Correlation between expression of cyclooxygenase-2 and angiogenesis in human gastric adenocarcinoma

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INTRODUCTION
COX is a key enzyme in the conversion of arachidonic acid to prostaglandin, and two isoforms of COX, namely COX-1 and COX-2, have been identified[1-3]. COX-1 is constitutively expressed in many tissues and is considered to be involved in various physiological functions, whereas COX-2 is induced by pathological stimuli, such as inflammation, various growth factors and cytokines produced by tumor cells[4-10].

Epidemiologic studies showed that nonsteroidal anti-inflammatory drugs (NSAIDs), known to inhibit COX, could reduce the incidence rate and mortality from digestive tract carcinomas[4-10]. In rodent models of FAP, a genetic disease leading to colonic carcinoma, blockade of COX-2, suppresses intestinal polyp formation[11]. Increased COX-2 expression has been reported in colorectal, pancreatic, hepatocellular and other cancers[12-20]. Taken together, these data provide strong evidence for the importance of COX-2 in oncogenesis.

It has been reported that tumor angiogenesis play an important role in tumor growth, invasion and metastasis[21-25]. We investigated the expression of COX-2, MVD in human gastric cancer. The aim of this study was to determine the relationship between COX-2 and tumor angiogenesis, and the development, progression of gastric cancer. The further understanding of oncogenesis might provide a new approach to tumor therapy.

Materials and Methods

Materials
45 patients with gastric adenocarcinomas confirmed pathologically underwent gastrectomy in our hospital from January 2000 to October 2001. From these subjects, gastric tumor and paracancerous tissues (more than 5 cm away from the lesion) were obtained from resected specimen. Among them, 35 were male, and 10 female, with a mean age of 57.51±10.73 (33 to 78). Patients who had received radiotherapy or chemotherapy before gastrectomy were excluded. Histologically, they were classified by the WHO criteria, 5 were highly differentiated adenocarcinoma, 10 moderately-differentiated, 27 poorly-differentiated, 3 undifferentiated. As regards to the size of cancer, 20 were <5 cm, 25≥5 cm. 33 tumors invaded to the serosa and 12 tumors not.36 cases had local lymph node metastasis.

Reagents and methods
Antibody against COX-2 was purchased from Santa Cruz Biotechnology, Inc; antibody against CD34 and ready to use SP immunohistochemical reagent box were purchased from Fijian Maixin CO, Ltd. Formalin-fixed, paraffin-embedded surgical specimens from 45 cases of gastric carcinoma were available and sliced sequentially with a thickness of 4 μm. The slices carrying the detected antigen were dyed with SP immunohistochemical reagent, and those in the control group were dyed according to the above method, with the first antibody substituted by PBS.

Statistical methods
The data were presented as x̄±σ; numerical variable by χ² test;
RESULTS

The cytoplasm of the gastric cancer cells stained with brown granules were identified to be COX-2 positive. Only nucleuses stained blue were identified to be COX-2 negative. COX-2 expression was scored semi-quantitatively according to the density and the percentage of positivity into score 0, 1, 2, 3. A minimum of 10 high power view were used to assess COX-2 expression level. If the sum of two scores was 1-3, the slice would be considered as low-expression of COX-2. If 4-6, it would be considered as high-expression of COX-2. Vascular endothelial cells were considered CD34-positive if their cytoplasm stained brown or brownish yellow. The microvessels were counted according to the number of single endothelial cell or endothelial cell clustor showing brownish yellow granules in the cytoplasm. The slices were observed first microscopically under the low power (×40), then selected the most dense area of microvessels was selected to be observed under high power (×200, the surface area of every visual field was 0.785 mm²), and the number of microvessels in 3 visual field were counted and took the average as MVD of this specimen[26].

