Cyclooxygenase-2 Expression Is Related to Prostaglandin Biosynthesis and Angiogenesis in Human Gastric Cancer

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ABSTRACT

Although recent studies have demonstrated that cyclooxygenase (COX)-2 is overexpressed in various cancers including gastric cancer, the mechanisms underlying the contribution of COX-2 to tumorigenesis and tumor promotion still remain unclear. To determine the role of COX-2, we investigated the COX-2 expression, the prostaglandin (PG) levels, and the microvessel density in 42 patients with primary gastric adenocarcinoma. COX-2 protein was overexpressed in 31 (74%) of 42 gastric cancers based on an immunoblot analysis. The intensity of COX-2 expression was found to significantly correlate with lymph node involvement. The COX-2 overexpressed cases showed significantly elevated levels of prostaglandin E_2 (PGE_2) in cancer tissues in comparison with the normal gastric mucosa by an immunoassay (201 ± 90 *versus* 161 ± 57 ng/mg protein; $P < 0.05$). However, the COX-2 overexpression was not related to the levels of thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$. The density of microvessel immunostained with CD34 was significantly higher in patients demonstrating COX-2 overexpression than in those without such expression (63 ± 21 *versus* 45 ± 17/200 ×; $P < 0.01$). Our data thus suggested COX-2 overexpression to be associated with increased PGE_2 biosynthesis and angiogenesis in gastric cancer, which indicates that COX-2 may play a role in the development of gastric cancer.

INTRODUCTION

COX[2] is a key enzyme in PG biosynthesis (1). Two isoforms of COX have been identified: constitutively expressed COX-1 and mitogen-inducible COX-2 (2–4). Recently, an enhanced expression of COX-2, but not COX-1, has been found in cases of colon cancer (5–8). Various epidemiological studies have previously revealed that the use of NSAIDs can reduce the risk of colon cancer (9–11). Because NSAIDs are known to inhibit COX, their beneficial effect in colon cancer is considered to be associated with COX-2 overexpression in this disease. COX-2 overexpression has been recently observed in many tumors other than the colon, such as the lung, the breast, and the esophagus (12–16). In gastric cancer, we and others have demonstrated an enhanced expression of COX-2 protein and mRNA in most cancer tissues compared with the accompanying normal mucosa (15, 16).

Recent studies have demonstrated that COX-2 could affect carcinogenesis via several different mechanisms. COX-2-mediated PG biosynthesis has been suggested to be involved in the development of cancer based on elevated levels of PGs, especially PGE_2, in cancer tissues (17–19). COX-2 has been also reported to induce angiogenesis, which might be essential for tumor growth (20). COX-2 may be related to the development of gastric cancer as well, however, its association with PG biosynthesis and angiogenesis still remains unclear. To determine the role of COX-2 expression in gastric cancers, we examined the PG levels and microvessel density in patients with gastric cancer, and then compared the findings with the expression of COX-2 protein.

MATERIALS AND METHODS

Patients. Forty-two patients undergoing surgery for primary gastric cancer at the National Defense Medical College Hospital from 1993 to 1998 were examined. Of these, 29 were male and 13 were female. The mean age was 62 years (range, 31–86). Paired samples of cancer tissue and normal gastric mucosa were obtained from each patient at the time of surgery. The samples were immediately frozen in liquid nitrogen and were stored at −80°C. The remaining tissue specimens were fixed in 10% formalin and embedded in paraffin for a routine histological examination and immunohistochemical analysis.

Western Blot Analysis for COX-2. Immunoblot analysis was performed as described previously (15). Briefly, cell lysates (20 μg/lane) were separated on 10% SDS polyacrylamide gel and then were electrophoretically transferred to a polyvinylidene difluoride membrane. COX-2 protein was detected by a rabbit polyclonal IgG (Immunobiological Laboratories, Fujioka, Japan) and visualized by the enhanced chemiluminescence system (Amersham, Arlington Heights, IL). The density of the bands was quantitated using the NIH image software package (Version 1.61). The intensity of COX-2 expression was judged by the ratio of its expression in cancer tissue (C) to its expression in paired normal gastric mucosa (N), and a ratio (COX-2 C:N) of more than 1.0 was considered to indicate an overexpression of COX-2 (15).

