ORIGINAL ARTICLE

Cyclooxygenase-2 expression correlates with poor prognosis in pancreatic cancer

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J Clin Pathol 2006;**59**:382–386. doi: 10.1136/jcp.2005.026831

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Accepted for publication 26 August 2005

Background: Cyclooxygenase-2 (COX-2) overexpression is related to poor outcome in several cancers. COX-2 is upregulated in 42–90% of pancreatic ductal adenocarcinomas and is a potential target for chemotherapy. Earlier studies have not shown the expression of COX-2 to be a prognostic factor in pancreatic cancer.

Objective: To evaluate the prognostic value of COX-2 in a series of patients with pancreatic adenocarcinoma.

Methods: 128 patients operated on for pancreatic adenocarcinoma at Helsinki University Central Hospital between 1974 and 1998 provided sections from primary tumours which were immunohistochemically stained with a COX-2-antihuman monoclonal antibody.

Results: Cytoplasmic COX-2 reactivity (>5%) occurred in 46 specimens (36%), correlating neither with age, sex, stage, size, tumour stage, nodal metastases, nor grade. Lack of COX-2 expression correlated with distant metastases ($p = 0.026$). In univariate survival analysis, COX-2 expression ($p = 0.0114$), stage ($p = 0.0002$), grade ($p = 0.0001$), and age ($p = 0.042$) had prognostic significance. One, two, and five year survival rates were 51%, 32%, and 8% in the COX-2 negative groups compared with 34%, 5%, and 5% in the COX-2 positive groups ($p = 0.011$). Prognostic significance was especially high for patients operated on with curative intent ($p = 0.004$). In multivariate analysis, COX-2 was an independent prognostic factor (hazard ratio = 1.6 (95% confidence interval, 1.1 to 2.3)).

Conclusions: Expression of COX-2 was associated with poor outcome from pancreatic ductal adenocarcinoma and was independent of tumour stage, grade, or age in multivariate analysis.

Rudolf Virchow suggested in 1863 that there was a connection between cancer and persistent inflammation.[1] In population based studies, the use of non-steroidal anti-inflammatory drugs (NSAIDs) protects from colorectal and possibly from other cancers.[2–4] Cyclooxygenase-2 (COX-2) is an integral membrane protein and the rate limiting enzyme in the biosynthesis of such prostanoids as prostaglandins, thromboxanes, and prostacyclins in acute inflammation. In most tissues, COX-2 is not physiologically expressed. However, hormones, cytokines, growth factors, and tumour promoters rapidly induce COX-2 expression.[5 6] At molecular level, a key role in the process that links inflammation to carcinogenesis seems to be activation of COX-2, although the intracellular pathways in that process are still mostly unknown.

Increased tissue levels of COX-2 occur in several human carcinomas. In tumorigenesis, COX-2 may take part in stimulation of proliferation, in inhibition of apoptosis, and in invasion by enhancing production of matrix metalloproteinases and by promoting angiogenesis.[7–9] Increased COX-2 expression is associated with a poor prognosis in oesophageal, gastric, colonic, breast, and ovarian carcinomas.[10–14] In the pancreas, COX-2 is expressed in the cytoplasm of ductal tumour cells but not in the surrounding stroma.[15] In pancreatic ductal adenocarcinomas, COX-2 is upregulated in 42% to 90% of cases.[15–18] The association of aspirin use with pancreatic cancer risk has been explored in several epidemiological studies, with controversial results.[19] However, several NSAIDs inhibit pancreatic cancer in hamster models.[20 21] NSAIDs also inhibit the growth of human pancreatic cancer cell lines.[22]

Our aim in this study was to investigate COX-2 expression in a series of cases of pancreatic ductal adenocarcinoma and compare immunohistochemical staining results with clinicopathological factors such as survival, histological grade, TNM stage, tumour size, tumour location, age, and sex.

