Expression of cyclooxygenase-2 in non-small cell lung cancer


Department of Pathology and Clinical Pathology*, College of Medicine, Soonchunhyang University, Cheonan 330-090, Korea

= ABSTRACT =

Background: Previous studies report that increased expression of cyclooxygenase 2 (COX-2) correlate with poor clinical outcome in several malignancies, including non-small cell carcinoma (NSCLC). We examined COX-2 expression in 95 cases of NSCLC to evaluate its prognostic significance.

Methods: Ninety-five NSCLC were selected and microarrayed in paraffin blocks. Immunohistochemical analysis was performed with antibody for COX-2. Overall and 3-year survival were compared between each group according to COX-2 expression.

Results: No relationship was found between COX-2 expression and patients' overall survival when the entire cohort was considered (p=0.57). But 3 year mean survival mean time were 32.1 and 23.3 months for COX-2 negative and COX-2 positive, respectively, which was statistically significant (p=0.04). Multivariate factors including gender, histologic type, stage, T stage and N stage were not correlated to poor outcome.

Conclusion: This result indicated that COX-2 expression in NSCLC showed no relationship with patients' overall survival, but showed significant relationship with 3 year survival as early survival. Therefore, COX-2 expression could be used as an early poor prognostic marker.

Key words: cyclooxygenase-2 (COX-2), non-small cell lung cancer, prognostic factors, survival

Introduction

There are two isoforms of cyclooxygenase (COX). The COX enzymes catalyze the first step in the synthesis of prostaglandins from arachidonic acid. COX-1 is constitutively expressed in most normal tissues and seems to mediate various physiologic functions. In contrast, COX-2 is absent in most normal tissues but is induced by a variety of mitogenic and proinflammatory stimuli. Increased levels of COX-2 and prostaglandin E2 (PGE2) have been observed in a variety of malignancies, including NSCLC. Several mechanisms can potentially account for the tumor promoting effects of COX-2 derived prostaglandins.
Prostaglandins can enhance tumor growth and metastasis by stimulating cell proliferation, angiogenesis, and invasiveness in addition to inhibiting apoptosis and immune surveillance. Importantly, the formation and growth of tumors is reduced in mice engineered to be COX-2 deficient. Moreover, selective inhibitors of COX-2 suppress the growth of experimental tumors. The aim of this study was to further investigate the potential relationship between COX-2 expression and prognosis in 95 patients with NSCLC.

Materials and methods

Tumor specimens

Ninety-five resected NSCLCs were retrospectively retrieved from the files of the Department of Pathology, Soonchunhyang University Cheonan Hospital from 1990 to 2004. The clinical information was obtained from the file of the pathologic requests and medical records. All pathological slides were reviewed and one appropriate paraffin block was selected from each case.

Construction of the tissue microarray

The representative tissue area from each case was taken by using 1.0 mm punch, and inserted into a recipient paraffin block to create a tissue microarray. Four micrometer-thick sections were cut from the completed array block and transferred to silanized glass slides.

Immunohistochemical staining

Immunohistochemical studies with mouse monoclonal anti-human COX-2 (1:1000 dilution; 4H12, Novocastra, Newcastle, UK) was used. Formalin-fixed, paraffin-embedded tissues were cut into 4-μm serial sections using a microtome and attached on Superfrost/Plus slides. Sections were deparaffinized in xylene, and rehydrated in graded alcohols, and washed in distilled water. Antigen retrieval was done by boiling the slides in pressure cooker containing 2.0L of 0.01M sodium citrate buffer (pH 6.0) for 20 minutes. The following procedures were performed in automated immunostainer (Vision BioSystems, Mount Waverley, Australia). The slides were then dehydrated with ethanol, rinsed with xylene, and mounted.

Analysis of immunohistochemical staining

A semiquantitative assessment for COX-2 expression was carried out according to the following criteria: negative (<10% positive staining of neoplastic cells) and positive (>10% positive staining of neoplastic cells) (Fig. 1).

Statistical analysis

The overall and 3-year survival curves were plotted using the Kaplan-Meier method, and differences between the survival curves were tested by using log-rank test and Breslow test. The multivariate analysis for covariates showing statistical significance in univariate analysis was performed using the Cox proportional hazards model. The results were considered to be statistically significant that the p-values were less than 0.05. All statistical analysis were conducted using the SPSS 12.0 statistical software program (SPSS Inc., Chicago, IL, USA).
Results

Patients
Patients included in this investigation underwent surgery between 1990 and 2004. Relationships between COX-2 expression & clinicopathologic profile are listed in Table 1. At the time of last follow-up (median follow-up, 22.6 months), 64 patients had died.

Immunohistochemical analysis
Of 95 patients, 29 (31%) showed negativity (less than 5%) and 66 (69%) showed positivity (more than 10%) for COX-2 immunoreactivity (Figure 1). All 66 of the samples exhibit a positive immunohistochemical reaction compared with a negative control reaction in which the primary antibody was omitted. All positive staining showed cytoplasmic staining. No relationship was found between COX-2 expression and patient sex, histologic type, TNM stage, T stage and N stage.

Survival
Survival analysis demonstrated no association between COX-2 expression and poor outcome when the proportion of positive neoplastic cells was considered. Mean survival time according to COX-2 negativity and positivity were 45.6 and 48.4 months, respectively ($p$=0.57), which revealed no significant difference. We also evaluated 3-year survival time that were 32.1 and 23.3 months for COX-2 negative and COX-2 positive, respectively, which revealed statistically significant difference ($p$=0.04) (Fig. 2). We also multivariate analysis to identify which factors would be significantly related to survival time in patients, using age, sex, histologic type, TNM stage, T stage and N stage. This analysis showed no significant correlation.