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[2] The abbreviations used are: COX, cyclooxygenase; PG, prostaglandin; PGE_2, prostaglandin E_2; TXB_2, thromboxane B_2; PGI_2, prostacyclin; 6-k-$PGF_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$; NSAID, nonsteroidal anti-inflammatory drug.

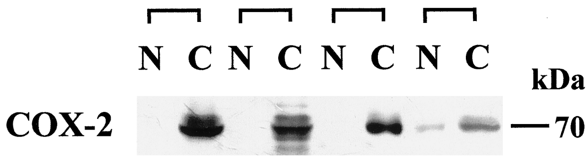

Fig. 1 A representative immunoblot analysis of COX-2 protein expression in the normal gastric mucosa (*N*) and cancer tissue (*C*). The cancer tissue showed intense immunoreactive bands located at M_r 70,000 (*70 kDa*), although the paired normal gastric mucosa showed weak bands. The ratio of COX-2 C:N was more than 1.0 in 31 (74%) of 42 cases.

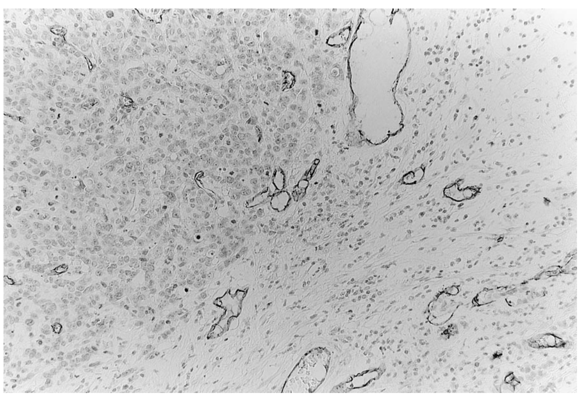

Fig. 2 Detection of microvessels in cancer tissue by immunostaining for CD34. The microvessels were found to be most numerous at the periphery of the tumor ("hot spot").

Immunoassay for PGs. The levels (ng/mg protein) of PGE_2, TXB_2, and 6-k-$PGF_{1\alpha}$ were examined using an immunossay kit (Amersham) in paired protein samples of gastric cancer and normal mucosa.

Immunohistochemistry. To detect microvessels on paraffin embedded samples, anti-CD34 (a monoclonal antibody to a transmembrane protein found on immature endothelial cells) was used (DAKO, Kyoto, Japan). Four-μm-thick sections were deparaffinized, microwaved for 15 min for antigen retrieval, immersed in 0.3% hydrogen peroxide for 30 min, and then immersed in normal goat serum for 30 min. The slides were incubated with anti-CD34 overnight at 4°C, and then stained by the standard streptavidin-biotin immunoperoxidase method. The areas containing a large number of microvessels or "hot spots" were identified at low magnification (×40) using a light microscope. Once hot spots were recognized, microvessels per field were counted at ×200. From the 10 fields counted, the highest number of microvessels was used to determine the density (21, 22).

Statistical Analysis. Differences between the groups were analyzed by the χ^2 test or Welch's *t* test. Pearson's correlation coefficient (*r*) was tested by the *F* test. A $P < 0.05$ was considered to be statistically significant.

RESULTS

COX-2 Protein Expression. The expression of COX-2 protein was up-regulated (COX-2 C:N > 1.0) in 31 (74%) of 42 gastric carcinomas by an immunoblot analysis (Fig. 1). The ratio of COX-2 C:N was statistically higher in the patients with lymph node involvement than in those without ($P < 0.05$). No significant differences in the ratio were found in either the histological type or the tumor invasion (Table 1).

