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• COLORECTAL CANCER •

Cyclooxygenase-2 expression and angiogenesis in colorectal cancer

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Abstract

AIM: Cyclooxygenase-2 is involved in a variety of important cellular functions, including cell growth and differentiation, cancer cell motility and invasion, angiogenesis and immune function. However, the role of cyclooxygenase-2 as an angiogenic factor in colorectal cancer tissue is still unclear. We investigated the relationship between cyclooxygenase-2 and angiogenesis by analyzing the expression of cyclooxygenase-2 in colorectal cancer tissue, as well as its association with vascular endothelial growth factor (VEGF) and microvascular density (MVD).

METHODS: The expression of cyclooxygenase-2, VEGF, as well as MVD was detected in 128 cases of colorectal cancer by immunohistochemical staining. The relationship between the cyclooxygenase-2 and VEGF expression and MVD was evaluated. Our objective was to determine the effect of cyclooxygenase-2 on the angiogenesis of colorectal cancer tissue.

RESULTS: Among 128 cases of colorectal cancer, 87 were positive for cyclooxygenase-2 (67.9 %), and 49 for VEGF (38.3 %), respectively. The microvessel counts ranged from 23 to 142, with a mean of 51.7 (standard deviation, 19.8). The expression of cyclooxygenase-2 was correlated significantly with the depth of invasion, stage of disease, metastasis (lymph node and liver), VEGF expression and MVD. Patients in T3-T4, stage III-IV and with metastasis had much higher expression of cyclooxygenase-2 than patients in T1-T2, stage I-II and without metastasis ($P<0.05$). 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 %, $P<0.05$). Also, the microvessel count (56±16) in cyclooxygenase-2 positive group was significantly higher than that in cyclooxygenase-2 negative group (43±12, $P<0.05$). The microvessel count in tumors with positive cyclooxygenase-2 and VEGF was the highest (60±18, 41-142, $P<0.05$), whereas that in tumors with negative cyclooxygenase-2 and VEGF was the lowest (39±16, 23-68, $P<0.05$).

CONCLUSION: Cyclooxygenase-2 may be associated with tumor progression by madulating the angiogenesis in colorectal cancer tissue and used as a possible biomarker.

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INTRODUCTION

Angiogenesis has been postulated to play an important role in the development of malignant tumors[1-5]. Increased vascularity may allow not only an increase in tumor growth but also a greater chance of hematogenous tumor embolization[6]. An association between poor prognosis and increase in microvascular density (MVD) of tumor has been reported in certain tumors[7-10]. This neoangiogenesis depends on the production of angiogenic factors by tumor cells and normal cells[11-15].

Cyclooxygenase (COX) is a key enzyme in prostaglandin biosynthesis[16]. 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[17-22]. An enhanced expression of COX-2 has been found in many tumors, such as the lung, the breast, the esophagus and colon cancer[23-26]. Recent studies have demonstrated that COX-2 could affect carcinogenesis via several different mechanisms[27-35]. COX-2 has been also reported to induce angiogenesis[36-39]. COX-2 may be related to development of colorectal cancer, however, its association with angiogenesis in colorectal cancer tissue still remains unclear. To determine the role of COX-2 expression in angiogenesis of colorectal cancer, we examined the VEGF and MVD in colorectal cancer tissue, and then compared the findings with the expression of COX-2 protein.

MATERIALS AND METHODS

Patients

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

Methods

Immunohistochemistry: All the tissue specimens were fixed in 100 $mL \cdot L^{-1}$ 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 $moL \cdot L^{-1}$ PBS, and incubated in endogenous peroxidase blocking solution. Non-specific antibody binding was blocked by pretreatment with PBS containing 5 $g \cdot L^{-1}$ bovin serum albumin. Sections were then rinsed in PBS and incubated overnight at 4 ℃ with diluted anti-COX-2, anti-VEGF and anti-CD34 antibodies. The steps were performed using immunostain kit according to the manufacturer' s

instructions. PBS was used as substitutes of antibody for negative control. The sections were examined under light microscope. Anti-VEGF polyclonal antibody and anti-CD34 monoclonal antibody were purchased from Bosden Co. (Wuhan, China). Anti-COX-2 polyclonal antibody was purchased from Santa Cruz Co. (USA). S-P detection kit was purchased from Fuzhou Maixin Co. (Fuzhou, China). Anti-COX-2 polyclonal antibody was diluted to 1:75. Anti-VEGF polyclonal antibody and anti-CD34 monoclonal antibody were impromptu type.

Results: Positive evaluation for COX-2 was performed according to the following scoring system[16]: staining intensity was graded as weak (1), moderate (2), or strong (3), and area of staining positivity as <10 percent (0) of all cells stained in the cytoplasm as viewed by microscope, 10 to 40 percent (1), 40 to 70 percent (2), or ⩾70 percent (3). A total for grade and area of 3 or more was defined as positive expression and less than 3 as negative. Positive signal for VEGF was located in the cytoplasm or/and cell membrane[2]. Immunoreactivity was graded as follows: +, ⩾10 percent stained tumor cells; -, <10 percent stained tumor cells[2]. The microvessel counting procedures have been described in the published studies[2]. Briefly, the stained sections were screened at a magnification of ×100 (×10 objective and ×10 ocular lens) under a light microscope to identify 3 regions of the section with the highest microvessel density. Microvessels were counted in these areas at a magnification of ×200, and the average numbers of microvessels were recorded. The average number is known as MVD of the tumor.

Statistical analysis

The difference between each group was analyzed by Chi-square 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 1). COX-2 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, metastasis (lymph node and liver). Patients in T3-T4, stage III-IV, with metastasis had much higher COX-2 expression than patients in T1-T2, stage I-II, without metastasis ($P<0.05$). The expression of COX-2 was not correlated with age, gender and differentiation degree of the tumor ($P>0.05$).