METHODS

Patients

The study involved surgical specimens from 128 consecutive patients undergoing surgery for pancreatic adenocarcinoma at Helsinki University Central Hospital between 1974 and 1998, and with a histological block available in the files of the Department of Pathology. The most representative sample of the primary tumour was chosen, and the diagnosis of pancreatic adenocarcinoma was confirmed from haematoxylin and eosin (H&E) and van Gieson stains by a pathologist (SN). The median age of the patients at diagnosis was 62 years (range 34 to 79); 72 (56%) were female and 56 (44%) male. Histological grade was re-evaluated by a pathologist (SN), revealing 14 well differentiated (grade 1), 77 moderately differentiated (grade 2), and 37 poorly differentiated (grade 3) tumours. Staging was done according to the UICC 1997 TNM classification, based on patient records, imaging methods, operation records, and histological evaluation. Patients comprised 25 at stage I, 39 at stage II, 28 at stage III, and 35 at stage IV. All patients underwent surgery, either curative (R0) pancreaticoduodenectomy (n = 85), non-curative (R1) pancreaticoduodenectomy (n = 33), palliative bypass (n = 6), or diagnostic laparotomy (n = 4). The operation was considered to be R0 pancreaticoduodenectomy when no macroscopic or microscopic residual tumour was present. Median survival for patients who underwent R0 pancreaticoduodenectomy was 15.1 months, for those

Abbreviations: COX-2, cyclooxygenase-2; HR, hazard ratio; NSAID, non-steroidal anti-inflammatory drug; TNM, tumour stage, node, metastasis classification; UICC, International Union Against Cancer

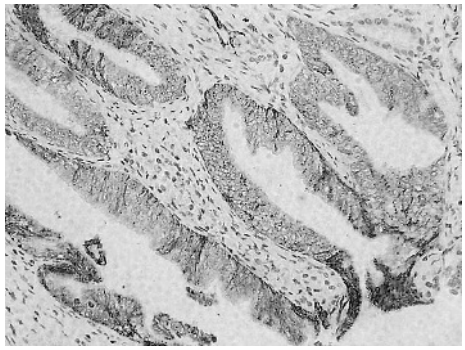

Figure 1 Immunohistochemical staining of COX-2 in pancreatic adenocarcinoma, showing a well differentiated ductal carcinoma with strong positivity for COX-2. The stain is evident in the cytoplasm of the tumour cells, whereas the adjacent stroma is negative. Original magnification ×20.

undergoing R1 pancreaticoduodenectomy 6.7 months, for those undergoing palliative bypass 2.4 months, and for those undergoing diagnostic laparotomy one month; 120 patients died of pancreatic cancer. Six patients were alive at the end of the study, and two died from diseases other than cancer. Survival data for the patients came from the patient records, Statistics Finland, and the Finnish Population Registry.

Staining

Our COX-2 immunohistochemical staining has been described in detail previously.[23] Briefly, archival formalin fixed, paraffin embedded tissue samples were freshly cut (4 μm), and deparaffinised, and microwave treated for antigen retrieval. For immunohistochemical staining, a COX-2 specific mouse antihuman monoclonal antibody (160112;5 Cayman Chemical, Ann Arbor, Michigan, USA) was used at a dilution of 1:200. Bound antibody was visualised by the avidin-biotin complex immunoperoxidase technique (Vectastain ABComplex, Vector Laboratories, Burlingame, California, USA). For each staining batch we used as a positive control a colon sample in which adenocarcinoma cells stained >50%, and adjacent epithelial non-neoplastic cells stained 5–10%. As an internal control we used pancreatic islet cells that consistently expressed COX-2.[24] As negative controls we used sections with phosphate buffered saline or non-immune antibody instead of primary antibody.

Interpretation of immunohistochemistry

Two independent pathologists (SN and AR) interpreted the staining results while unaware of the clinical data. COX-2 expression was considered negative if fewer than 5% of the tumour cells expressed COX-2, weak if 5–10% of the cells were positive, moderate if 10–50% of the cells were positive, and strong if more than 50% of the cells were positive. Cells were considered positive only if COX-2 intensity was moderate (granular cytoplasmic stain) or strong (diffuse +++ staining). Samples with 0–5% of any intensity were considered negative. The six samples given scores by the pathologists that differed by two categories were re-evaluated, and the consensus score served for further analysis. For dichotomic analysis, we chose 5% as the cut off line for COX-2 positive tumours.

