Expression of Cyclooxygenase Enhances Tumor Invasion and Metastasis in Human Gastric Carcinoma

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ABSTRACT

Background: Expression of COX-2 in vitro has been shown to have a number of cellular effects including increasing proliferation, reducing apoptosis promoting angiogenesis, decreasing E-cadherin expression and increasing invasive/metastatic potential.

Aims: To determine the role of COX-2 in the development and metastasis potential of gastric carcinoma in human subjects.

Methods: Tissue samples were obtained from surgically removed specimens of 48 patients with primary gastric adenocarcinoma who underwent gastrectomy from January 1998 to December 1999. The specimens were stained for HE while COX-2 expressions in cancer fold and antrum site were evaluated immunohistochemically. Expression of COX-2 was defined as positive when either one of cancer lesion or antrum site showed immunoreactivity.

Results: Preliminary result from 12 out of 48 cases, COX-2 immunoreactivity was detected in 50% (6 of 12 specimens). Expression of COX-2 were more frequent in tumor with serosal invasion (5 of 6 specimens), lymph node metastases (3 of 3 specimens), tumor size more than 4 cm and were significant, statistically ($p<0.05$). The expression of COX-2 in well differential carcinoma type was similar with in poorly differentiated carcinoma type.

Conclusion: COX-2 expression in gastric carcinoma tissue is correlated closely with tumor size, serosal invasion and lymph node metastases, indicating that COX-2 is involved in the growth and metastases of gastric carcinoma.

Keywords: *Gastric carcinoma, cyclooxygenase-2, invasion, immunochemistry*

INTRODUCTION

Cyclooxygenase is a key enzyme in prostaglandin biosynthesis.[1] Two isoform of COX has been recognized. COX-1 is a constitutively expressed enzyme in many tissues, including the gastrointestinal tract,[2,3] while COX-2 which is normally undetectable in most tissues, is induced in response to a number of stimuli including the cytokines interleukine-1 (IL-1), interferon-γ (INF-γ), tumor necrosis factor (TNF)[4] and activation of epidermal factor receptor (EGF-r).[5]

Expression of COX-2 had been reported in colorectal cancers,[6,7] pancreatic cancer,[8] hepatocellular carcinoma,[9,10] esophageal cancers[11,12] and gastric cancer.[13,14] COX-2 expression is especially prominent in gastrointestinal cancers, suggesting its important role in gastrointestinal cancers.[6,7,11,15]

Over expression of COX-2 in vitro has been shown to have a number of cellular effects including increasing proliferation, reducing apoptosis[16] promoting angiogenesis,[17,18] decreasing E-cadherin expression[16] and increasing invasive/metastatic potential.[19]

Recent studies have been demonstrated that COX-2 could affect carcinogenesis via several mechanisms. COX-2 mediated PG biosynthesis has been suggested to be involved in the development of cancers based on elevated level of PGs, especially PGE2, in cancer

tissues.[20-22]

In papilomas and carcinomas in mouse skin, overexpression of COX-2 is associated with large increase in PGE2 and PGF2α.[23] COX-2 is detected in majority of colon tumor samples[6] and is involved in early events of colon carcinogenesis.[24,25] However the association between COX-2 and the metastatic potential of stomach carcinoma in human has been not yet studied. We investigated the involvement of COX-2 in the development and metastasis of human gastric carcinoma.

MATERIALS AND METHODS

Patients and Samples

Tissue samples were obtained from surgically removed specimens of 48 patients with primary gastric adenocarcinoma who underwent gastrectomy from January 1998 to December 1999 at Yamanashi University/Hospital, Japan. Patients with multiple organ malignancy have been excluded from this series. The surgical specimens were fixed in 10% buffered formaldehyde, embedded in paraffin, sectioned and stained with H & E. Specimens from antrum; outside of cancer lesion were also obtained from the same patients. These specimens were subjected to detailed pathological examination, which identified severity of gastritis, depth of invasion, nodal status, marginal involvement and histological type of the tumors. The clinicopathologic data were analyzed according to the Japanese classification system for gastric carcinoma outlined by the Japanese Gastric Cancer Association.[26] Well differentiated gastric carcinoma (WGC) included papillary and tubular adenocarcinoma, poorly differentiated medullary carcinoma and well differentiated mucinous carcinoma; whereas poorly differentiated gastric carcinoma (PGC) included poorly differentiated scirrhous carcinoma, signet ring cell carcinoma and poorly differentiated mucinous carcinoma.[27] Gastritis pattern in the antrum site was determined according to Update Sydney System.