COX-2, MVD expression and distribution

77.78 % (35/45) cases of gastric carcinomas showed COX-2 positive expression while high-expression was detected in 22 cases, low-expression in 13. 33.33 % (15/45) cases of paracancerous tissues showed COX-2 positive expression while high-expression was only detected in 3 cases. The rate and density of COX-2 expression in cancerous tissues were significantly higher that in paracancerous tissues (χ²=18, χ²=6,09, P<0.005, P<0.05, respectively). The positive COX-2 staining was mainly diffusely located as brownish yellow stained granules in the cytoplasm. Immunohistological analysis revealed cytoplasmic staining in the neoplastic cells (Figure 1), atypical hyperplasia and intestinal metaplasia. In addition, COX-2 was also detected in the angiogenic vasculature present within the tumors and preexisting vasculature adjacent to cancer lesions (Figure 2). In contrast, normal epithelium or stroma occasionally showed weak staining pattern or didn’t.

The mean MVD in gastric carcinoma was significantly higher than that in para-cancerous tissues (58.13±19.99, 24.02±10.28, t=10.18, P<0.001). The positive expression of CD34 was mainly presented as brownish yellow or brownish granules in the cytoplasm of vascular endothelial cell within gastric adenocarcinoma. New blood vessles in the cancerous lesions had no regular contour and were not evenly distributed.

The relationship between the rate of COX-2 expression and MVD

The result showed that MVD (61.29±14.31) in the COX-2-positive gastric cancerous tissues was higher than that (45.38±12.43) in the COX-2-negative one (t=5.64, P<0.001). The expression of COX-2 was positively correlated with MVD (r=0.63, P<0.05).

The relationship between the expression of COX-2, MVD and pathological features of gastric carcinoma

In Table 1, the associations between COX-2, MVD expression and the pathological features were shown. Both COX-2 and MVD were not correlated with tumor size, tumor histological type. However, there was a correlation between COX-2, MVD and depth of invasion and lymph-node metastasis of gastric carcinoma respectively.

| Pathological characteristics | Positive COX-2(%) | MVD | Total |
|-----------------------------|------------------|-----|-------|
| Tumor size                   |                  |     |       |
| <5 cm                        | 15(75.0)         | 60.64±18.55 | 20   |
| ≥5 cm                        | 20(80.0)         | 55.68±17.98 | 25   |
| Depth of invasion            |                  |     |       |
| Invading serosa              | 29(87.88)a       | 57.01±18.79 | 33   |
| Noninvasion to serosa        | 6(50.0)          | 42.35±14.65 | 12   |
| Degree of differentiation    |                  |     |       |
| Well differentiated           | 12(80.0)         | 52.45±17.67 | 30   |
| Poorly differentiated        | 23(76.67)        | 57.32±18.20 | 15   |
| Lymph-node metastasis        |                  |     |       |
| Positive                     | 31(86.11)b       | 58.60±18.24 | 36   |
| negative                     | 4(44.44)         | 43.54±15.05 | 9    |

Note: well differentiated cancer cells include highly and moderately differentiated ones; poorly differentiated cancer
cells include poorly differentiated and undifferentiated ones. \( p < 0.05 \) (\( \chi^2 = 5.23 \)), vs the rate of COX-2 expression in gastric carcinomas not involving serosa; \( p < 0.05 \) (\( \chi^2 = 5.08 \)), vs the rate of COX-2 expression in gastric carcinoma without lymph-node metastasis; \( p < 0.05 \) (\( t = 2.44 \)), vs MVD in gastric carcinomas not involving serosa; \( p < 0.05 \) (\( t = 2.28 \)), vs MVD in gastric carcinomas without lymph-node metastasis.

**DISCUSSION**

Human gastric mucosa normally expresses no detectable levels of COX-2 protein\(^{27,28}\). In the current study, we found that the rate of COX-2 expression in gastric cancer was significantly increased, compared with that in the paracancerous tissues, the expression of COX-2 showed cytoplasmic staining, not only in cancerous cells but also in precancerous lesion such as atypical hyperplasia and intestinal metaplasia. A similar pattern of COX-2 expression has previously been found in human gastric cancer\(^{29-34}\). The above data demonstrated that COX-2 was up-regulated in human gastric cancer, suggesting COX-2 may play an important role in occurrence of gastric cancer, being a relatively early event in the carcinogenesis of stomach.