Statistical analysis

The associations between factors were calculated by the χ^2 test and Fisher's exact test in cases of very small expected frequencies. For life tables we used the Kaplan–Meier product limit method. Differences in survival were compared by the log-rank or log-rank for trend test when appropriate. Disease specific overall survival was from the date of diagnosis to death from pancreatic cancer, with patients dying of other causes censored. Multivariate survival analysis was with the COX proportional hazards model, entering the following covariates: histological grade, TNM stage, COX-2 (negative *v* positive), tumour location (head of pancreas *v* other), tumour size (⩽2 cm *v* 2–4 cm *v* >4 cm), and age (⩽62 *v* >62 years). Cox regression was done by a backward stepwise selection of variables, and a probability (p) value of 0.05 was adopted as the limit for inclusion of a covariant. Statistical analyses were done using SPSS 11.0.1 software (SPSS Inc, Chicago, Illinois, USA).

Table 1 Distribution of COX-2 according to preoperative characteristics in 128 patients with pancreatic cancer

Clinicopathological variable	n	COX-2 >5% (n (%))	χ^2	p Value
Age (years)				
⩽62	64	18 (28%)	3.4	0.066
>62	64	28 (44%)		
Sex				
Female	72	26 (36%)	0.002	0.096
Male	56	20 (36%)		
Grade				
1	14	4 (28%)	3.7	0.158
2	77	24 (31%)		
3	37	18 (49%)		
Grades 1 and 2 v 3				
1 and 2	91	28 (31%)	3.6	0.056
3	37	18 (49%)		
Stage				
I	25	7 (28%)	4.0	0.261
II	39	15 (38%)		
III	28	14 (50%)		
IV	35	10 (29%)		
Unavailable	1	–		
Primary tumour (T)				
1	7	1 (14%)	2.4	0.495
2	28	9 (32%)		
3	63	26 (41%)		
4	29	10 (34%)		
Unavailable	1	–		
Regional nodes (N)				
0	78	27 (35%)	8.9	0.345
1	39	17 (44%)		
Unavailable	11	–		
Distant metastasis (M)				
0	118	46 (39%)	3.9	0.026
1	9	0 (0)		
Unavailable	1	–		
Tumour size				
⩽2 cm	19	6 (32%)	0.3	0.864
2–4 cm	66	25 (38%)		
>4 cm	29	10 (34%)		
Unavailable	14	–		
Curability				
Intent to cure	85	31 (36%)	0.03	0.860
Non-curative	43	15 (35%)		
Location				
Head	113	39 (35%)	1.1	0.225
Other location	13	7 (54%)		

COX-2, cyclooxygenase-2.

RESULTS

Immunoreactivity of COX-2 in 128 pancreatic ductal adenocarcinomas showed 82 (64%) to be negative, 16 (13%) weakly positive, 27 (21%) moderately positive, and three (2%) strongly positive. COX-2 expression was evident in cytoplasmic granules of ductal tumour cells, whereas the stroma was negative (fig 1). Islet cells stained positive in all samples, including those with no COX-2 expression in the tumour.

No correlation appeared between COX-2 expression and sex, grade, stage, nodal status, tumour size (<2 cm *v* 2–4 cm

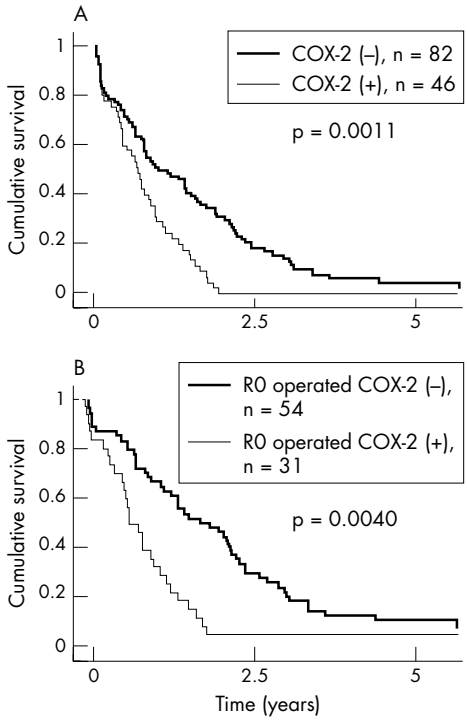

Figure 2 Cumulative survival curves for 128 patients with pancreatic cancer. Survival of those with COX-2 negative tumours was significantly better than that of patients with COX-2 positive tumours. This was true in (A) the whole patient group and (B) in patients undergoing R0 pancreaticoduodenectomy (n = 85).

v >4 cm), curability, or tumour location (head *v* other). COX-2 expression was associated with distant metastases (n = 9); none of the primary tumours with distant metastases (p = 0.026) showed any COX-2 expression. COX-2 was expressed more often in samples of older patients (>62 years), although not significantly so (p = 0.0655) (table 1).

