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Expression and significance of cyclooxygenase-2 in human lung cancer

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Abstract ***Objective:*** We aimed to examine the expression of cyclooxygenase-2 and the relationship with the pathological types, TNM stage, lymph node metastasis, the degree of differentiation, smoking and the survival. ***Methods:*** Immunohistochemical staining method was used to examine the expression of cyclooxygenase-2 of 121 cases of lung cancer and three control groups. The data were statistically analyzed. ***Results:*** Compared with the health group, cyclooxygenase-2 was over expressed in the inflammatory tissue (P = 0.036), lung adenocarcinoma (P = 0.005) and squamous carcinoma (P = 0.047). Compared with patients without lymph node metastasis, cyclooxygenase-2 was over expressed (P = 0.033) in patients with lymph node metastasis. Compared with high differentiation group, cyclooxygenase-2 was over expressed (P = 0.004) in low differentiation group. Compared with non-smokers, the expression of cyclooxygenase-2 increased in smokers (P = 0.000). The median survival time of patients that the expression of cyclooxygenase-2 were negative was 9 months (95% CI, 5.6–12.4 months). The median survival time of patients that the expression of cyclooxygenase-2 were positive was 5 months (95% CI, 3–7 months). They was statistical difference (P = 0.001). ***Conclusion:*** Overexpression of cyclooxygenase-2 is associated with pathological types, TNM stage, lymph node metastasis, degrees of differentiation, smoking and prognosis in lung cancer.

Key words cyclooxygenase-2; lung neoplasms; immunohistochemistry

A connection between chronic inflammation and carcinogenesis has long been suspected [1]. With improved characterization of infiltrating immune cells, the precise role of inflammation in cancer is being elucidated, leading to a resolution of the initial contradiction that inflammation is protective in certain tumors [2–4], yet detrimental in others [5]. Overall, it appears that chronic inflammation more often stimulates than inhibits tumor development [6].

Cyclooxygenase-2 (COX-2) is a rate-limiting enzyme involved in the conversion of arachidonic acid into prostaglandin (PG) and other eicosanoids, including PGD2, PGE2, PGF2, PGI2, and thromboxane A2. COX-2 is involved in a wide range of inflammatory reactions. Recent studies have found that COX-2 is responsible for producing large amounts of PGE2 in tumor tissues [7–10] and plays an important role in the development of many tumors except for involving in inflammation [11–16, 7]. COX-2 inhibitors have produced encouraging results in preventing progression of and even curing some digestive system cancers [17–19]. However, previous reports of the associations between COX-2 and clinicopathologic factors have not been consistent for lung cancer.

In this study, we hypothesized that COX-2 is correlated with cell malignancy in lung cancer. Therefore, we investigated the association between COX-2 expression and clinicopathologic factors in lung cancer.

Patients and methods

Patients

A cohort of 121 lung cancer patients and three control groups were enrolled at Beijing Chest Hospital (China) between May 2006 and June 2007. The three control groups consisted of a group of healthy subjects, an inflammatory disease group and an atypical hyperplasia group, which were composed of males and females ranging from 26 to 88 years (mean of 53.4 years), 30 to 76 years (mean of 50 years) and 34 to 72 years (mean of 52.6 years) respectively. The case group was composed of males and females ranging from 24 to 83 years (mean of 59 years), who were monitored for five years, and the endpoint was

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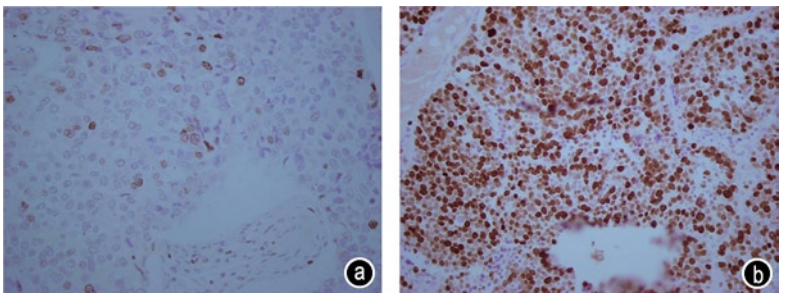

Fig. 1 Level of COX-2 expression in lung cancer specimens, as determined by immunohistochemistry (× 100). (a) high positive; (b) negative

overall survival (OS). All survival times were taken at the time of last contact or on 1 August 2013. Age and sex did not differ significantly between the different groups, and all samples were confirmed by pathology.

Immunohistochemistry and assessment

Tumor tissue samples of lung cancer patients and nude mice were fixed in 10% neutral-buffered formalin and then embedded in paraffin. Four-μm-thick sections were immersed in 0.3% hydrogen peroxide for 10 min to block endogenous peroxidase activity, microwaved in citrate phosphate buffer (pH 6.0) for antigen retrieval, and incubated with 10% normal goat serum for 30 min to block non-specific binding. The slides were incubated with monoclonal anti-COX-2 antibody (Zhongshan Company, China) for 12 h at 4 ℃ in a moist chamber. Subsequently, the slides were sequentially incubated with biotinylated rabbit anti-mouse immunoglobulin antibody at a concentration of 1:100 for 30 min at 37 ℃ and then with a streptavidin-peroxidase conjugate for 30 min at 37 ℃ with diaminobenzidine as the chromogen substrate. The nucleus was counterstained using Meyer's hematoxylin. The negative control was obtained by replacing the primary antibody with PBS. All slices were reviewed by two experienced pathologists independently. Information on cell shape, atypia, interstitial constituents and the invasion of surrounding tissues was collected. The assessment of immunohistochemical stains of the slices were interpreted as either positive or negative; more than 10% of cells that stained brown particles in nests were considered positive (Fig. 1).