Immunohistochemical Staining

Paraffin-embedded blocks were sectioned at about 5 mm thickness, deparaffinized and rehydrated. Slides were immersed in methanol and 30% hydrogen peroxide for 15 minutes to block the endogenous peroxidase activity. After rinsing with PBS the specimens were immersed in normal goat serum for 30 minutes. After rinsing three times with PBS in 20 minutes, slides were incubated overnight in room temperature with the polyclonal antibody against COX-2 (Nichirei, Tokyo, Japan) in a dilution of 1:100. All the slides were rinsed with PBS three times in 20 minutes and incubated with an avidin biotin conjugated anti Rabbit IgG (Nichirei, Tokyo, Japan) for 2 hours. After rinsing the slides were incubated with peroxidase-conjugated streptavidin (Nichirei, Tokyo, Japan) for another two hours and followed by DAB staining (Histofine Kit; Nichirei, Tokyo, Japan).

A senior morphologist examined the immuno-histochemical expression of COX-2. Expression of COX-2 was defined as positive when either one of cancer lesion or antrum site from the same patient showed immunoreactivity. The expression of COX-2 immuno-reactivity was graded semi quantitatively and each sample was assigned of the following categories: negative (-), small (+), moderate (++) and many (+++). The intensity of immunostaining was determined as 0 (negative), 1 (weak), 2 (intermediate) and 3 (strong). The immunoreactive score was calculated by multiplication of the grade of positive cells and the staining intensity.

Statistical Analysis

The score of COX-2 expression was compared within pathological parameters such as: histopathological type, stage grouping, venous vessel invasion and lymphatic vessel invasion in gastric wall, serosal invasion and the presence of lymph node metastasis using Mann-Whitney U test.

RESULTS

Preliminary result from 12 out of 48 cases was demonstrated in Table 1. COX-2 immunoreactivity was detected in 50% cases (6 of 12 specimens). The score of COX-2 expressions were related to the tumor size more than 4 cm, serosal invasion and lymph node metastasis ($p<0.05$). The score of COX-2 expression showed no correlation with histopathologic type, lymphatic and vascular permeation, UICC TNM stage, the presence of Helicobacter pylori, gastric atrophy, intestinal metaplasia and gastritis score ($p>0.05$).

Table 1. Characteristic of 12 Gastric Carcinoma Cases and COX-2 Expression

Case	Age/ sex	G	pT	Ser	pN	Size (cm)	Stage Group	v	Ly	IM	Atrophy	Hp	COX-2
1	48/M	1	3	1	0	3.75	III a	- ve	+ ve	2	1	+ ve	No
2	49/M	1	1	0	0	1.50	I a	- ve	- ve	2	2	+ ve	No
3	51/M	2	2	0	0	1.25	I b	- ve	- ve	2	2	+ ve	No
4	65/F	2	0	0	0	2.30	I a	- ve	- ve	2	2	+ ve	No
5	48/M	2	1	0	0	1.50	I a	- ve	- ve	0	1	- ve	No
6	59/M	1	2	0	0	3.75	I b	- ve	- ve	2	2	+ ve	No
7	64/M	1	0	0	0	2.50	1a	- ve	- ve	2	1	+ ve	Yes
8	71/M	1	4	1	1	5.30	III b	- ve	- ve	2	2	+ ve	Yes
9	67/M	2	4	1	1	12.25	IV b	+ve	+ ve	2	2	+ ve	Yes
10	68/M	1	4	1	0	6.75	II	- ve	+ ve	0	1	- ve	Yes
11	69/M	2	3	1	1	2.75	II	+ ve	+ ve	2	1	- ve	Yes
12	55/M	2	3	1	0	7.50	III a	- ve	- ve	0	1	- ve	Yes

Abbreviations: M = male; F = female; G = histopatological grading; G1 = well differentiated; G2 = poorly differentiated; pT = primary tumor invasion; Ser = serosal involvement; pN= regional lymph node; v = vascular permeation; + ve = positive; - ve = negative; Ly = lymphatic permeation, Hp = Helicobacter pylori