Survival among patients with COX-2 negative tumours was (p = 0.011) than among those with COX-2 positive tumours (fig 2, table 2): one, two, and five year survival rates were 34%, 5%, and 5% in COX-2 positive categories, compared with 51%, 32%, and 8% in the COX-2 negative category. Median survival for patients with COX-2 positive tumours was 8.1 months, compared with 13.2 months for COX-2 negative tumours. Low histological grade, low TNM stage, no distant metastases, and curability showed a strong association with better survival in univariate survival analysis (p<0.001). Young age (p = 0.042), low stage (p = 0.045), small tumour size (p = 0.045), and tumour in head of pancreas (0.045) were also associated with better prognosis (table 2). Within the group of patients undergoing R0 pancreaticoduodenectomy, COX-2 expression in univariate analysis correlated with survival (p = 0.004). One, two, and five year survival rates were 40%, 7%, and 0% in COX-2 positive categories, compared with 67%, 46%, and 11% in the COX-2 negative category. Median survival was 10 months for COX-2 positive patients, compared with 20 months for patients with COX-2 negative tumours (fig 2B).

In multivariate analysis, COX-2 retained its independent prognostic significance (p = 0.018). TNM stage and histological grade (HR 3.5) were the strongest independent prognostic factors, followed by COX-2 (HR = 1.6).

DISCUSSION

In this retrospective study of 128 pancreatic adenocarcinoma patients, increased COX-2 expression was associated with a poor prognosis and was independent of stage and grade (HR = 1.6). COX-2 thus seems to be a promising prognostic marker, especially for patients undergoing R0 pancreaticoduodenectomy. Patients with COX-2 expression in their tumours had a strikingly poor prognosis; only two patients survived more than two years, even in the group undergoing surgery for cure. In our earlier studies on other cancer forms, COX-2 was also associated with poor outcome.[10 13 14 23 25]

In the present study COX-2 expression was less (36%) than in most published studies (42% to 90%).[15–18] One reason could be the use of different antibody preparations. For immunohistochemical staining we used a COX-2 specific mouse antihuman monoclonal antibody. Before choosing this, we tested other antibodies to find the optimal tool, as described in an earlier study.[26] To avoid intra-assay or interassay variability, we used the positive control described in Methods. This also helped us to score the trial specimens. For example Merati *et al*[27] used a polyclonal antibody, and Okami *et al*[28] used polyclonal rabbit antihuman COX-2 antibodies and had a 74% to 90% positivity rate. Our experience is that in antigenic blocking experiments polyclonal antibodies are more sensitive but do not show as high a specificity as the monoclonal antibody.[26] Using this same method we have reported the prognostic significance of COX-2 in other cancer forms, such as oesophageal, breast, ovarian, and gastric carcinoma (accepted for publication).[10 13 14]

The difference between our results and others may also depend in part on different cut off values. Our 5% cut off for positivity and only moderate (granular cytoplasmic stain) to strong (diffuse +++ staining) intensity meant that samples with 0 to 5% of any intensity were considered negative.

Three studies on COX-2 in pancreatic cancer show no significant association between COX-2 and prognosis.[27–29] One study on 120 patients showed only a tendency for association within the whole patient group and within a patient group that received chemotherapy.[28] One reason for differing results could be differences in patient series. Our patients received no chemoradiation but mostly underwent R0 pancreaticoduodenectomy. We had a more significant correlation between COX-2 and survival within patients operated on for cure. Another reason why results may differ regarding COX-2 association with survival may be the antibody used. In two reports including 50 and 72 patients, study power was unassessed.[28 29] Had the patient group been larger, results might have been similar to ours.

