

Expression of COX-2 proteins in gastric mucosal lesions

Lian-Zhen Yu, Heng-Jun Gao, Jian-Feng Bai, Gu Sun, Han-Lin Zhao, Liang Sun, Kun Miu, Zhi-Quan Zhao

Lian-Zhen Yu, Heng-Jun Gao, Liang Sun, Kun Miu, Zhi-Quan Zhao, Department of Gastroenterology, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China

Jian-Feng Bai, Gu Sun, Han-Lin Zhao, Department of General Surgery, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China

Supported by the Natural Science Fund of the Educational Committee of Jiangsu Province, No.125FA9608 and Fund of Nanjing Medical University for Outstanding Young Faculty

Correspondence to: Heng-Jun Gao, Department of Gastroenterology, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China

Received: 2003-04-02 **Accepted:** 2003-05-17

Abstract

AIM: To investigate the expression of COX-2 proteins in gastric mucosal lesions and to assess the relationship between COX-2 expression and type, pathologic stage, differentiation, or lymph node metastasis in gastric cancer and the relationship between COX-2 expression and *H pylori* infection in gastric mucosal lesions.

METHODS: Thirty patients with gastric carcinoma underwent surgical resection. Samples were taken from tumor site and paracancerous tissues, and ABC immunohistochemical staining was used to detect the expression of COX-2 proteins. *H pylori* was determined by rapid urea test combined with pathological staining/¹⁴C urea breath test.

RESULTS: The positive rate and staining intensity of mutant COX-2 gene expression in gastric cancer were significantly higher than those in paracancerous tissues (66.7% vs 26.7%) ($P<0.01$, $P<0.001$). There was a significant correlation between COX-2 and pathologic stage or lymph node metastasis type of gastric carcinoma (76.0% vs 20.0%, 79.2% vs 16.7%) ($P<0.05$). No correlation was found between COX-2 expression and type or grade of differentiation ($P>0.05$). COX-2 expression of intestinal metaplasia (IM) or dysplasia (DYS) with positive *H pylori* was significantly higher than that with negative *H pylori* (50.6% vs 18.1%, 60.0% vs 33.3%) ($P<0.05$).

CONCLUSION: COX-2 overexpression was found in a large proportion of gastric cancer tissues compared with matched non-cancerous tissues and was significantly associated with advanced tumor stage and lymph node metastasis. Overexpression of COX-2 plays an important role in tumor progression of gastric cancer. COX-2 may also play a role in the early development/promotion of gastric carcinoma and is associated with *H pylori* infection.

Yu LZ, Gao HJ, Bai JF, Sun G, Zhao HL, Sun L, Miu K, Zhao ZQ. Expression of COX-2 proteins in gastric mucosal lesions. *World J Gastroenterol* 2004; 10(2): 292-294
<http://www.wjgnet.com/1007-9327/10/292.asp>

INTRODUCTION

Recently, a number of researches show that COX-2 was expressed at a very high level in gastrointestinal tumors. However, we know less about COX-2 expression in gastric cancer, especially the relationship between COX-2 overexpression and typing, degree, differentiation, lymphonic metastasis of gastric cancer. In this paper, we investigated the expression of COX-2 proteins in gastric mucosal lesions and assessed the relationship between COX-2 expression and the type, pathologic stage, differentiation, or lymph node metastasis in gastric cancer and the relationship between expression of COX-2 and *H pylori* infection in gastric mucosal lesions.

MATERIALS AND METHODS

Materials

Tissue samples were acquired from 30 patients with gastric cancer diagnosed between April 1996 and March 1998 in our hospital, including a piece of tumor tissue and a piece of paracancerous tissue obtained from the surgery. Samples were then fixed quickly into formalin solution at pH 7.0, embedded in paraffin and cut into slices (4 μ m thick). Slides were used for HE staining and ABC immunohistochemical staining, the latter was used to detect the expression of COX-2 proteins. *H pylori* was determined by rapid urea test combined with pathological staining/¹⁴C urea breath test.

