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• GASTRIC CANCER •

Expression of cyclooxygenase-2 and clinicopathologic features in human gastric adenocarcinoma

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Abstract

AIM: To study the expression of cyclooxygenase-2 (COX-2) gene in gastric cancer and the relationship between COX-2 expression and clinicopathologic features of gastric cancer.

METHODS: With reference to the expression of β-actin gene, COX-2 mRNA level was examined in cancerous tissues and adjacent noncancerous mucosa from 33 patients by semiquantitative reverse transcription- polymerase chain reaction (RT-PCR). Quantitation of relative band Adj volume counts was performed using molecular Analyst for windows software. The COX-2 index was determined from the band Adj volume counts ratio of COX-2 to constitutively expressed actin.

RESULTS: The COX-2 index in gastric carcinoma was significantly higher than that in normal mucosa (0.5966±0.2659 *vs* 0.2979±0.171, u=5.4309, P<0.01). Significantly higher expression of COX-2 mRNA was also observed in patients with lymph node involvement than that in those without (0.6775±0.2486 *vs* 0.4105±0.2182, t=2.9341, P<0.01). Furthermore, the staging in the UICC TNM classification significantly correlated with COX-2 overexpression (F=3.656, P<0.05), the COX-2 index in stage III and IV was significantly higher than those in stage I and II(q=3.2728 and q=3.4906, P<0.05). The COX-2 index showed no correlation with patient's age, sex, blood group, tumor location, gross typing, depth of invasion, differentiation, and the greatest tumor dimension (P>0.05).

CONCLUSION: Expression of COX-2 mRNA in gastric carcinoma was significantly higher, which may enhance lymphatic metastasis in patients with gastric carcinoma. The staging in the UICC TNM classification was significantly correlated with COX-2 over-expression. COX-2 may contribute to progression of tumor in human gastric adenocarcinoma.

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INTRODUCTION

Cyclooxygenase-2 (COX-2) is a rate-limiting enzyme in conversion of arachidonic acid to prostaglandins, also referred to as prostaglandin endoperoxide synthase (PGHS). Epidemiological and clinical results have suggested that non-steroidal anti-inflammatory drugs (NSAIDs) may reduce the risk of digestive tract carcinomas, including gastric and colon lesions. COX-2, which has been identified as being overexpressed in colorectal and gastric cancers, is one of the major targets of NSAIDs. Not only specific inhibitors of COX-2 significantly suppressed proliferation of gastric cancer cell lines[1, 2], but also a recent in-vitro study suggested that specific inhibitors of COX-2 significantly suppressed proliferation of gastric cancer xenografts in nude mice[3]. This evidence supports the hypothesis that COX-2 is an important factor in the growth and development of gastric cancer. We used the semiquantitative reverse transcription- polymerase chain reaction (RT-PCR) to examine the expression of COX-2 mRNA in gastric cancerous tissues and adjacent noncancerous mucosa, and to study the relationship to the clinicopathologic features.

MATERIALS AND METHODS

Patients and samples

Thirty-three patients undergoing surgery for primary gastric cancer at the Third Clinical Hospital of Harbin Medical University from 2001 to 2002 were examined. Of these, 27 were male and 6 were female. The mean age was 58.6 years (range, 32-76). The clinicopathologic features showed in Table 1. Paired samples of cancer tissue and normal gastric mucosa (the distance to border of tumor is beyond 5 cm) were obtained from each patient at the time of surgery. The samples were immediately frozen in liquid nitrogen. All specimens were verified by the same pathologists.

Methods

Total RNA was isolated from 50-100 mg of the tissues according to the method of User Manual TRIzol reagent (Life Science) and was quantitated by reading absorbance at 260 nm. The total RNA solution was subjected to RT-PCR analysis using the TITANIUM™ one-step RT-PCR kit (CLONTECH Laboratories, Inc, USA). The total RNA specimens (1 μg) were reverse transcripted and amplified in 25 μl of reaction mixture, and the reaction conditions for COX-2 and β-actin was identical.

Oligonucleotide primers for COX-2 used were 5' -TGA AAC CCA CTC CAA ACA CAG-3' (sense) and 5' -TCA TCA GGC ACA GGA GGA AG-3' (antisense), the PCR product length was 232 bp; those for β-actin were 5' -GTT TGA GAC CTT CAA CAC CCC-3' (sense) and 5' -GTG GCC ATC TCT CTT GCT CGA AGT C-3' (antisense), the PCR product length was 320 bp. The RT-PCR procedures were as following: reverse transcription at 55 ℃ for 60 min, inactivation of reverse transcriptase at 94 ℃ for 5 min, amplification for 30 cycles of denaturation at 94 ℃ for 30 sec, annealing at 55 ℃ for 30 sec, and extension at 68 ℃ for 60 sec.