Many studies report somewhat improved survival rates in recent patient series, probably because of factors such as surgical techniques, postoperative care, and adjuvant protocols. Our patients experienced no major changes in surgical techniques or strategy during follow up. In Helsinki, extended lymphadenectomy was not initiated until 1999. In our series, no patients received neoadjuvant therapy and a few received postoperative chemotherapy. There certainly has been improvement in preoperative and postoperative care, but we find no reason to believe that changes in treatment would have affected COX-2 figures or our conclusions.

Both the hereditary and sporadic forms of chronic pancreatitis are associated with an increased risk developing pancreatic cancer,[30–32] which often shows a strong desmoplastic reaction around the tumour.[33] These cells produce cytokines, growth factors, and inflammation mediators[34] known to induce COX-2 expression. In chronic pancreatitis, COX-2 expression is increased in pancreatic acinar and hyperplastic ductal cells. Likewise in pancreatic cancer, the ductal expression of COX-2 is markedly upregulated.[35] It is reasonable to hypothesise that as COX-2 is inducible and implicated in epithelial tumour development, its expression in pancreatic tumour results in a poor prognosis. Our results are in accordance with this hypothesis, showing the

Table 2 Univariate analysis of the relation between preoperative characteristics and survival of 128 patients with pancreatic cancer

Clinicopathological variable	n	%	1 year CS (%)	95% CI	2 year CS (%)	95% CI	5 year CS (%)	95% CI	χ^2	p Value
COX-2										
Negative ⩽5%	82	64	51	40 to 62	32	22 to 42	8	2 to 14	3.18	0.074
Weak 5 to 10%	16	13	14	0 to 32	7	0 to 20	7	0 to 20		
Moderate 10 to 50%	27	21	44	26 to 63	0	0 to 0	0	0 to 0		
Strong >50%	3	2	33	0 to 88	33	0 to 88	33	0 to 88		
COX-2										
⩽5%	82	64	51	40 to 62	32	22 to 42	8	2 to 14	6.4	0.011
>5%	46	36	34	20 to 47	5	0 to 11	5	0 to 11		
Sex										
Female	72	56	45	34 to 57	23	13 to 32	7	1 to 13	0.12	0.727
Male	56	44	45	32 to 58	21	11 to 32	6	0 to 13		
Age (years)										
⩽62	64	50	51	39 to 63	25	15 to 36	8	1 to 15	4.1	0.042
>62	64	50	39	27 to 51	19	9 to 28	6	0 to 12		
Grade of differentiation										
1	14	11	79	57 to 100	43	17 to 67	29	5 to 52	15.0	<0.001
2	77	60	48	36 to 59	25	15 to 35	7	1 to 12		
3	37	29	27	13 to 41	8	0 to 17	0	0 to 0		
TNM stage										
I	25	20	64	45 to 83	36	17 to 55	12	0 to 25	13.6	<0.001
II	39	30	51	36 to 67	33	19 to 48	8	0 to 16		
III	28	22	41	22 to 60	19	4 to 33	7	0 to 17		
IV	35	27	26	11 to 40	3	0 to 8	0	0 to 0		
Unavailable	1	1	–	–	–	–	–	–		
Primary tumour (T)										
T1	7	5	71	38 to 100	29	0 to 62	0	0 to 0	4.0	0.045
T2	28	22	46	28 to 65	32	15 to 49	11	0 to 22		
T3	63	49	47	35 to 59	26	15 to 37	8	1 to 15		
T4	29	23	31	14 to 48	3	3 to 10	3	3 to 10		
TX	1	1	–	–	–	–	–	–		
Regional nodes (N)										
N0	78	61	55	44 to 66	28	18 to 38	8	2 to 14	2.5	0.115
N1	34	27	34	19 to 50	16	4 to 28	8	1 to 17		
NX	11	9	–	–	–	–	–	–		
Distant metastasis (M)										
M0	118	92	48	39 to 57	24	16 to 32	7	3 to 12	12.5	<0.001
M1	9	7	11	9 to 32	0	0 to 0	0	0 to 0		
MX	1	1	–	–	–	–	–	–		
Tumour size										
⩽2 cm	19	15	67	46 to 89	23	3 to 42	0	0 to 0	3.89	0.049
2 to 4 cm	66	52	53	41 to 65	27	17 to 38	11	4 to 19		
>4 cm	29	23	17	4 to 31	10	1 to 21	3	0 to 10		
Unavailable	14	11	–	–	–	–	–	–		
Tumour location										
Head	113	88	47	38 to 57	24	16 to 32	8	3 to 13	4.01	0.045
Other location	13	6	31	6 to 56	8	0 to 22	0	0 to 0		
Curability										
Intent to cure	85	66	57	47 to 68	32	22 to 42	10	3 to 16	23.11	<0.001
Non-curative	43	34	21	9 to 33	2	2 to 7	0	0 to 0		

