

Advances in Brief

Prognostic Significance of Elevated Cyclooxygenase 2 Expression in Primary, Resected Lung Adenocarcinomas¹

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Abstract

Recently, we demonstrated that elevated expression of cyclooxygenase 2 (COX-2) is frequently seen in a specific type of lung cancer, *i.e.*, adenocarcinoma, and is possibly associated with its invasion and metastasis. Here, the prognostic significance of elevated COX-2 expression was evaluated in a cohort of 130 adenocarcinoma patients who had consecutively undergone potentially curative resections. Immunohistological examination showed the presence of tumor cells with markedly increased COX-2 immunoreactivity in 93 of 130 (72%) cases. No relationship was found between the increase in COX-2 expression and clinical outcomes when the entire cohort was considered ($P = 0.099$). Reasoning that the influence of the increase in COX-2 expression may have been obscured by the clinical and molecular pathogenetic complexities in cases with an advanced disease, we also separately analyzed the prognostic significance of increased COX-2 expression after stratification according to the disease stage. A significant relationship between elevated COX-2 expression and shortened patient survival was observed only in a cohort of patients with stage I disease ($P = 0.034$). These findings suggest that an increase in COX-2 expression may be clinically significant for the prognosis of patients undergoing surgical resection of early-stage adenocarcinomas and, thus, warrant further conclusive studies involving a larger cohort.

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Introduction

Lung cancer currently claims more than 40,000 lives annually and is expected to become the leading cause of cancer deaths in Japan in the very near future (1). Although surgical resection can provide lung cancer patients with the hope of a cure, the long-term survival rate, even in surgically treated cases, remains unsatisfactory. Identification of genetic markers associated with a distinct prognostic outcome would, therefore, be useful for defining a subset of lung cancer patients as candidates for new investigational adjuvant therapies, leading to an improvement in prognosis.

Recent studies have suggested that an increase in the expression of COX-2,³ a key inducible enzyme involved in the production of prostaglandins and other eicosanoids, may play a significant role in carcinogenesis in addition to its well-known role in inflammatory reactions (2–11). Oshima *et al.* (8) recently provided direct genetic evidence that formation of intestinal polyps in Apc^{Δ716} knockout mice was dramatically suppressed by crossing with COX-2 knockout mice, indicating that induction of COX-2 represents an early rate-limiting step. Moreover, a number of clinical and epidemiological studies suggest that nonsteroidal anti-inflammatory drugs induce a significant and often complete regression of colonic polyps in patients with familial adenomatous polyposis and also reduce the risk of colon cancer in nonfamilial adenomatous polyposis subjects (12–17). Although previous studies have been largely confined to colorectal tumorigenesis, we and another group recently reported that an increased expression of COX-2 is also frequently seen in a specific type of lung cancer, *i.e.*, adenocarcinoma (18, 19) and is possibly associated with its invasion and metastases (18).

This study was conducted to evaluate the prognostic significance of an increase in COX-2 expression in a cohort of 130 adenocarcinoma patients who had consecutively undergone potentially curative resections between January 1986 and December 1990.

Materials and Methods

Patients and Tissue Samples. Between January 1986 and December 1990, 131 adenocarcinoma cases successfully underwent potentially curative operations at Aichi Cancer Center Hospital (Nagoya, Japan) and were considered appropriate for inclusion in this study, which was designed to evaluate the prognostic significance of elevated COX-2 expression in surgically treated patients with adenocarcinoma. Exclusion of one case (0.8%) because of a lack of adequate pathology specimens yielded a cohort of 130 patients who were fully assessable for increased COX-2 expression. Complete clinical and follow-up information was available for all

³ The abbreviation used is: COX-2, cyclooxygenase 2.

