

Expression of c-erbB-2 and Cyclooxygenase-2 in Pancreatic Ductal Adenocarcinoma

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Background : Carcinoma of the pancreas is a fatal malignant disease with limited therapeutic options. Cyclooxygenase-2 (COX-2) and c-erbB-2 are known to be involved in the carcinogenesis, differentiation and invasiveness of various neoplasms. We studied the immunohistochemical expressions of c-erbB-2 and COX-2 and the correlation between these expressions and the clinicopathologic parameters and the relation between the expressions. **Methods** : Immunohistochemical staining for c-erbB-2 and COX-2 were performed on the paraffin embedded sections of 36 cases of surgically resected ductal adenocarcinoma of the pancreas and 10 cases of non-neoplastic pancreas tissue. **Results** : The non-neoplastic control group showed a c-erbB-2 expression in the acini (8/10) and ducts (2/10), and a COX-2 expression in the acini (6/10) and ducts (3/10). The overexpression of c-erbB-2 was observed in 58% (21/36) of the carcinoma specimens. No significant correlation was found between c-erbB-2 and age, gender, tumor size, gross type, histologic grade, vascular invasion, perineural invasion, lymph node metastasis, and the TNM stage. The overexpression of COX-2 was observed in 41.7% (15/36) of the carcinoma specimens. The COX-2 expression was significantly high in the lymph node metastasis group ($p < 0.05$), but it was not correlated with the other clinicopathologic parameters. Also there was no significant correlation between the c-erbB-2 and COX-2 expressions. **Conclusions** : In pancreatic ductal adenocarcinomas, c-erbB-2 and COX-2 were frequently overexpressed, and COX-2 overexpression was correlated with lymph node metastasis.

Key Words : Carcinoma; Pancreatic ductal; c-erbB-2 Protein; Cyclooxygenase-2; Immunohistochemistry

Pancreas carcinoma is one of the most deadly malignant neoplasms due to the difficulty to detect it early and the limited choices for treatment. The only effective therapy is surgical excision. Adjuvant chemo- and radiotherapy provide only a minimal survival advantage.¹ *K-ras* mutations are found in the majority of pancreatic ductal adenocarcinomas, and the additional loss of the p53, p16 and DPC4/SMAD functions in carcinogenesis has been suggested.² Yet the exact pathogenic mechanism and progression of this neoplasm remain unexplained and effective neo-chemopreventive or chemotherapeutic modalities are needed.

Recent studies have highlighted the potential role of cyclooxygenase-2 (COX-2) in carcinogenesis. COX-2 is an inducible enzyme that is encoded in chromosome 9, with a cDNA of 4.3 kb. COX enzymes catalyze the rate-limiting step in arachidonic acid metabolism, resulting in prostaglandin H₂ production. This molecule is the precursor of other prostaglandins, prostacyclin, and thromboxanes. The COX-2 gene is dissimilar to COX-1,

which is constitutively expressed, and the COX-2 gene is regulated at the site of transcription in response to inflammation, ovulation and a variety of other mitogens, cytokines and growth factors.³ In 1994, the induction of COX-2 was reported in carcinoma of colon, and in subsequent years, increased levels of COX-2 were found in carcinomas of the stomach, esophagus, and lung.⁴⁻⁷ Importantly, the overexpression of COX-2 in human carcinomas appears to be of functional significance because there is accumulating evidence that selective COX-2 inhibitors prevent carcinogenesis in experimental animals and these compounds induce apoptosis in several types of carcinoma cells.⁸⁻¹⁰

The *ErbB-2* gene encodes a 185-kDa transmembrane receptor with tyrosine kinase activity that belongs to the family of receptors for epidermal growth factors.¹¹ The overexpression of c-erbB-2 is found in various types of neoplasm. It has been suggested that the overexpression of c-erbB-2 is associated with invasiveness and a poor prognosis.

The link between c-erbB-2 and COX-2 has been described in breast, colorectal and prostate cancers.¹²⁻¹⁴ High levels of COX-2 have been detected in c-erbB-2 positive tumors. This observation suggests that c-erbB-2 mediates the induction of COX-2 gene transcription.

In the present study, we examined the expression of COX-2 and c-erbB-2 by performing immunohistochemistry in pancreatic ductal adenocarcinomas and normal pancreatic tissue. In addition, we studied the relationship between these expressions and the clinicopathologic parameters and between these expressions.