CI, confidence interval; COX-2, cyclooxygenase-2; CS, cumulative survival.

independent prognostic significance of COX-2 expression. The evidence of pancreatic tumour growth inhibition by COX-2 inhibitors also supports this hypothesis.

Kokawa *et al*[36] showed that COX-2 correlated with inhibition of cell growth by aspirin in four pancreatic cancer cell lines and proposed chemoprevention by COX inhibitors. Other groups have demonstrated tumour growth inhibition by selective COX-2 inhibitors, but this was COX-2 independent.[18 37] In preclinical studies, COX-2 inhibitors enhance the antitumoral efficacy of gemcitabine.[22] Our pancreatic cancer

Table 3 Backward stepwise Cox proportional hazard model figures for 128 patients with pancreatic cancer

Covariate	Coefficient	χ^2	p Value	HR	95% CI
Grade 1				1.0	
Grade 2	0.788	12.138	0.002	2.2	1.1 to 4.3
Grade 3	1.246			3.5	1.7 to 7.1
TNM stage 1				1.0	
TNM stage 2	0.188	20.961	<0.0001	1.2	0.7 to 2.0
TNM stage 3	0.395			1.5	0.8 to 2.7
TNM stage 4	1.248			3.5	1.9 to 6.4
COX-2	0.484	5.562	0.018	1.6	1.1 to 2.4
Age >62 years	0.411	4.011	0.045	1.5	1.0 to 2.3

Tumour location, NS.
CI, confidence interval; COX-2, cyclooxygenase-2; HR, hazard ratio.

Take home message

- Expression of COX-2 was associated with a poor outcome in pancreatic ductal adenocarcinoma and was independent of tumour stage, grade, and age in multivariate analysis

patients received no adjuvant therapy. Earlier studies and the present findings support efforts to initiate clinical trials to discover whether tumours with COX-2 expression could distinguish those patients who benefit from neoadjuvant treatment combined with surgery.

Because our study was based on patients operated on for pancreatic cancer, only nine had distant metastases, and interestingly, none of these patients' primary tumour samples expressed COX-2. This could reflect the small number of patients or the biology of the disease. Pancreatic cancer is known to invade surrounding tissues at an early phase. COX-2 seems to enhance cell proliferation and the production of matrix metalloproteinases and to promote angiogenesis, facilitating local tumour growth in pancreatic cancer.[8 9] At later phases, other factors join the biological process, leading to metastases.

Results for COX-2 expression and the differentiation of tumour cells in previous studies on pancreatic cancer are inconsistent, with most showing no correlation of grade of pancreatic cancer with COX-2 expression.[28 29 36] In a study by Merati *et al*,[27] increased COX-2 expression was associated with well differentiated glandular components of the pancreatic tumour. When we reassessed the histological grade of all tumours and compared the results with the COX-2 expression, COX-2 failed to correlate with grade.

In conclusion, based on our study of 128 patients with pancreatic adenocarcinoma, COX-2 seems to be an independent prognostic factor. The possibility of including COX-2 inhibitors in treatment of pancreatic cancer deserves evaluation.

ACKNOWLEDGEMENTS

The technical assistance of Elina Laitinen, Päivi Peltokangas, and Elina Malkki is greatly appreciated. This study was supported by grants from the Finnish Cancer Society, Finska Läkaresällskapet, Medicinska Understödsföreningen Liv och Hälsa, Helsinki University Central Hospital Research Funds, the Academy of Finland, and the Sigrid Juselius Foundation.

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