Here, we also analyzed the relationship between COX-2 expression and clinicopathological features in gastric carcinoma. It was shown that the rate of COX-2 expression was correlated closely with the depth of tumor invasion, indicating COX-2 may contribute to invasive growth of gastric carcinoma. The rate of COX-2 expression of gastric carcinoma with lymph-node metastasis was higher than that without suggesting the increase of its expression in gastric cancer tissue can promote lymph-node metastasis. It seemed more likely that COX-2 probably heightened viability and increased infiltrative potential of gastric cancer. The mechanism was not clear. Tsujii concluded that overexpression of the COX-2 gene as a result of transfection promoted invasiveness in wild type human colon carcinoma cell lines through the induction of metalloproteinase-2 and membrane-type metalloproteinase\(^{35}\). Rat intestinal epithelial cells that overexpressed COX-2 protein were found to be resistant to butyrate-induced apoptosis and had elevated bcl-2 protein expression and decrease expression of both E-cadherin and the transforming growth factor-\( \beta \) receptor\(^{36,37}\). Each of these changes has been linked to enhanced tumorigenic potential and increased tumor invasiveness. Therefore, the above data further indicated that COX-2 might play an important role in gastric tumorigenesis and tumor progression.

Recently the relation of COX-2 and tumor angiogenesis is emphasized. One of the mechanisms by which PGE\(_2\) supports tumor growth is by inducing the angiogenesis necessary to supply oxygen and nutrients to tumors \( > 2 \text{ mm in diameter} \).\(^{37,38}\), Masferrer\(^{39}\) reported that SC-236, a COX-2-selective inhibitor, was effective in reducing angiogenesis driven by bFGF in the matriel rat model, whereas SC-560, a COX-1-selective inhibitor was ineffective. He also observed COX-2 expression in newly formed blood vessels within tumors grown in animals, whereas under normal physiological conditions the quiescent vasculature expressed only the COX-1 enzyme, indicating COX-2-derived prostaglandins contributed to tumor angiogenesis\(^{40}\). In our study, COX-2 expression was also detected in the angiogenic vasculature present within the tumors and preexisting vasculature adjacent to cancer lesions, suggesting COX-2 may induce newly formed blood vessels to sustain tumor cell viability and growth. COX-2 was also expressed within atypical hyperplasia, intestinal metaplasia and neovascularization in the paracancerous tissue, indicating COX-2 may promote precancerous lesion to cancer by new blood vessel formation.

MVD is a reliable index of tumor angiogenesis\(^{41}\). We found that the MVD in COX-2 positive tumors was significantly higher than that in COX-2 negative tumors, MVD in gastric carcinoma was higher than that in paracancerous tissues, suggesting its distribution was similar to the pattern of COX-2 in gastric carcinoma. A close correlation was present between MVD and COX-2 (\( P < 0.01 \)), indicating COX-2 was closely related to tumor angiogenesis further, and may be one of important factors involved in gastric carcinoma angiogenesis. In addition, MVD in the specimens with lymph node metastasis was significantly higher than that without and it was also correlated closely with the depth of tumor invasion, suggesting that tumor angiogenesis in gastric carcinomas might result in cancer cells entering blood circulation, and the lymph node metastasis could be promoted when the gastric cancer cells invade lymphatic vessels. Both COX-2 and MVD were associated with the depth of invasion and lymph-node metastasis, suggesting the effect of COX-2 on angiogenesis can promote metastatic potential as well as tumor invasiveness. Therefore, inducing tumor angiogenesis may be one of mechanisms which COX-2 promotes the development and metastasis of gastric cancer.

In conclusion, COX-2 expression in gastric adenocarcinoma was higher than that in the paracancerous tissues, and was related to lymph node metastasis and the depth of invasion, suggesting COX-2 might correlate with the occurrence and advancement of gastric carcinoma; COX-2 expression in gastric carcinoma was closely related to MVD, suggesting COX-2 might be involved in tumor angiogenesis in gastric carcinoma, it is likely that COX-2 inducing angiogenesis may be one of mechanisms which COX-2 promotes the invasion, metastasis of tumor in gastric carcinoma. These findings suggest that COX-2 may be a new therapeutic target for anti-angiogenesis.

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