Methods

ABC immunohistochemical staining Polyclonal antibody against COX-2 was obtained from Gene Company Limited, ABC immunohistochemical kits and DAB substrate solution were from Vector Laboratories Inc, USA. Slides were treated with 0.01 mol/L citric acid buffer to recover the antigen activity, and developed with routine ABC immunohistochemical staining at an antibody concentration of 1:50, and the second antibody with labeled biotin at 1:200. The negative control was used with PBS buffer replacing the polyclonal antibody, and positive control was also set up with a tissue sample with a known positive reaction. The criteria for positive reactions were as follows: positive staining of COX-2 protein located within cytosol, but without stain in the nucleus, being pale yellow to deep pale yellow, even pale red. In evaluation of the positive activity, the positive cell number and reaction level were two useful parameters. When over 10% cells were dyed, it could be considered as a positive expression, and the positive reaction levels were shown as weakly positive (+), moderately positive (++) and strongly positive (+++).

Statistics

Statistical analysis system (SAS) software package was used for χ^2 test, and rank sum test for the degree of group data.

RESULTS

COX-2 expression in gastric cancer tissue and paracancerous tissue

The positive rate and intensity of COX-2 expression in gastric cancer tissue were all significantly higher than those in paracancerous tissues ($P<0.01$, $P<0.001$, Table 1).

Table 2 COX-2 expression in gastric mucosa with *H pylori* infection, *n* (%)

	CG (n=30)		IM (n=19)		DYS (n=11)		GC (n=30)	
	Hp+	Hp -	Hp+	Hp -	Hp +	Hp -	Hp +	Hp -
<i>n</i>	25	5	9	11	5	6	11	19
COX-2	2(8.0%)	1(0%)	5(50.6%) ^a	2(18.1%)	3(60.0%) ^a	2(33.3%)	8(72.7%)	12(63.5%)

^a $P < 0.05$, IM or DYS (*H pylori* positive) vs IM or DYS (*H pylori* negative).

Table 1 COX-2 expression in gastric cancer and paracancerous tissues

	Number	COX-2 expression intensity ^b				Positive rate ^d <i>n</i> (%)
		-	+	++	+++	
Gastric cancer	30	10	4	8	8	20 (66.7)
Paracancerous tissue	30	22	6	2	0	8 (26.7)

^b $P < 0.001$ gastric cancer vs paracancerous tissue; ^d $P < 0.01$ gastric cancer vs paracancerous tissue.

COX-2 expression in gastric mucosa with *H pylori* infection

COX-2 expression of IM or DYS with positive *H pylori* was significantly higher than that with negative *H pylori* ($P < 0.05$), (Table 2).

Relationship between COX-2 expression and type, pathologic stage, differentiation, or lymph node metastasis of gastric cancer

The relationship between COX-2 expression and type, pathologic stage, differentiation, or lymph node metastasis of gastric cancer is shown in Table 3. COX-2 positive expression in gastric cancer tissue at the developing stage (76.0%) was significantly higher than that at the early stage (20.0%) ($P < 0.05$). The positive rate in gastric cancer with lymph node metastasis (79.2%) was significantly higher than that without lymph node metastasis (16.7%) ($P < 0.05$). But the COX-2 positive expression in intestinal gastric cancer (66.7%) was the same as that in gastric type of gastric cancer (66.7%). The positive rate in gastric cancer with low or no differentiation (80%) was not higher than that with high or moderate differentiation (57.1%) ($P > 0.05$), (Table 3).

Table 3 COX-2 expression in gastric cancer tissues

Groups	Number	COX-2 <i>n</i> (%)
Type		
Intestinal type	24	16 (66.7)
Gastric type	6	4 (66.7)
Stage		
Early stage	5	1 (20.0)
Developing	25	19 (76.0) ^a
Differentiation (Intestinal type)		
High and moderate	14	8 (57.1)
Low and no differentiation	10	8 (80.0)
Lymph node metastasis		
Without metastasis	6	1 (16.7)
With metastasis	24	19 (79.2) ^a

^a $P < 0.05$, developing stage vs early stage; metastasis vs no metastasis.