MATERIALS AND METHODS

Patients and tissue specimens

Thirty-six patients with ductal adenocarcinoma of the pancreas and who underwent pancreaticoduodenectomy at Yeungnam University Hospital were selected for this study. The pancreatic resection specimens were entirely submitted for histological evaluation, and blocks harboring infiltrating carcinomas were selected for the immunohistochemistry. TNM staging was performed according to the American Joint Committee of Cancer (AJCC) manual.¹⁵ Ten cases of non-neoplastic normal pancreas were obtained from trauma patients or from surgical operations for other organ diseases.

Immunohistochemistry

Immunohistochemical staining of c-erbB-2 and COX-2 was performed using a modification of the standard avidin biotin-peroxidase complex method. The slides were deparaffinized, and the endogenous peroxidase activity was blocked by incubating the slides in 3% H₂O₂ in methanol for 10 min at room temperature. The sections were then microwaved in phosphate-buffered saline for 4 min for antigen retrieval and next they were incubated with avidin and then biotin (Vector Laboratories, Burlingame, CA) for 15 min each to block nonspecific binding. An immunoperoxidase technique was performed using the Vectastain ABC Elite kit (Vector Laboratories). A mouse monoclonal antibody against human COX-2 (Cayman Chemical Co., Ann Arbor, MI) and mouse monoclonal IgG1 anti-HER2 (Clone CB11, Zymed Lab, South San Francisco, CA) were then applied at a dilution of 1:50 and 1:100, respectively, overnight at 4°C. The latter antibody recognizes a 19-amino acid sequence at the COOH terminus of COX-2, which is absent in COX-1. Following rinsing with PBS, the biotinylated secondary IgG antibody was applied for 30 min at room temperature. Hematoxylin was used as a counterstain. More than 10% complete membranous staining was considered positive for c-erbB-2 and more than 10% cytoplasmic staining was considered positive for the COX-2 staining (Fig. 1).

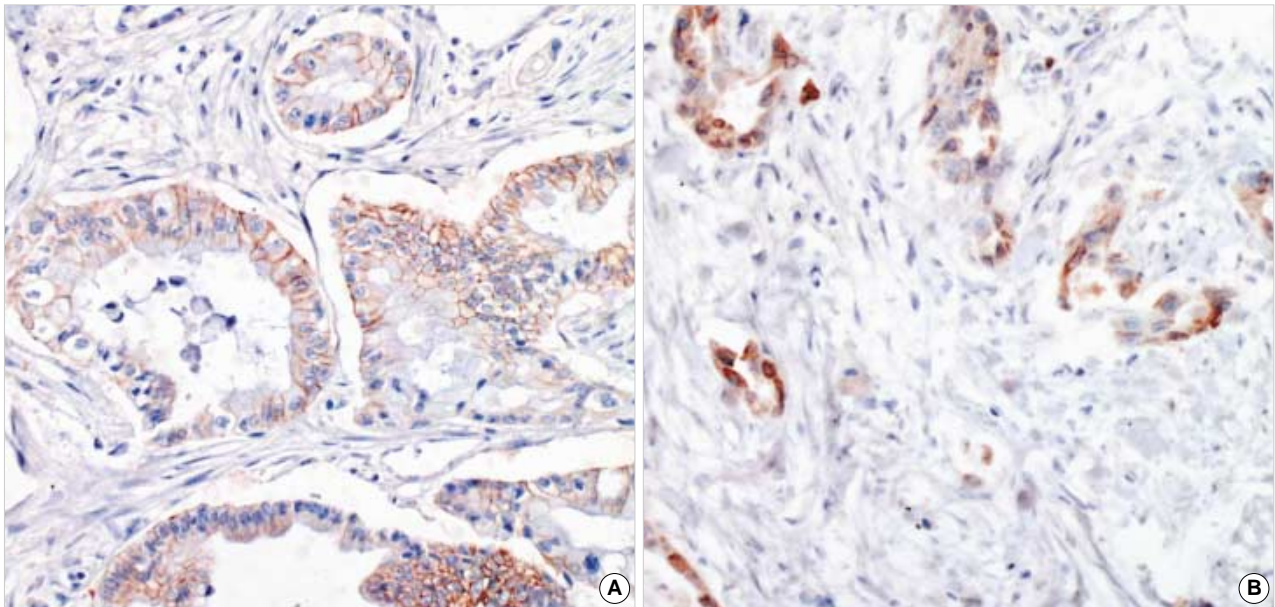


Fig. 1. Immunohistochemical stain for c-erbB-2 and COX-2. (A) Tumor cells show membranous positivity for c-erbB-2 in well differentiated adenocarcinoma. (B) Tumor cells show cytoplasmic positivity for COX-2 in moderately differentiated adenocarcinoma.