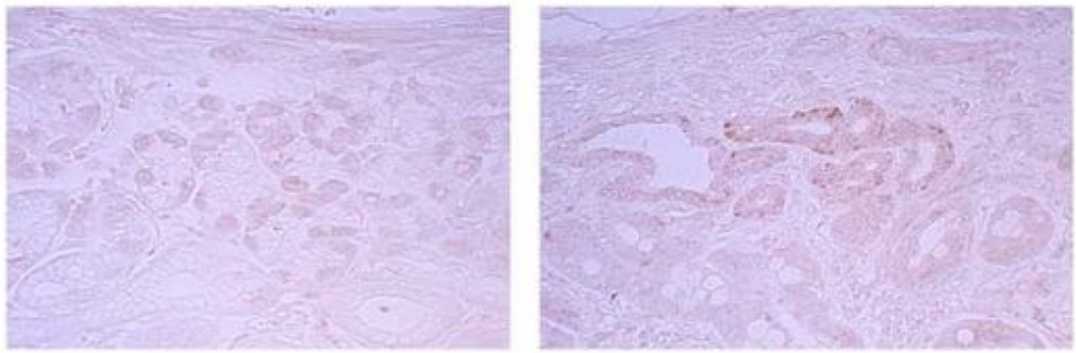

Figure 1. Immunopositive cells in the antrum

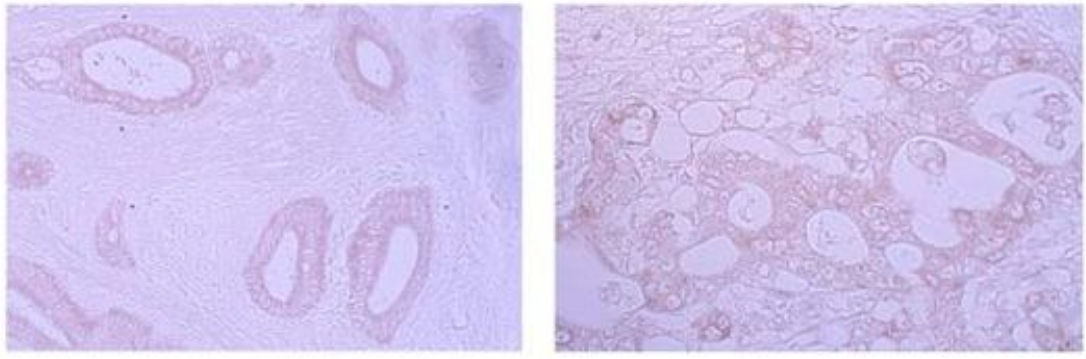

Figure 2. Immunopositive cells in the cancer fold

DISCUSSION

The present study demonstrated the involvement of COX-2 in gastric carcinoma. Recently Ohno et al,[28] reported that the degree of COX-2 mRNA elevation was related to the depth of primary tumor invasion. Similar findings also reported by Murata et al,[29] that carcinoma tissue expressed remarkably higher levels of COX-2 in advanced carcinoma compare to early gastric carcinoma. Present study revealed the expression of COX-2 related to the serosal invasion but not to stage of diseases (stage I and II vs. stage III and IV, $p>0.05$) in gastric carcinoma patients. Both of these factors were claimed to be a prognostic factors.[27]

Recent studies have found a correlation between the levels of COX-2 expression and lymph node metastasis and lymphatic permeation in patients with gastric carcinoma. Murata et al,[29] found that tumor over expression of COX-2 protein, according to Western blot analysis, was associated significantly with invasion into gastric wall lymphatic vessels as well as with metastasis to lymph node. Yamamoto et al, also reported that over expression of COX-2 protein was associated significantly with lymphatic involvement and lymphatic metastasis. On the contrary Ohno et al,[28] using RT-PCR analysis reported that the correlation between the levels of COX-2 mRNA and lymph node metastasis were not significant. Present study showed COX-2 expression were associated to lymph node metastasis, but did not attain a significant association with lymphatic permeation in gastric carcinoma patients.

The size of tumor more than 4 cm has been proposed as an independent prognostic factor previously.[27] Interestingly, our result revealed the expression of COX-2 was related to the size of tumor more than 4 cm.

In the human stomach, Helicobacter pylori infection is associated with chronic gastritis and leads to mucosal atrophy and intestinal metaplasia, whish are pre-cancerous lesion. Furthermore, Helicobacter pylori infection is associated with a Ca-fold increased risk of gastric carcinoma.[30] Sung et al,[31] reported that COX-2 protein is expressed in the gastric epithelium throughout the multistep gastric carcinogenesis cascade. The present study failed to demonstrated any association between the expression of COX-2 and the presence of Helicobacter pylori, gastric atrophy, intestinal metaplasia and the severity of gastritis.

CONCLUSION

COX-2 expression in gastric carcinoma tissue is correlated closely with tumor size, serosal invasion and lymph node metastases, indicating that COX-2 is involved in the growth and metastasis of gastric carcinoma.

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