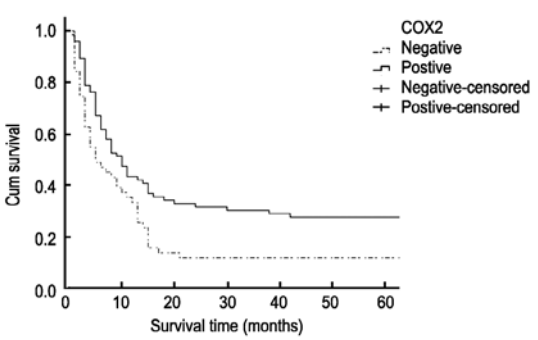

Fig. 2 Association between COX-2 expression and survival

Statistical analysis

SPSS 13.0 software was used to analyze the data and plot curves. Correlation analyses used the Chi-square test. Kaplan-Meier survival curves were generated and log-rank tests were used to evaluate the differences between OS rates. A Cox regression model was applied for the multivariate analysis. The boundary for statistical significance was $P \leq 0.05$.

Results

Association between COX-2 expression and clinicopathologic parameters

Table 1 showed that COX-2 expression was increased in inflammatory control tissue ($P = 0.036$), adenocarcinoma tissue ($P = 0.005$) and squamous cell carcinoma tissue ($P = 0.047$) compared with healthy controls. COX-2 expression did not differ between small cell carcinoma and healthy control tissue, but was significantly different between stages III ($P = 0.036$), and IV ($P = 0.049$) and atypical hyperplasia and carcinoma in situ. However, COX-2 expression was not significantly different between stages I and II and atypical hyperplasia and carcinoma in situ. COX-2 expression was increased in tissues with lymph node metastasis compared with those that did not have lymph node metastasis ($P = 0.033$). COX-2 expression in poorly differentiated tissues was increased compared with that in highly differentiation tissues ($P = 0.004$), while it was increased in smoking patients compared with those patients who were non-smokers ($P = 0.000$).

Association between COX-2 expression and survival

The median overall survival of the patients with negative COX-2 expression was 9 months (95% CI: 5.6–12.4 months), in contrast to the median overall survival of the patients with positive COX-2 expression, which was 5 months (95% CI: 3.0–7.0 months), with a significant difference ($P = 0.001$; Fig. 2).

Table 1 Association between COX-2 expression and clinicopathologic parameters (*n*)

Clinicopathologic parameters	COX-2 expression		χ^2 value	*P* value
	Positive	Negative		
Pathological types				
Healthy control	1	9		
Atypical hyperplasia and carcinoma in situ	4	13		0.621††
Adenocarcinoma	35	24	8.336	0.005†
Squamous cell carcinoma	22	29	3.909	0.047†
Small cell carcinoma	1	10		1.000††
TNM stage				
Atypical hyperplasia and carcinoma *in situ*	4	13		
I	12	15	1.972	0.208†
II	11	11		0.112††
III	19	16	4.388	0.036†
IV	14	12	3.882	0.049†
Lymph node metastasis				
No	17	33		
Yes	41	36	4.526	0.033†
Degree of differentiation				
High	5	15		
Medium	24	24	3.607	0.058†
Low	27	15	8.373	0.004†
Smoking				
No	13	32		
Yes	47	15	23.303	0.000†

† Chi-square test; †† Exact tests

Discussion

Recently, many studies have shown that COX-2 is involved in tumorigenesis and development except for involving in inflammation. Overexpression of COX-2 is a normal phenomenon in tumor tissues. However, previously published results of the associations between COX-2 and clinicopathologic factors are not consistent for lung cancer, thus in this study, we first sought to clarify this anomaly. Our results showed COX-2 expression was associated with malignant pathology, TNM stage, lymph node metastasis, degree of differentiation and smoking. COX-2 expression was an independent prognosis factor and had an effect on the overall survival of lung cancer patients. Some of these results were consistent with those reported in the literature but conflicted with other studies [20–23]. We found that COX-2 expression was increased in smoking patients with lung cancer and in individuals with inflammation. Long-term smoking can damage the bronchial mucosa cilia and cause submucosal gland hyperplasia, hypertrophy and mucus secretion. Importantly, smoking-induced lung inflammation is a key factor in the promotion of tumor growth [24, 25]; our results indirectly confirmed this result.

In conclusion, COX-2 overexpression is associated with TNM stage, lymph node metastasis, degree of differentiation, smoking and prognosis in lung cancer.

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