DISCUSSION

New COX isozyme-COX-2, is not expressed in normal tissues, but expressed at a high level in inflammatory tissues. It has

been shown in animal studies that COX-2 expression can enhance PGE2 production, which induces cell proliferation and bcl-2 expression. These can destroy the balance between proliferation and apoptosis and induce tumors. More and more studies have shown that COX-2 could express at a high level in human colorectal tumor^[1-5] and other gastrointestinal tumors^[6-8]. COX-2 overexpression was found in well-differentiated epidermoid carcinoma of the esophagus. Ratnasinghe^[9] studied the COX-2 expression in epidermoid carcinoma of the esophagus and found that COX-2 expressed at a high level in well-differentiated tissues, at a low positive level in the normal esophagus, and negative in poorly-differentiated tissues. Hao^[10] found COX-2 protein expressed at a high level in adenocarcinoma and adenoma of colon, compared with normal mucosal tissues. COX-2 mRNA expressed in tumor tissues at a significantly higher level than that in normal tissues. There was neither a relationship between COX-2 protein expression and proliferation degree or volume of adenoma, nor a relationship between COX-2 expression and tumor differentiation, Duke's stage as well as lymph node metastasis ($P > 0.05$). Interestingly, COX-2 expressed in the tissues near adenocarcinoma or adenoma at a higher level than in normal mucosal tissues ($P < 0.0001$), but lower than that in adenocarcinoma or adenoma itself ($P < 0.001$, $P < 10^5$).

It has been found that the positive rate of COX-2 expression in gastric cancer tissue was 60%-70%^[6-8]. Ratnasinghe^[6] found that COX-2 expressed positively in 36% cardia adenocarcinoma and 60% gastric body adenocarcinoma in his research on 19 patients with cardia adenocarcinoma and 15 patients with gastric body adenocarcinoma. COX-2 overexpression was found in most of gastric body adenocarcinoma and some cardia adenocarcinoma tissues. It is necessary to further confirm the status of COX-2 expression in gastric cancer tissues, especially the characteristics of COX-2 overexpression related to typing, degree, differentiation and lymph node metastasis^[11-15]. We studied the COX-2 expression at gene and protein levels in tissues with gastric mucosal lesion, and explored the relationship between COX-2 expression and gastric carcinoma and *H pylori* infection at pathological and pathophysiological levels.

Our study based on 30 tissue samples with gastric cancer as well as paracancerous tissues showed that COX-2 protein expressed at a high level in tumor tissues, which was significantly higher than that in paracarcinoma tissues ($P < 0.01$), and also significantly higher in tumor tissues ($P < 0.01$). COX-2 positive expression in gastric cancer tissues at the developing stage was significantly higher than that at early stage, the positive rate in gastric cancer with lymph node metastasis was significantly higher than that without lymph node metastasis ($P < 0.05$), but the COX-2 positive expression in intestine type of gastric cancer was the same as that in gastric type of gastric cancer. The positive rate in gastric cancer with low or no differentiation was not higher than that with high or moderate differentiation ($P > 0.05$). Our results were similar to those of foreign investigators^[7,8]. In conclusion, abnormal expression of COX-2 protein was related to the progress of gastric carcinoma as well as lymph node metastasis, while it was not significantly related to the type of gastric cancer and degree of pathological differentiation^[13,15].

We also found that COX-2 expression in tissues with *H pylori* positive intestinal metastasis or dysplasia was significantly higher than that in tissues with *H pylori* negative infection. *H pylori* could induce acute and chronic inflammation of gastric mucosa, and the production of cell factors such as IL-8 and IL-1 β , and the secondary high COX-2 expression which caused gastric mucosal lesions. *H pylori* infection could also induce gastric mucosal cell proliferation by COX-2 expression. COX-2 gene expression was one of the related factors mediating the progress from gastritis with *H pylori* infection to pre-carcinoma lesions even gastric carcinoma^[16,17]. Based on this study, treatment of *H pylori* infection and special COX-2 inhibitor could be useful for the prevention of gastric carcinoma^[18].