The PCR products were electrophoresed in 2 % agarose gels with 0.5 μg/ml ethidium bromide and visualized under UV light. Quantitation of relative band volume counts was

performed using Molecular Analyst for windows software. To estimate COX-2 expression levels, a COX-2 index was designated as a band volume counts ratio of COX-2 to constitutively expressed β-actin, because β-actin mRNA is expressed constitutively in both the normal gastric mucosa and tumor tissues. The higher COX-2 index showed the higher expression level of COX-2 mRNA in tissues.

Statistical analysis

Statistical significance was calculated with the Students *t* test, Students *u* test, one-way ANOVA and Student-Newman-Keuls. $P<0.05$ was selected as the statistically significant value. All results are shown as means ±SE.

RESULTS

The total RNA was electrophoresed in 1 % agarose gels with 0.5 μg/ml ethidium bromide and visualized under UV light, then showed three bands: 5s, 18s and 28s. The total RNA was quantitated by reading absorbance at 260 nm, and A260/280 that ranged from 1.7 to 2.0.

COX-2 mRNA and β-actin mRNA were amplified by RT-PCR, and their products length were 232bp and 320bp. The β-actin mRNA expressed constitutively in all tissues, including normal gastric mucosa and tumor tissues (Figure 1). COX-2 mRNA expressed in 29 of 33 (87.88 %) human gastric cancer specimens, over-expression was seen in 26 of 33 (78.79 %) cases (COX-2 index was 0.5966±0.2659), and weak or negative COX-2 expression was seen in 25 of 33 (75.8 %) normal mucosa (COX-2 index was 0.2979±0.171) (Figure 2). COX-2 index in gastric carcinoma was significantly higher than that in normal mucosa ($u=5.4309$, $P<0.01$). Significantly higher expression of COX-2 mRNA was also observed in patients with lymph node involvement than that in those without (0.6775±0.2486 *vs* 0.4105±0.2182, $t=2.9341$, $P<0.01$). Furthermore, the staging in the UICC TNM classification (1985) significantly correlated with COX-2 overexpression ($F=3.656$, $P<0.05$), COX-2 index in stages III and IV was significantly higher than those in stages I and II ($q=3.2728$ and $q=3.4906$, $P<0.05$). COX-2 index showed no correlation with patient's age, sex, blood group, tumor location, gross typing, depth of invasion, differentiation, and the greatest tumor dimension ($P>0.05$, Table 1).

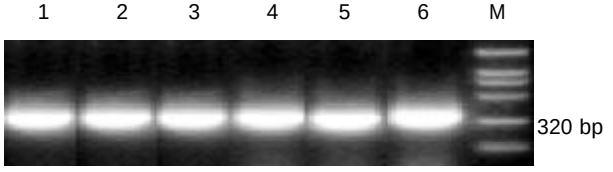

Figure 1 Representative β-actin mRNA expression in samples analyzed by RT-PCR. Lane M, molecular marker, from above down: 2 000, 1 000, 750, 500, 250, 100 bp.

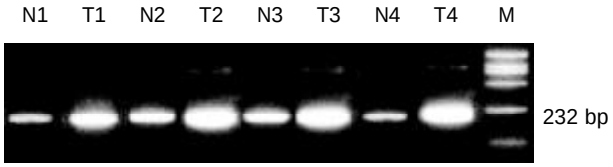

Figure 2 Representative COX-2 mRNA expression in gastric carcinoma tissues analyzed by RT-PCR. Lane M, molecular marker, from above down: 2 000, 1 000, 750, 500, 250, 100 bp. Lane N, noncancerous tissue; lane T, cancerous tissue.

Table 1 Clinicopathologic features in these patiens and COX-2 index ($\bar{x}\pm s$)

Parameter	$\bar{x}\pm s$	*P*
Sex		
male ($n=27$)	0.5798±0.2754	NS[a]
female ($n=6$)	0.6722±0.2012	
Age (range)		
≤60 ($n=16$)	0.6144±0.2655	NS
>60 ($n=17$)	0.5799±0.2651	
Blood group		
A type ($n=10$)	0.5928±0.2647	NS
B type ($n=11$)	0.6415±0.264	
O type ($n=7$)	0.5906±0.2614	
AB type ($n=5$)	0.6221±0.2551	
Greatest tumor dimension		
<5 cm ($n=11$)	0.4929±0.2807	NS
≥5 cm ($n=22$)	0.6485±0.242	
Histopathologic type		
Middling differentiation ($n=7$)	0.5772±0.2645	NS
Low differentiation ($n=9$)	0.5563±0.249	
Middling and low differentiation ($n=6$)	0.4278±0.2911	
Signet-ring / mucous cell ($n=11$)	0.7341±0.186	
Gross type		
Borrmann I ($n=8$)	0.4897±0.28	NS
Borrmann II III ($n=22$)	0.6244±0.2672	
Borrmann IV ($n=3$)	0.6785±0.0443	
Depth of invasion[b]		
pT2 ($n=10$)	0.5198±0.2516	NS
pT3 ($n=11$)	0.5682±0.2526	
pT4 ($n=12$)	0686±0.2633	
Lymph node[b]		
pN0 ($n=10$)	0.4105±0.2182	<0.05
pN1 and pN2 ($n=23$)	0.6775±0.2486	
TNM stage[b]		
I and II ($n=9$)	0.4054±0.2275	<0.05
III ($n=13$)	0.6561±0.2233	
IV ($n=11$)	0.6829±0.2631	
Tumor location		
Antrum ($n=18$)	0.5678±0.314	NS
Corpus ($n=6$)	0.6771±0.1488	
Cardiac orifice ($n=9$)	0.6006±0.203	

[a]NS, not significant. [b]Each factor of pT, pN and TNM stage was determined according to the UICC TNM classification, published in 1985.