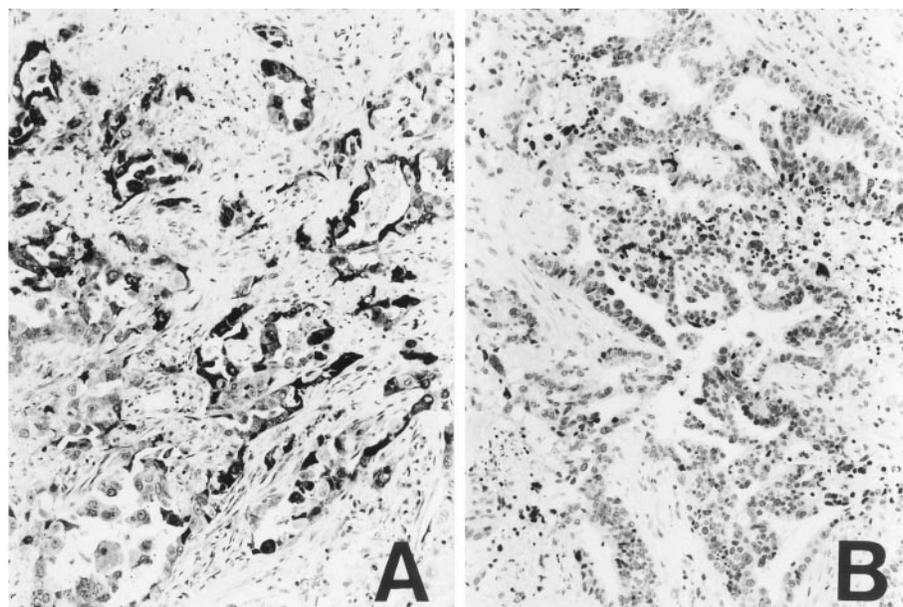


Fig. 1 Representative results of immunohistochemical staining of COX-2. A, a representative case with elevated COX-2 expression. B, a representative case without an increase in COX-2 expression.

Table 1 Relationships between elevated COX-2 expression and clinical characteristics

Clinical feature	No. of cases	Elevated COX-2 expression		P
		Positive	Negative	
All cases	130	93	37	
Age (yr)				
≤62	73	49	24	0.207
>63	57	44	13	
Sex				
Male	72	53	19	0.560
Female	58	40	18	
Primary tumor (pT)				
1	54	35	19	0.310
2	62	49	13	
3	10	6	4	
4 ^a	4	3	1	
Nodal involvement (pN)				
0	89	63	26	0.853
1	9	6	3	
2	32	24	8	
Disease stage				
I	81	57	24	0.983
II	8	6	2	
IIIA	37	27	10	
IIIB ^a	4	3	1	
Smoking history				
Never-smokers ^b	60	41	19	0.453
Ever-smokers ^c	70	52	18	

^a One case with invasion to a vertebral body and three cases with an ipsilateral metastasis in the nonprimary tumor lobe.

^b Seven male and 53 female cases without any history of active smoking.

^c Current smokers and ex-smokers.

130 patients, with a median follow-up duration of 85 months (range, 2–144 months). Histological classification was performed according to the criteria of the WHO, and postoperative pathological staging was performed according to those of the international staging system for lung cancer (20, 21).

Immunohistochemistry. Four- μ m-thick formalin-fixed and paraffin-embedded tissue sample sections were deparaffinized in xylene, treated with 0.3% hydrogen peroxide in methanol for 20 min to block endogenous peroxidase activity, microwave in citrate-phosphate buffer (pH 6.0) for antigen retrieval, and incubated with 10% normal goat serum for 30 min to block nonspecific binding. Rabbit polyclonal antibody specific for human COX-2 (Immuno-Biological Laboratories Co., Ltd., Gunma, Japan) was then applied as the primary antibody at a dilution of 1:25 at 4°C overnight, followed by a standard staining procedure using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA). Nonimmunized rabbit serum was used for the negative control.

Evaluation of COX-2 Immunostaining. The results were evaluated independently by three observers (H. A., Y. Y., and T. H.) and repeated three times each. In cases of occasional discrepancy in the interpretation, consensus was achieved after discussion of findings obtained with the aid of a multiheaded microscope. Reactions in smooth muscles and vascular endothelial cells, which were present in all specimens, were used as internal built-in controls, and cases with tumor cells showing significantly more intense staining than the internal control cells were recorded as positive.