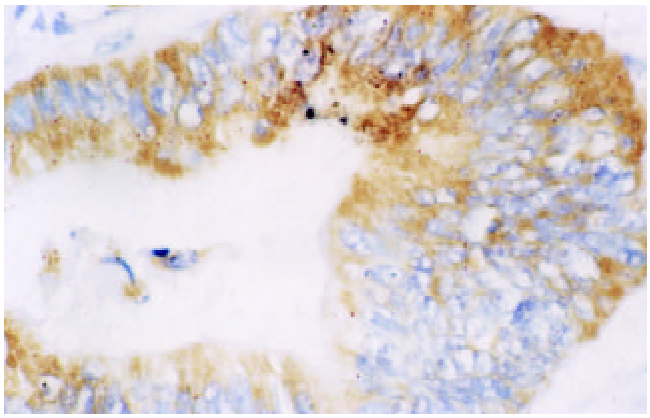

Figure 1 Expression of COX-2 mainly in cytoplasm of tumor cells, S-P, ×400.

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(y)		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]
Duke's stage			
A	37	15(40.5)	22(59.5)
B	41	28(68.3)	13(31.7)
C+D	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]
MVD ($\bar{x}\pm s$)		56±16	43±12[a]

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

Relationship between COX-2 and VEGF expression and MVD

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

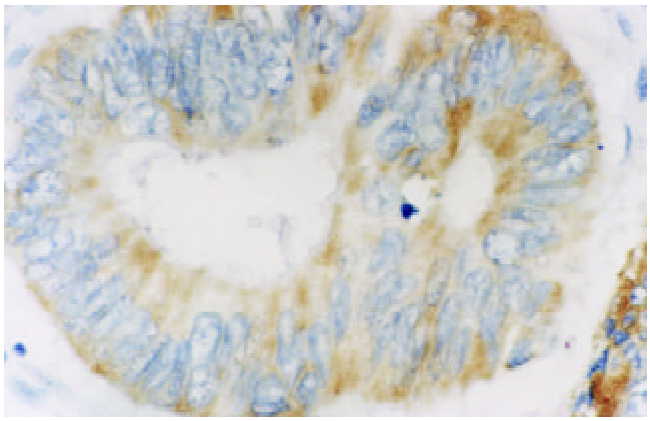

Figure 2 VEGF expression mainly in cytoplasm and membrane of tumor cell, S-P, ×400.

The number of microvessel counts in all cases was 23-142 ($\bar{x}\pm s$, 50±19). Moreover, the microvessel counts were 56±16 in COX-2 positive tumors and 43±12 in COX-2 negative tumors ($P<0.05$, Table 1). COX-2 expression, VEGF expression and MVD were significantly correlated with one another (r=0.5635, 0.2812, 0.5253, respectively. $P<0.05$). The microvessel counts in tumors with both positive COX-2 and VEGF were the highest (60±18, 41-142; $P<0.05$).The

microvessel counts in tumors with both negative COX-2 and VEGF were the lowest (39±16, 23-68; $P<0.05$). The microvessel counts in tumors with positive COX-2 and negative VEGF were 50±16 (29-130), and that in tumors with negative COX-2 and positive VEGF were 51±18 (30-132), lower than that in tumors with both positive COX-2 and VEGF ($P<0.05$). The microvessel counts in tumors with positive COX-2 and negative VEGF were not different from that in tumors with negative COX-2 and positive VEGF ($P>0.05$).

DISCUSSION

Angiogenesis is essential for tumor growth and metastasis. The process of angiogenesis is the outcome of an imbalance between positive and negative angiogenic factors produced by both tumor cells and normal cells. Numerous angiogenic factors have been described. Of these, VEGF plays a key role in the angiogenesis in colorectal cancer[1-15]. VEGF is a multifunctional cytokine, and has direct relationship with angiogenesis. The factors that regulate VEGF expression in tumor and non-tumor cells have now been elucidated[10-28]. COX-2 is an inducible enzyme catalyzing 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 tissue from patients with different carcinoma, and is believed to play a role in tumor transformation and progression, as well as in tumor regression[23-33]. Recent experimental studies showed that COX-2 could inhibit cell apoptosis, regulate angiogenesis, and was associated with matrix metalloproteinases (MMP)[16-48]. Uefuji *et al*[45] studied the correlation of COX-2 and angiogenesis of gastric cancer, and found COX-2 might regulate angiogenesis.

COX-2 was overexpressed in approximatly 80 percent of colorectal cancer cases[16], but the role of COX-2 in angiogenesis of colorectal cancer tissue has not been identified yet. This study found that the expression of VEGF and MVD in positive COX-2 group was significantly higher than that in COX-2 negative group. The expression of COX-2 was significantly correlated with the expression of VEGF. It demonstrated that COX-2 might be correlated indirectly with angiogenesis through an up-regulation of the expression of VEGF. The expression of COX-2 was also significantly correlated with MVD in colorectal cancer. It indicates that COX-2 may modulate angiogenesis directly or indirectly through up-regulating the expression of other angiogenic factors. The microvessel counts in tumors that were both positive COX-2 and VEGF were the highest of all. It suggests that COX-2 and VEGF may co-modulate angiogenesis.

COX-2 expression was detected in 87 tumors (67.9 %). The expression of COX-2 was correlated significantly with the depth of invasion, stage of disease and metastasis (lymph node and liver). Patients in T3-T4, stage C-D and with metastasis had much higher expression of COX-2 than patients in T1-T2, stage A-B and without metastasis ($P<0.05$). It suggests that COX-2 is closely related to the invasion and metastasis of colorectal cancer, and COX-2 may be used as a possible biomarker.

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