DISCUSSION

Two isoforms of COX have been identified: COX-1 expressed constitutively in a number of cell types, which take part in sustaining physiologic function of body; COX-2 is a inducing immediate-early gene, and human gastric mucosa normally expresses detectable levels of COX-2. COX-2 is induced by a variety of cytokines, hormones, and tumor promoters, leading to more PGs producing. Initial association of COX-2 with tumor has shown in studies for colorectal cancer, and then for other tumor[4-10]. A series of studies confirmed that COX-2 levels elevated in colorectal carcinoma, and over-expression of COX-2 in colorectal cancer was associated with carcinogenesis, develop ment[11-17] and poor prognosis[18, 19]. In recent years, a lot of researchers studied the association of COX-2 expression with gastric carcinoma[20-26], but those conclusions were not identical, with a variety of causes.

In the current study, we found that elevated levels of COX-2 mRNA in human gastric adenocarcinoma tissues, and the COX-2 index in gastric carcinoma was significantly higher than that in normal mucosa (u=5.4309, P<0.01), which is identical to the data published[20-26]. The mechanism of the COX-2 involved in the pathogenesis of tumor is that over-expression of COX-2 may promote PGs biosynthesis in gastric cancer cells, and PGs shows a potent immunosuppression effect by inhibiting the T-cell or natural killer cell activity[27]. PGs thus provide a selective advantage for cancer cell survival; in addition, COX-2 can also suppress cell apoptosis[28-32], prolong cell cycle G1[33,34], and decrease level of cyclin D1[35, 36], which lead to that cells can not enter the cycle and proliferate continuously. Meantime, COX-2 enhances adhesion of cells[37, 38] and promotes tumor angiogenesis[22,39], which may finally lead to the carcinogenesis and progression of the tumor.

Many researchers[20-40] found that COX-2 mRNA expression in gastric carcinoma is correlated closely with depth of invasion, indicating that COX-2 is involved in the growth of the tumor. But, our studies did not find a correlation between COX-2 expression and the extent of primary tumor invasion. Our recent studies have found a correlation between the level of COX-2 expression and lymph node metastasis in patients with gastric carcinoma. Murata *et al*[21] and Leung *et al*[41] found that tumor with the overexpression of COX-2 protein by Western blot and immunohistochemical analysis was associated significantly with invasion into gastric wall lymphatic vessels as well as with metastasis to lymph nodes (P<0.05). Uefuji *et al*[40] detected level of COX-2 mRNA expression by RT-PCR analysis, and the conclusion was in agreement with this study. We demonstrated that significantly higher expression of COX-2 mRNA was also observed in patients with lymph node involvement than that in those without (t=2.9341, P<0.01). It is suggested that COX-2 may influence lymphatic involvement by the way of increasing tumor invasiveness. Some studies have found that over-expression of COX-2 decreased the expression of both E-cadherin and the transforming growth factor-β receptor, which has been linked to enhancing tumorigenic potential and increasing tumor invasiveness[37,38,42-45]. Meantime, the overexpression of the COX-2 promotes invasiveness in gastric cancer through the induction of metalloproteinase-2 and membrane-type metalloproteinase[11,21,46].

Although several investigators[20] reported that the COX-2 level was not associated with UICC TNM stage, but majority of them[21, 23] have reported a significant relation between the levels of COX-2 protein over-expression and UICC TNM stage. Our results are in agreement with previous ones. The COX-2 level in Stage III and IV was significantly higher than in Stage I and II (q=3.2728 and q=3.4906, P<0.05); but the difference of COX-2 level between Stages III and IV showed no statistical significance (q=0.3702, P>0.05). Consequently, we can infer that COX-2 may be independent or synergistic with other factors to promote growth of gastric cancer, and to enhance the lymph node metastasis and involvement. But, in advanced gastric cancer, when COX-2 expression is up-regulated to a certain level, COX-2 can not increase continuously, which suggests that there are some much more complicated mechanisms in the regulation of COX-2 expression.

In conclusion, COX-2 mRNA shows elevated expression in gastric carcinoma tissues, and the degree of COX-2 mRNA elevation is related to the lymphatic metastasis, UICC TNM stage, and poor prognosis. These findings suggest that COX-2 is involved in the carcinogenesis and growth of gastric carcinoma and that the inhibition of COX-2 activity may prove to have an important therapeutic benefit in the control of gastric carcinoma.

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