REFERENCES

- 1 **Eberhart CE**, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. Up-regulation of cyclooxygenase-2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology* 1994; **107**: 1183-1188
- 2 **Sano H**, Kawahito Y, Wilder RL, Hashiramoto A, Mukai S, Asai K, Kimura S, Kato H, Kondo M, Hla T. Expression of cyclooxygenase-1 and -2 in human colorectal cancer. *Cancer Res* 1995; **55**: 3785-3789
- 3 **Reddy BS**, Rao CV, Seibert K. Evaluation of cyclooxygenase-2 inhibitor for potential chemopreventive properties in colon carcinogenesis. *Cancer Res* 1996; **56**: 4566-4569
- 4 **Tsuji M**, Kawano S, DuBios RN. Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. *Proc Natl Acad Sci U S A* 1997; **94**: 3336-3340
- 5 **Watson AJ**. Chemopreventive effects of NSAIDs against colorectal cancer: regulation of apoptosis and mitosis by COX-1 and COX-2. *Histol Histopathol* 1998; **13**: 591-597
- 6 **Ratnasinghe D**, Tangrea JA, Roth MJ, Dawsey SM, Anver M, Kasprzak BA, Hu N, Wang QH, Taylor PR. Expression of cyclooxygenase-2 in human adenocarcinomas of the gastric cardia and corpus. *Oncol Rep* 1999; **6**: 965-968
- 7 **Murata H**, Kawano S, Tsuji S, Tsuji M, Sawaoka H, Kimura Y, Shiozaki H, Hori M. Cyclooxygenase-2 overexpression enhances lymphatic invasion and metastasis in human gastric carcinoma. *Am J Gastroenterol* 1999; **94**: 451-455
- 8 **Yamamoto H**, Itoh F, Fukushima H, Hinoda Y, Imai K. Overexpression of cyclooxygenase-2 protein is less frequent in gastric cancers with microsatellite instability. *Int J Cancer* 1999; **84**: 400-403
- 9 **Ratnasinghe D**, Tangrea J, Roth MJ, Dawsey S, Hu N, Anver M, Wang QH, Taylor PR. Expression of cyclooxygenase-2 in human squamous cell carcinoma of the esophagus; an immunohistochemical survey. *Anticancer Res* 1999; **19**(1A): 171-174
- 10 **Hao X**, Bishop AE, Wallace M, Wang H, Willcocks TC, Macclouf J, Polak JM, Knight S, Talbot IC. Early expression of cyclooxygenase-2 during sporadic colorectal carcinogenesis. *J Pathol* 1999; **187**: 295-301
- 11 **Ristimaki A**, Honkanen N, Jankala H, Sipponen P, Harkonen M. Expression of cyclooxygenase-2 in human gastric carcinoma. *Cancer Res* 1997; **57**: 1276-1280
- 12 **Sawaoka H**, Kawano S, Tsuji S, Tsujii M, Murata H, Hori M. Effects of NSAIDs on proliferation of gastric cancer cells *in vitro*: possible implication of cyclooxygenase-2 in cancer development. *J Clin Gastroenterol* 1998; **27**(Suppl 1): S47-52
- 13 **Saukkonen K**, Nieminen O, van Rees B, Vilkkilä S, Harkonen M, Juhola M, Mecklin JP, Sipponen P, Ristimaki A. Expression of cyclooxygenase-2 in dysplasia of the stomach and in intestinal-type gastric adenocarcinoma. *Clin Cancer Res* 2001; **7**: 1923-1931
- 14 **Van Rees BP**, Saukkonen K, Ristimaki A, Polkowski W, Tytgat GN, Drilenburg P, Offerhaus GJ. Cyclooxygenase-2 expression during carcinogenesis in the human stomach. *J Pathol* 2002; **196**: 171-179
- 15 **Yamagata R**, Shimoyama T, Fukuda S, Yoshimura T, Tanaka M, Munakata A. Cyclooxygenase-2 expression is increased in early intestinal-type gastric cancer and gastric mucosa with intestinal metaplasia. *Eur J Gastroenterol Hepatol* 2002; **14**: 359-363
- 16 **Walker MM**. Cyclooxygenase-2 expression in early gastric cancer, intestinal metaplasia and *Helicobacter pylori* infection. *Eur J Gastroenterol Hepatol* 2002; **14**: 347-349
- 17 **Wambura C**, Aoyama N, Shirasaka D, Sakai T, Ikemura T, Sakashita M, Maekawa S, Kuroda K, Inoue T, Ebara S, Miyamoto M, Kasuga M. Effect of *Helicobacter pylori*-induced cyclooxygenase-2 on gastric epithelial cell kinetics: implication for gastric carcinogenesis. *Helicobacter* 2002; **7**: 129-138
- 18 **Sung JJ**, Leung WK, Go MY, To KF, Cheng AS, Ng EK, Chan FK. Cyclooxygenase-2 expression in *Helicobacter pylori*-associated premalignant and malignant gastric lesions. *Am J Pathol* 2000; **157**: 729-735

Edited by Ma JY and Wang XL