Discussion
This study examined the immunohistochemical expression of COX-2 in matched in NSCLC. The main focus of our study was to determine whether COX-2 expression is a poor prognostic marker or not. Our results are somewhat different with previous reports that COX-2 expression is related to poor outcome. In a evaluation of the prognostic significance of COX-2 in lung cancer, Achiwa et al. evaluated the expression of COX-2 by immunohistochemistry in surgically resected adenocarcinomas of the lung. Their data indicate that an increase in COX-2 expression may be clinically significant for the prognosis of patients undergoing surgical resections, particularly of stage I NSCLC. In their study, elevated COX-2 expression was associated with shortened survival time of patients with stage I disease ($p$=0.034). But most of them, the subject of study was early stage (stage I or II) of NSCLC. Therefore, further subdivided study will be necessary concerning to TNM stage, histologic type and metastasis, etc.

Numerous in vitro studies have shown that the either oncogene activation or tumor suppressor gene inactivation result in enhanced expression of COX-2 and increased synthesis of PGE_2. An effort has been made to translate these in vitro findings to human tumors. For example, both overexpression of HER-2/neu and mutation of TP53 has been associated with elevated levels of COX-2 in human malignancies. Based on the findings in this study, it is clear that the ability to identify molecular
determinants of COX-2 expression will be compromised if samples are obtained from subjects with varying
treatment histories. Therefore, future correlative studies need to be done with a detailed knowledge
of any treatment that was given before the tumor sample was obtained. Also, the other limitation of this
study is that the specimen is too small to be representative by using of 1-mm core of tissue microarray.
There is little doubt that improved insight into the biology of cancer has greatly expanded the spectrum
of potential therapeutic targets.\textsuperscript{42} Drug that target the epidermal growth factor receptor family, for
example, have yielded symptomatic and survival benefits in breast, lung, and colon cancers, often with
less toxicity than that occasioned by standard chemotherapy.\textsuperscript{43-47} COX-2 is another potentially attractive
molecular target for theses common malignancy. Considerable attention has been gazed on COX-2 expression
in colorectal adenocarcinomas. It is well known that NSAIDs, the best-known COX enzyme inhibitor,
obviously decrease the number and size of colorectal adenomas in patients with familial adenomatous
polyposis (FAP) and reduce the risk of developing colorectal adenocarcinomas even in non-FAP individuals.
However, the precise role and clinicopathological significance of COX-2 expression has not been
sufficiently elucidated in human bronchial carcinomas. So many clinical trials currently underway are
exploring the potential of targeting COX-2 in lung cancer.\textsuperscript{48} Recently, prolonged use of selective COX-2
inhibitors has been associated with an increased risk of cardiovascular complications including
myocardial infarction and stroke. In every clinical situation, the potential risk/benefit ratio of a
medication needs to be considered. Although the current study was not designed to evaluate clinical
efficacy, it did show the ability of celecoxib to effectively suppress intratumoral COX-2 activity.
Clinical trials are needed to determine whether the promising anticancer activity of COX-2 inhibitors
shown in preclinical studies\textsuperscript{7} translates into clinical benefit for cancer patients.\textsuperscript{48,49}

Conclusion
This result indicated that COX-2 expression in NSCLC showed no relationship with patients' overall survival,
but showed significant relationship with 3 year survival as early survival. Therefore, COX-2 expression could
be used as an early poor prognostic marker.

References
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Table 1. Relationships between Cox-2 expression & clinicopathologic profile

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<th>No. of cases</th>
<th>positive</th>
<th>negative</th>
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<td>66</td>
<td>29</td>
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<td>Age (year)</td>
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<tr>
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<td>74</td>
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Fig. 1. Representative sections of non-small cell lung carcinoma with negative (left) and positive (right) immunoreactivity for cyclooxygenase-2.
Fig. 2. A, Survival curve for all 95 patients according to negativity and positivity for COX-2 expression. Overall survival showed no difference between two groups \((p=0.57)\). B, In three year survival curve for 53 patients, we found a statistically significant difference between two groups \((p=0.04)\).

= 국문 요약 =

배경: COX-2의 발현이 비소세포 폐암종을 포함한 다양한 악성종양에서 발현되며 이는 환자의 불량한 예후와 관련이 있다는 내용이 최근 보고되고 있으나 아직까지 예후에 미치는 영향에 대해서는 일치된 결론에 이르지 못하고 있다. 이에 저자들은 종양의 COX-2 발현과 임상병리학적 특징과의 관계를 관찰하였다.

재료 및 방법: 1990년부터 2004년까지 순천향대학교 천안병원에서 수술로 절제된 95명 환자의 포르말린에 고정한 파라핀 포매 조직을 이용하여 COX-2 항체를 이용한 면역조직화학 검사를 시행하였으며 이의 결과를 환자의 임상병리학적 소견 및 전체 생존기간과 3년 생존기간과 각각 비교 분석하였다.

결과: COX-2 발현과 환자의 전체 생존기간과는 상관관계를 보이지 않았으나 \((p=0.57)\) 3년 생존기간의 경우는 COX-2 양성군이 23.3개월, COX-2 음성군이 32.1개월로 유의한 차이를 보였다 \((p=0.04)\). 그 외에 성별, 조직학적 유형 TNM-병기, T-병기와 N-병기 등은 COX-2 발현 및 생존기간과 상관관계를 보이지 않았다.

결론: 비소세포 폐암종에서 COX-2의 발현은 환자의 전체 생존과의 관련은 미약하나 초기 생존기간에 해당하는 3년 생존기간과는 유의한 상관관계를 보이므로 초기 예후인자로서의 가능성이 있을것으로 사료된다.

간추린 제목: 비소세포 폐암종에서 Cox-2의 발현
연락처: 순천향대학교 천안병원 병리과 오미혜
041-570-3582