Table 1 The association of COX-2 expression with the histological findings

	COX-2 C:N[a]	P[b]
Histological type[c]		
Intestinal (n = 17)	3.7 ± 3.3	NS[d]
Diffuse (n = 25)	2.9 ± 3.2	
Depth of invasion[e]		
T_1, T_2 (n = 24)	2.7 ± 2.4	NS
T_3, T_4 (n = 18)	4.1 ± 4.0	
Lymph node involvement		
Negative (n = 13)	1.9 ± 1.4	<0.05
Positive (n = 29)	3.9 ± 3.7	

[a] The values are expressed as the mean ± SD.
[b] According to Welch's *t* test.
[c] According to Lauren's classification.
[d] NS, not significant.
[e] According to the tumor-node-metastasis classification.

PG Levels. The levels of PGE_2 were higher in the cancer tissues than in the normal gastric mucosa ($P < 0.05$). The differences in the levels of TXB_2 and 6-k-$PGF_{1\alpha}$ between the normal and the cancer tissue specimens were not statistically significant. No significant association was seen between the levels of any of the three PGs and the histological type, depth of invasion, or lymph node involvement (Table 2).

The cases with COX-2 overexpression showed significantly higher levels of PGE_2 in the cancer tissues than in the normal gastric mucosa ($P < 0.05$; Table 2), whereas the cases without COX-2 overexpression did not. The levels of TXB_2 and 6-k-$PGF_{1\alpha}$ did not differ between the normal and cancer tissue specimens in either the patients with COX-2 overexpression or those without (Table 2).

Microvessel Density. The mean microvessel density of all of the cases was 58 (SD, 20), with a range of 31–121 (Fig. 2). No significant relationship was observed between the microvessel density and the histological type, depth of invasion, or lymph node involvement (data not shown).

The microvessel density was significantly higher in patients with COX-2 overexpression than in those without (63 ± 21 *versus* 45 ± 17; ×200 microfield; $P < 0.01$). A significant correlation was observed between the ratio of COX-2 C:N and the microvessel density ($P < 0.0001$; Fig. 3*A*). However, the PGE_2 levels in the cancer tissue specimens showed no correlation with the microvessel density (Fig. 3*B*).

DISCUSSION

In the present study, we provided a profile of an enhanced expression of COX-2 protein and elevated levels of PGE_2 in human gastric cancer tissues compared with the normal mucosa. Increased levels of PGE_2 in the cancer tissue specimens were prominent in patients with COX-2 overexpression but not in those without. We previously demonstrated by immunohistochemical staining (15) that the main source of increased COX-2 protein in the cancer tissue was the cancer cells themselves.

Table 2 The levels of PGs in human gastric cancers

PG levels[a] (ng/mg protein)	PGE_2 N	PGE_2 C	TXB_2 N	TXB_2 C	6-k-$PGF_{1\alpha}$ N	6-k-$PGF_{1\alpha}$ C
Total (n = 42)	166 ± 63	198 ± 83[b]	54 ± 23	59 ± 26	61 ± 25	71 ± 24
Histological type[c]						
Intestinal (n = 17)	152 ± 70	210 ± 104	49 ± 23	61 ± 23	57 ± 29	71 ± 25
Diffuse (n = 25)	175 ± 58	190 ± 65	57 ± 22	57 ± 28	64 ± 22	70 ± 24
Depth of invasion[d]						
T_1, T_2 (n = 24)	170 ± 73	211 ± 96	50 ± 23	55 ± 26	61 ± 27	69 ± 28
T_3, T_4 (n = 18)	160 ± 48	180 ± 59	58 ± 23	63 ± 26	62 ± 22	72 ± 19
Lymph node involvement						
Negative (n = 13)	176 ± 79	215 ± 67	57 ± 28	63 ± 27	66 ± 29	75 ± 25
Positive (n = 29)	161 ± 56	190 ± 88	52 ± 20	57 ± 25	59 ± 23	69 ± 25
COX-2 overexpression[e]						
Positive (n = 31)	161 ± 57	201 ± 90[b]	55 ± 20	60 ± 26	62 ± 21	71 ± 26
Negative (n = 11)	179 ± 80	189 ± 58	50 ± 31	54 ± 24	59 ± 34	68 ± 20

[a] Mean ± SD; N, normal gastric mucosa; C, cancer tissue.
[b] $P < 0.05$, according to Welch's t test.
[c] According to Lauren's classification.
[d] According to the Tumor-Node-Metastasis classification.
[e] Positive, COX-2 C:N > 1.0; Negative, COX-2 C:N < 1.0.