Table 1. Correlation between COX-2, c-erbB-2 overexpression and clinicopathologic parameters

Parameters	No. patients	COX-2 expression			c-erbB-2 expression		
		Positive (%)	Negative (%)	p value	Positive (%)	Negative (%)	p value
Age							
<40	1	0 (0)	1 (100)	0.740	1 (100)	0 (0)	0.217
40-49	8	3 (37.5)	5 (62.5)		6 (75)	2 (25)	
50-59	12	5 (41.7)	7 (58.3)		4 (33.3)	8 (66.7)	
60-69	11	6 (54.5)	5 (45.5)		8 (72.7)	3 (27.3)	
>70	4	1 (25)	3 (75)		2 (50)	2 (50)	
Sex							
Male	19	7 (36.8)	12 (63.2)	0.548	11 (57.9)	8 (42.1)	0.611
Female	17	8 (47.1)	9 (52.9)		10 (58.8)	7 (41.2)	
Tumor size							
<3 cm	3	1 (33.3)	2 (66.7)	0.665	2 (66.7)	1 (33.3)	0.904
3-5 cm	29	12 (41.4)	17 (58.6)		17 (58.6)	12 (41.4)	
>5 cm	4	2 (50)	2 (50)		2 (50)	2 (50)	
Differentiation							
Well	12	5 (41.7)	7 (58.3)	0.812	6 (50)	6 (50)	0.182
Moderate	18	7 (38.9)	11 (61.1)		13 (72.2)	5 (27.8)	
Poorly	6	3 (50)	3 (50)		2 (33.3)	4 (66.7)	
Vascular invasion							
Negative	7	1 (14.3)	6 (85.7)	0.111	6 (85.7)	1 (14.3)	0.111
Positive	29	14 (48.3)	15 (51.7)		15 (51.7)	14 (48.3)	
Perineural invasion							
Negative	16	4 (25)	12 (75)	0.069	8 (50)	8 (50)	0.285
Positive	20	11 (55)	9 (45)		13 (65)	7 (35)	
Lymph node metastasis							
Negative	22	4 (18.2)	18 (81.8)	0.001	12 (54.5)	10 (45.5)	0.411
Positive	14	11 (78.6)	3 (21.4)		9 (64.3)	5 (35.7)	
Stage							
I	2	0 (0)	2 (100)	0.308	2 (100)	0 (0)	0.200
II	32	14 (43.8)	18 (56.2)		17 (53.1)	15 (46.9)	
III	1	0 (0)	1 (100)		1 (100)	0 (0)	
IV	1	1 (100)	0 (0)		1 (100)	0 (0)	

Statistical analysis

The categorical variables were compared using the Fisher exact two-tailed test and χ^2 tests, and results were analyzed with SPSS statistical software V10.0 (SPSS inc., Chicago, IL, USA). Throughout this study, a 5% significance level was used for the statistical tests.

RESULTS

There were 19 (52.8%) males and 17 (47.2%) females (age range: 32 and 76). Twelve (33.3%) cases were well differentiated, 18 (50.0%) cases were moderately differentiated, and 6 cases (16.7%) were poorly differentiated. We used a grading system that was based on combined assessment of the histological and cytological features and the mitotic activity.¹⁶

The normal pancreas showed positive COX-2 reactivity in

Table 2. Correlation between c-erbB-2 and COX-2 expression in pancreatic ductal adenocarcinoma

	COX-2 expression		p value
	Positive (%)	Negative (%)	
c-erbB-2 expression			
Positive	11 (52.4)	10 (47.6)	0.123
Negative	4 (26.7)	11 (73.3)	

the acini (6/10) and ducts (3/10). There was no positive reactivity in the islet. The normal pancreas tissues showed positive c-erbB-2 reactivity in the acini (8/10), ducts (2/10) and islets (3/10). The overexpression of COX-2 was observed in 41.7% (15/36) of the pancreatic ductal adenocarcinomas. The COX-2 expression was significantly higher in the lymph node metastasis group ($p < 0.05$) and it tended to be higher in the perineural invasion group, but it was not correlated with the other clinicopathologic parameters (Table 1).

