 was significantly correlated with the depth of invasion, stage of disease, and metastasis (lymph node and liver). Patients in T3-T4, stages III-IV and with metastasis had much higher expression of cyclooxygenase-2 than ones in T1-T2, stages I-II and without metastasis ($P<0.05$). Among 45 cases of colorectal cancer with lymph node metastasis, the COX-2-positive rate was 86.7% (39/45) for primary lesions and diffuse cytoplasmic staining for COX-2 protein was detected in cancer cells in 100% of metastatic lesions of the lymph nodes. VEGF expression was detected in 49 tumors (38.3%), and VEGF expression was closely correlated with COX-2 expression. The positive expression rate of VEGF (81.6%) in the cyclooxygenase-2-positive group was higher than that in the cyclooxygenase-2-negative group (18.4%, $P<0.05$). MMP-2 expression was detected in 88 tumors (68.8%), and MMP-2 expression was closely correlated with COX-2 expression. The positive expression rate of MMP-2 (79.6%) in the positive COX-2 group was higher than that in the negative COX-2 group (20.4%, $P<0.05$).

CONCLUSION: Cyclooxygenase-2 may be associated with tumor progression by modulating the angiogenesis and cancer cell motility and invasive potential in colorectal cancer and it can be used as a possible biomarker.

Key words: Cyclooxygenase-2; Colorectal cancer; Immunohistochemical

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INTRODUCTION

Cancer has been described as a disease of aberrant signal transduction. Carcinogenesis is a multistep process characterized by progressive changes in the amounts or activity of proteins that regulate cellular proliferation, differentiation, and survival. These changes can be mediated through both genetic and epigenetic mechanisms. Cyclooxygenase (COX) is a rate-limiting enzyme in prostaglandin biosynthesis[1]. Evidence suggests that nonsteroidal anti-inflammatory drugs reduce the risk of colorectal cancer and that this effect is mediated through COX inhibition[2-4]. Two COX isoforms, COX-1 and COX-2, have been identified. COX-1 is constitutively expressed and involved in general cell functions, whereas COX-2 is an inducible enzyme that is up-regulated in response to various stimuli, including growth factors and mitogens[5-8]. An enhanced expression of COX-2 has been found in many tumors, such as the lung, breast, esophageal, and colon cancers[2-4,9-11]. Recent studies have demonstrated that COX-2 could affect carcinogenesis via several different mechanisms[1,12-15]. Overexpression of COX-2 leads to phenotypic changes involving increased adhesion to the extracellular matrix and inhibition of apoptosis in rat intestinal epithelial cell, which could enhance their tumorigenic potential[3,8,10,15-18]. Constitutive expression of COX-2 can also lead to alterations in the invasive potential of colorectal cancer cells, and COX-2 may be involved in tumor angiogenesis[1,11,13,17-20]. COX-2 may be related to the development of colorectal cancer, but the precise role of COX-2 in colorectal cancer is not yet fully known.

In this study we compared COX-2 expression in primary and metastatic lesions by immunohistochemical staining in a group of colorectal cancer patients. Our objective was to

determine the clinical significance of COX-2 in advance of colorectal cancer.

MATERIALS AND METHODS

Patients

A total of 128 cases of colorectal adenocarcinoma that had undergone surgical resection were collected in the Affiliated Zhongnan Hospital of Wuhan University (Wuhan, China) from January 1999 to September 2002, and COX-2, VEGF and MMP-2 immunohistochemical staining were performed. There were 73 men and 55 women, and their age ranged from 23 to 74 years (mean, 56±11 years). Among 128 patients, 26 were well-differentiated adenocarcinoma, 57 moderately differentiated adenocarcinoma and 45 poorly differentiated adenocarcinoma. According to Dukes' staging criteria, 37 cases were stage I, 41 stage II, 39 stage III and 11 stage IV.

Methods

Immunohistochemical staining All the tissue specimens were fixed in 100 mol/L neutral formalin and embedded in paraffin. Five-μm thick sections were dewaxed in xylene and dehydrated in ethanol. Tissue sections were washed three times in 0.05 mol/L PBS, and incubated in endogenous peroxidase blocking solution. Non-specific antibody binding was blocked by pretreatment with PBS containing 5 g/L bovine serum albumin. Sections were then rinsed in PBS and incubated overnight at 4 ℃ with diluted anti-COX-2 (Santa Cruz), anti-VEGF (Bosden, Wuhan, China) and anti-MMP-2 (Santa Cruz) antibodies. The steps were performed using S-P detection kit (Maxin, Fuzhou, China) according to the manufacturer's instructions. PBS was used as substitutes of antibody for negative control. The sections were examined under light microscope.

Evaluation of the staining Evaluation for COX-2 was performed according to the following scoring system[1]: staining intensity was graded as weak (1), moderate (2), or strong (3), and area of staining positivity as <10% (0) of all cells stained in the cytoplasm, 10-40% (1), 40-70% (2), or ⩾70% (3). Total scores for grade and area of three or more was defined as positive expression and less than three as negative. Positive signal for VEGF and MMP-2 was located in the cytoplasm or/and cell membrane[11,21-24]. Immunoreactivity was graded as follows: +, ⩾10% stained tumor cells; and −, <10% stained tumor cells[18,25-28].