Statistical Analysis. All statistical analyses were carried out with the Statistical Analysis System software (Version 6.12, SAS Institute Inc., Cary, NC) after completion of the immunohistological evaluation. The χ^2 test was used to examine the association between increased COX-2 expression and various clinicopathological characteristics. The Kaplan-Meier method was used to estimate survival as a function of time, and survival differences were analyzed with the log-rank test. Cox proportional hazards modeling of factors potentially related to survival was performed to identify which independent factors might jointly have a significant influence on survival.

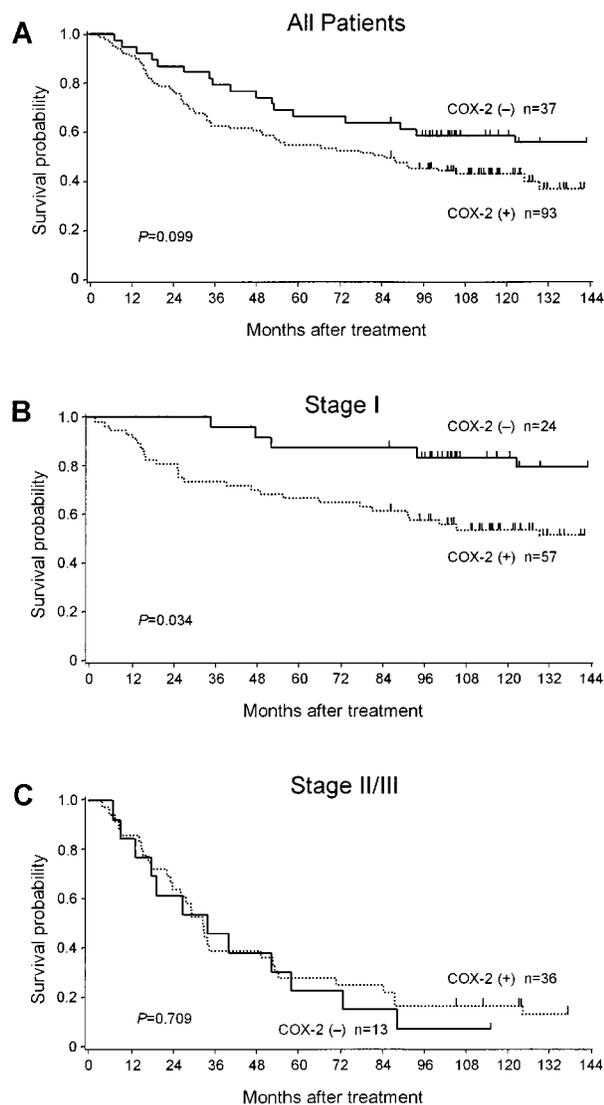


Fig. 2 Survival curves according to the presence or absence of increased COX-2 expression for the entire cohort (A) as well as for patients with stage I (B) or stage II/III (C) disease.

Results

Relationship between the Expression Status of COX-2 and Clinicopathological Characteristics. Of the 130 patients, 93 (72%) exhibited markedly more intense COX-2 immunoreactivities in tumor cells than in the internal control cells, whereas the remaining 37 cases did not show such an increase in COX-2 expression (Fig. 1). There was no significant association between elevated COX-2 expression and various clinicopathological features, including age ($P = 0.207$), sex ($P = 0.560$), tumor size ($P = 0.310$), nodal involvement ($P = 0.853$), disease stage ($P = 0.983$), and smoking history ($P = 0.453$; Table 1).

Relationship between COX-2 Expression and Survival. Kaplan-Meier survival curves demonstrated no association between increased COX-2 expression and a poor prognosis when

the entire cohort was considered ($P = 0.099$ by log-rank test; Fig. 2A). We also analyzed the prognostic significance of an increase in COX-2 expression after stratification according to the disease stage, reasoning that the influence of elevated COX-2 expression may have been obscured by the clinical and molecular pathogenetic complexities in cases with an advanced disease. Kaplan-Meier survival curves showed that stage I patients without an increase in COX-2 expression had a 88% 5-year survival rate, in contrast to the 66% of those with such an increase. Furthermore, a statistically significant survival difference was observed in patients with stage I disease between those with and without an increase in COX-2 expression ($P = 0.034$ by log-rank test; Fig. 2B). In contrast, there was no such difference in patients with stage II/III disease ($P = 0.709$ by log-rank test; Fig. 2C). These findings suggest that the absence of an increase in COX-2 expression may be indicative of a better prognosis.