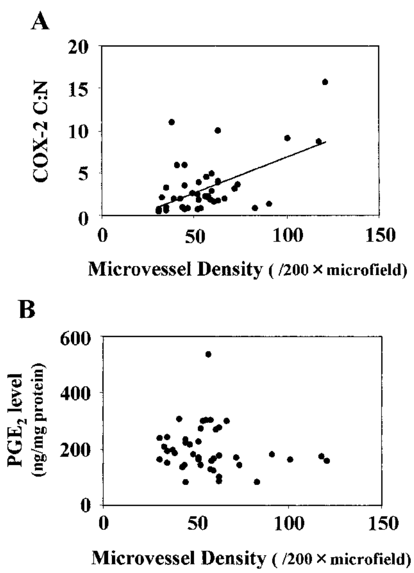

Fig. 3 Correlation between the microvessel density and the ratio of COX-2 C:N (*A*) and PGE_2 levels in the cancer tissue specimens (*B*). The correlation coefficient between the microvessel density and the ratio of COX-2 C:N was 0.581 ($P < 0.0001$), whereas the coefficient between the microvessel density and PGE_2 levels was −0.147 ($P = 0.3555$).

These findings suggest that gastric cancer cells overexpressing COX-2 may promote PGE_2 biosynthesis. PGE_2 shows a potent immunosuppression effect by inhibiting the T-cell or natural killer cell activity (23–26). Elevated PGE_2 levels may, thus, provide a selective advantage for cancer cell survival, which may lead to the development of gastric cancer.

TXA_2 has been reported to facilitate tumor metastasis (27, 28), whereas PGI_2 has been shown to be an antimetastatic agent (29). The levels of TXA_2 (assayed as its product TXB_2) and PGI_2 (assayed as its product 6-k-$PGF_{1\alpha}$) were not associated with COX-2 expression in this study. The effects of TXA_2 and PGI_2, thus, may not be involved in the role that COX-2 plays in the progression of gastric cancer.

The present study demonstrated that the intensity of COX-2 expression correlated with the metastatic involvement of the lymph nodes. In contrast, the levels of PGE_2 did not correlate with lymph node involvement. COX-2-expressing colon cancer cells have been reported to enhance the metastatic potential by activating metalloproteinase (30). COX-2 overexpression may thus enhance the lymphatic invasion in gastric cancer by a mechanism different from PGE_2 biosynthesis, possibly because of the activation of metalloproteinase.

Angiogenesis is well recognized to be essential for the growth of solid tumors (31, 32). In the present study, we confirmed that the microvessel density correlated with the intensity of COX-2 expression. There was no significant correlation between the PGE_2 levels and the microvessel density in the cancer tissue specimens, which, thus, suggests that PGE_2 biosynthesis—mediated via the COX pathway—may not act directly on endothelial cells in gastric cancer. COX-2 has been shown in colon cancer cell lines to stimulate angiogenesis by inducing such angiogenic factors as vascular endothelial growth factor and transforming growth factor β (20). These mechanisms may, therefore, play a role in the association between COX-2 overexpression and angiogenesis in gastric cancer.

In conclusion, our data suggest that COX-2 overexpression leads to increased PGE_2 biosynthesis and angiogenesis, which may be mechanisms underlying the contribution of COX-2 to the development of gastric cancer. Thanks to the recent advances in the development of selective COX-2 inhibitors, COX-2 may, thus, provide an attractive target for chemopreventive strategies in the treatment of gastric cancer.

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