Statistical analysis

The difference between each group was analyzed by χ^2 test. $P<0.05$ was considered significant.

RESULTS

COX-2 expression in colorectal cancer and clinicopathologic findings

COX-2 was expressed in the cytoplasm of cancer cells (Figure 1A) and the expression in primary tumor was noted in 67.9% (87/128). The correlation between COX-2 expression and the clinicopathologic findings is shown in Table 1. The expression of COX-2 was significantly correlated with depth of invasion, stage of disease and metastasis (lymph node and liver). Patients in T3-T4, stages III-IV and metastasis had much higher COX-2 expression than ones in T1-T2, stages I-II and without metastasis ($P<0.05$). The expression of COX-2 was not correlated with age, gender and differentiation degree of the tumor ($P>0.05$).

Table 1 Clinicopathologic characteristics of colorectal cancer with expression of COX-2

Variable	*n*	COX-2 positive *n* (%)	COX-2 negative *n* (%)
Sex			
Male	73	50 (68.5)	23 (31.5)
Female	55	37 (67.3)	18 (32.7)
Age (yr)		54±12	56±15
Histological differentiation			
Well	26	17 (65.4)	9 (34.6)
Moderate	57	40 (70.2)	17 (29.8)
Poor	45	30 (66.7)	15 (33.3)
Depth of invasion			
T1-T2	81	48 (59.3)	33 (40.7)
T3-T4	47	39 (83.0)	8 (17.0)[a]
Metastasis			
Present	50	42 (84.0)	8 (16.0)
Absent	78	45 (57.7)	33 (42.3)[a]
Dukes stage			
I	37	15 (40.5)	22 (59.5)
II	41	28 (68.3)	13 (31.7)
III+IV	50	44 (88.0)	6 (12.0)[a]
VEGF expression			
Positive	49	40 (81.6)	9 (18.4)
Negative	79	47 (59.5)	32 (40.5)[a]
MMP-2 expression			
Positive	88	70 (79.6)	18 (20.4)
Negative	40	17 (42.5)	23 (57.5)[a]

[a]$P<0.05$ *vs* positive.

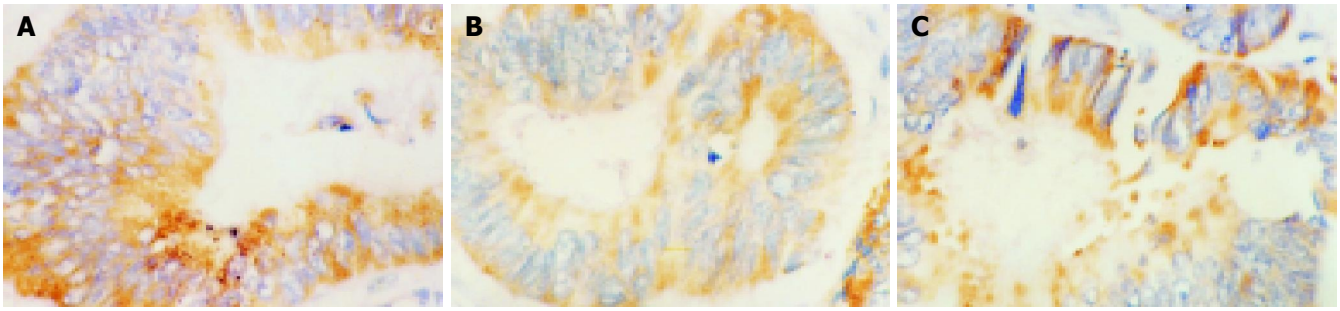

Figure 1 Expression of COX-2, VEGF and MMP-2 in well-differentiated colon adenocarcinoma. A: The staining of COX-2 is mainly in the cytoplasm of tumor cells. S-P, ×400; B: The staining of VEGF is mainly in the cytoplasm and membrane of tumor cells. S-P, ×400; C: The staining of MMP-2 is mainly in cytoplasm and membrane of tumor cells. S-P, ×400.

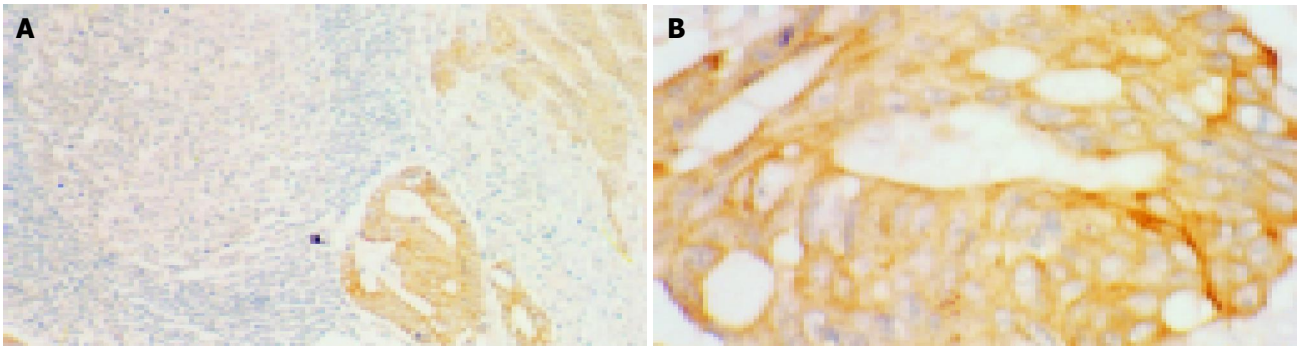

Figure 2 COX-2 expression in lymph node metastatic lesions. A: The staining is mainly in cytoplasm of tumor cells. S-P, ×200; B: The staining is mainly in cytoplasm of tumor cells. S-P, ×400.