We further carried out multivariate analysis to identify which independent factors would jointly have a significant influence on the survival of patients with stage I disease. Using age, sex, tumor size, smoking history and COX-2 expression as variables showed that, in addition to the significant effect of primary tumor size [hazard ratio (pT_2/pT_1) = 2.982; 95% confidence interval, 1.374–6.475; $P = 0.057$], there was a trend toward poorer prognosis in patients with elevated COX-2 expression (hazard ratio (positive/negative) = 2.500; 95% confidence interval, 0.945–6.610; $P = 0.0648$; Table 2).

Discussion

We previously showed frequent occurrence of increased COX-2 expression specifically in adenocarcinomas and the presence of significantly more intense COX-2 immunoreactivity in tumor-invasive lesions and in lymph node metastases (18). Here, therefore, we investigated 130 patients with adenocarcinomas who underwent consecutive surgical resections to determine whether an increase in COX-2 expression could have prognostic significance. A correlation between elevated COX-2 expression and a shortened survival of adenocarcinoma patients was found for stage I disease but not for advanced stage II/III disease, possibly reflecting the clinical and molecular pathogenetic complexities of the latter. In this regard, it is noteworthy that a similar difference between these two subgroups of adenocarcinoma cases was observed in our previous study on the prognostic significance of p53 abnormalities (22). This is, to our knowledge, the first demonstration of the possibility of the prognostic significance of COX-2 expression not only in lung cancers but also in other types of human cancers.

In addition to our previous demonstration of the possible involvement of COX-2 in lung cancers (18), there are several lines of experimental evidence supporting such involvement in the process of tumor progression. For example, overexpression of COX-2 reportedly suppresses apoptosis, resulting in the enhanced tumorigenic potential of rat intestinal epithelial cells (9), whereas COX-2 has been found to possibly play a role in inducing more potent invasiveness of colon cancer cells *in vitro* (10) and in the chemotactic response of vascular endothelial cells (11). Although further *in vitro* and

Table 2 Cox proportional hazards model for various potential prognostic factors of patients with stage I disease of adenocarcinoma of the lung

Variable	Hazard ratio (95% CI) ^a	Unfavorable/favorable	P
Age (yr)	0.684 (0.320–1.460)	>62/≤62	0.3263
Sex	1.435 (0.396–5.194)	Male/Female	0.5825
Primary tumor (pT)	2.982 (1.374–6.475)	pT ₂ /pT ₁	0.0057
Smoking history	1.617 (0.450–5.816)	Smoker/never-smoker	0.4616
Elevated COX-2 expression	2.500 (0.945–6.610)	Positive/negative	0.0648

^a CI, confidence interval.

also animal model studies of lung carcinogenesis and tumor progression are required to examine whether COX-2 is, indeed, the responsible molecule, the availability of COX-2 inhibitors, or nonsteroidal anti-inflammatory drugs, makes these results more interesting than those of previous studies on other prognostic factors because increased COX-2 expression might represent a direct therapeutic target in such cases with an unfavorable prognosis. The potential use of COX-2 inhibitors in adjuvant setting in early-stage adenocarcinoma cases may be of special interest considering their much less adverse effects than conventional cytotoxic anticancer agents. In this regard, it is interesting that in a preliminary study of ours COX-2-specific inhibitors were found to elicit apoptosis in lung cancer cell lines *in vitro*.⁴

These findings of the prognostic significance of an increase in COX-2 expression in stage I patients were obtained by analysis of a relatively large number of consecutively operated cases and, thus, should be of considerable clinical interest. However, these findings need to be confirmed with larger independent groups of patients because careful interpretation of findings based on subset analyses is particularly important in avoiding the attachment of significance to results by chance alone. In conjunction with the recent development of potent COX-2-specific inhibitors (23), further studies are warranted to gain more insight into the biological roles of COX-2 in the development and progression of adenocarcinoma of the lung. Such insight would be especially significant for future clinical applications, which may ultimately lead to a reduction in the high death toll caused by this fatal disease.

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