The overexpression of c-erbB-2 was observed in 58% (21/36)

of the adenocarcinoma specimens (21/36). No significant correlation was found between c-erbB-2 and age, gender, tumor size, gross type, histological grade, vascular invasion, perineural invasion, lymph node metastasis, and the TNM stage (Table 1).

There was no significant correlation between the c-erbB-2 and COX-2 expressions (Table 2).

DISCUSSION

Pancreatic carcinoma is one of the most deadly types of neoplasm, and pancreatic ductal adenocarcinoma accounts for 85-90% of all pancreatic neoplasms.

The present study investigated the involvement of COX-2 and c-erbB-2 in ductal adenocarcinomas of the pancreas by an immunohistochemical method and by comparing the findings with the normal pancreatic tissue expression. There have been only a limited number of studies concerned with the expressions of COX-2 or c-erbB-2 in the normal pancreas.

We observed the acinar (6/10) and ductal (3/10) COX-2 expression, and mainly the acinar expression (8/10) of c-erbB-2 expression in normal pancreas tissue. We also noted the ductal (2/10) and islet (3/10) c-erbB-2 expressions. Though Okami *et al.*¹⁷ observed that the expression of COX-2 was exclusively in the islets, Albazaz *et al.*¹⁸ reported on the acinar, ductal and islet cell COX-2 expression in normal pancreas. The latter research group observed an increased COX-2 expression in pancreatic adenocarcinoma and pancreatic intraepithelial neoplasia, as compared to normal tissue. They evaluated the immunostaining in a semiquantitative manner by assessing the intensity and extent of staining in the positive cells. Apple *et al.*¹⁹ reported non-positive c-erbB-2 staining in the normal ducts out of 30 non-malignant and malignant specimens of pancreas. A progressive increase was reported in the intensity of staining and the frequency of c-erbB-2 positivity in hyperplasia, dysplasia, and adenocarcinoma. Apple and coworkers showed much higher positivity (82%) than our result (58%) in the ductal adenocarcinomas. It seemed that normal ducts reveal positive reactions to COX-2 and c-erbB-2, though this is a much lower and weaker reaction.

There are several studies on c-erbB-2 and/or COX-2 overexpression in ductal adenocarcinoma of the pancreas, but the rate of overexpression varies between 20% and 80% and the prognostic significance is also variable.¹⁷⁻²⁴ These large variations might be partly caused by the different methodology used, the selected cases and the scoring system for the immunohistochemical results in those studies. Lee *et al.*²⁵ reported a 41.7% COX-2

positive rate and this was correlated with the proliferation index. Kang *et al.*²⁶ reported 40.4% c-erbB-2 overexpression and positive correlation with the p53 overexpression. Their positive rate is similar to our results. In the present study, the COX-2 expression was significantly high in lymph node metastasis and it was much higher in the perineural invasion group.

Vadlamudi *et al.*¹³ has shown that COX-2 is up-regulated in human colorectal carcinoma cells as a result of autocrine/paracrine activation of erbB-2/erbB-3 heterodimers. Though statistically insignificant, our study showed a doubled COX-2 expression rate in the c-erbB-2 expression group compared to the non-expression group. A larger number of cases might reveal the correlation between COX-2 and c-erbB-2.

Many lines of evidence indicate that the antitumor effects of NSAIDs may be due to the inhibition of COX activity. Tseng *et al.*²⁷ reported that the selective COX-2 inhibitor rofecoxib induces the expression of cell cycle to arrest genes and it slows tumor growth in human pancreatic cancer, according to in vitro and in vivo mice experiments. Yip-Schneider *et al.*²⁸ reported that COX-2 positive pancreatic tumor cell lines showed significantly suppressed tumor growth compared to a COX-2 negative pancreatic tumor cell line, which suggests that COX-2 may be a promising chemotherapeutic target for the treatment of pancreatic cancer. Further anticancer therapies targeting the c-erbB family of receptor tyrosine kinase receptors have been shown to be clinically effective in recent years.

The present study showed the frequency of the COX-2 and c-erbB-2 expressions, and this could be part of the baseline data for creating possible new chemopreventive and adjuvant therapeutic agents for pancreatic carcinoma.

In summary, we have shown that the overexpressions of the COX-2 and c-erbB-2 are relatively common events in pancreatic cancer, and the COX-2 expression is correlated with lymph node metastasis.

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