Relationship between COX-2, VEGF and MMP-2 expression

VEGF was mainly localized in the cytoplasm and cell membrane of the tumor cells (Figure 1B). VEGF expression was detected in 49 tumors (38.3%), and VEGF expression was closely correlated with COX-2 expression (Table 1). The positive expression rate of VEGF (81.6%) in the positive COX-2 group was higher than that in the negative COX-2 group (18.4%, $P<0.05$).

MMP-2 was mainly localized in the cytoplasm and cell membrane of the tumor cells (Figure 1C). MMP-2 expression was detected in 88 tumors (68.8%), and MMP-2 expression was closely correlated with COX-2 expression (Table 1). The positive expression rate of MMP-2 (79.6%) in the positive COX-2 group was higher than that in the negative COX-2 group (20.4%, $P<0.05$).

Relationship of COX-2 expression between primary and lymph node metastatic lesions

Among 45 cases of colorectal cancer with lymph node metastasis, the COX-2 positive rate was 86.7% (39/45) for primary lesions and 100% for metastatic lesions in the lymph nodes. All cases with no staining in the primary lesion showed COX-2 staining in the metastatic lesion in the lymph nodes (Figures 2A, B).

DISCUSSION

Epidemiological and experimental studies have demonstrated the effect of non-steroidal anti-inflammatory drugs in the prevention of human cancers[1,5-7,16-18]. These drugs block endogenous prostaglandin synthesis through inhibition of COX enzymatic activity[17,20,23-26]. COX-2 is an inducible enzyme that catalyzes the conversion of arachidonic acid to biologically active prostanoids. COX-2 modulates the growth and function of many cells, including those with malignant transformation. The over-expression of COX-2 has been reported in tissues from patients with different carcinoma, and is believed to play a role in tumor transformation and progression, as well as in tumor regression[1,4-7,18,29-32]. Recent experimental studies showed that COX-2 inhibits cell apoptosis, regulates angiogenesis, and is associated with matrix metalloproteinases (MMP)[16,26,33-35].

COX-2 was over-expressed in approximate 80% of colorectal cancer cases[1,18,30,36], and may be related to the development of colorectal cancer. However, the precise role of COX-2 in colorectal cancer is not yet fully known. Yamauchi *et al*[1] reported that COX-2 expression correlated significantly with histologic type, depth of invasion, pathologic stage, and metachronous liver metastasis of colorectal cancer. Multivariate analysis for factors associated with metachronous liver metastasis of colorectal cancer showed that COX-2 expression was one of the independent risk factors, second only to lymph node metastasis[1]. COX-2 expression in the primary lesion may be a useful marker for evaluating prognosis and liver metastasis in patients with colorectal cancer[1]. In our study, COX-2 expression was detected in 87 tumors (67.9%). The expression of COX-2 was significantly correlated with the depth of invasion, stage of disease and metastasis (lymph node and liver). Patients in T3-T4, stages III-IV and with metastasis had much higher expression of COX-2 than ones in T1-T2, stages I-II and without metastasis ($P<0.05$). Among 45 cases of colorectal cancer with lymph node metastasis, the COX-2-positive rate was 86.7% (39/45) for primary lesions and diffuse cytoplasmic staining for COX-2 protein was detected in cancer cells in 100% of metastatic lesions in the lymph nodes. The preferential expression of COX-2 in lymph node metastases suggests a clonal selection of tumor cells with COX-2 expression, specific for the higher potential of lymph node metastasis in tumor advance, and COX-2 plays a role related to the malignant progression of colorectal cancer.

Masferrer *et al*[31] reported that COX-2 stimulates endothelial cell migration and vessel tube formation, which are inhibited by NSAIDs. They also reported that COX-2 affects MMP-2 and activated collagenase levels. This study found that the expression of VEGF and MMP-2 in COX-2-positive group is significantly higher than that in COX-2-negative group. The expression of COX-2 is significantly correlated with the expression of VEGF. It demonstrated that COX-2 might be indirectly correlated with angiogenesis through an up-regulation of the expression of VEGF. The expression of COX-2 is also significantly correlated with MMP-2 in colorectal cancer. It indicates that COX-2 can also lead to alterations in the invasive potential of colorectal cancer cells through an up-regulation of the expression of MMP-2. It suggests that COX-2 is closely related to the invasion and metastasis of colorectal cancer and it may be used as a possible biomarker.

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