Expression of epidermal growth factor receptor (EGFR) in non-small cell lung cancer
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ABSTRACT

Background: The prognostic role of epidermal growth factor receptor (EGFR) remains controversial in patients with non-small cell lung cancer (NSCLC). Histopathologic findings cannot adequately predict disease progression, so we investigated the relationship between EGFR and clinicopathologic feature.

Methods: Using immunohistochemical techniques, we retrospectively analyzed formalin-fixed, paraffin-embedded samples from 95 patients with resected pathological NSCLCs. Then, we correlated these data with patients' clinical outcome and pathologic findings.

Results: No relationship was found between EGFR overexpression and patient survival when the entire cohort was considered. Multivariate factors including gender, histologic type, TNM stage, T stage and N stage were not correlated to poor outcome. Also, no factors revealed correlation with EGFR overexpression except histologic type. Squamous cell carcinoma showed positive reaction in 43 out of 47 patients (p=0.005).

Conclusion: This result indicated that EGFR overexpression in NSCLC showed no relationship with patients' survival. Therefore, EGFR overexpression could not be used as a poor prognostic marker.

Key words: epidermal growth factor receptor, non-small cell lung cancer, prognostic factors, survival

Introduction

Lung cancer has become the leading cause of cancer mortality in both men and women. Global statistics on cancer indicate that in the year 2000 there were 10.1 million new cases, 6.2 million deaths, and 22 million people living with the disease. Human lung cancers included both a biologically and histopathologically heterogenous group of tumors. The two major types are small cell cancer (SCLC) and non-small cell cancer (NSCLC), the latter consisting of several types, mainly squamous cell carcinoma and adenocarcinoma. Previously, squamous cell carcinoma was the predominant from NSCLC, but in the last few decades it has been replaced by adenocarcinoma. NSCLC is generally less sensitive to chemotherapy than SCLC. However, chemotherapy and/or radio therapy are often used for advanced or recurrent cases. Although complete surgical resection is the only curative therapy for NSCLC patients, the survival rate, even of patients with early stage NSCLC, is not satisfactory. This has
led to a search for prognostic parameters of lung cancer from clinical data or histologic examination. Recently, the potential prognostic roles of cancer cell proliferation and molecular biological alterations in oncogenes and tumor suppressor genes have been investigated in NSCLC, but none has been found to yield unambiguous results. The epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase and overexpression of EGFR has been observed in many types of human malignancies, including breast, gastric, colorectal, and bladder cancer, as well as in 30% to 70% of NSCLCs. Although some studies have reported an association between enhanced EGFR expression and poor prognosis in patients with lung cancer, the prognostic role of EGFR remains yet controversial.

The epidermal growth factor receptor (EGFR) gene, which is located on chromosome 7p12, encodes a 170-kD membrane glycoprotein. Upon binding of specific ligands, such as epidermal growth factor and transforming growth factor-α, the receptor dimerizes, which leads to receptor autophosphorylation and activation of a signal cascade. This results in changes in gene and protein expression that are crucial to tumor progression, including proliferation, decreased apoptosis, and angiogenesis, and invasion.

In the present study, we assayed immunohistochemically EGFR expression in human NSCLC tissues, and we analyzed the relationships between EGFR overexpression and several prognostic variables, and investigated the prognostic role of EGFR overexpression in relation to overall survival.

Materials and methods

Tumor specimens

Ninety-five resected non-small cell lung cancer 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 EGFR (1:800 dilution; 31G7, Zymed, San Francisco, U.S.A.) 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 Wavertey, Australia). The slides were then dehy drated with ethanol, rinsed with xylene, and mounted.

Analysis of immunohistochemical staining

A semiquantative 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.
Statistical analysis

The overall survival curves were plotted using the Kaplan-Meier method, and differences between the survival curves were tested by using log-rank 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 when 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 EGFR expression & clinicopathologic profile are listed in Table 1. At the time of follow-up (median follow-up, 22.6 months), 64 patients had died.

Immunohistochemical analysis

Of 95 patients, 21 (22%) showed negativity (less than 10%) and 74 (78%) showed positivity (more than 10%) for EGFR immunoreactivity (Fig. 1). All 74 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 and membranous staining. The median age was 63 years and range was 36 to 76 years. No relationship was found between EGFR overexpression and patient sex, TNM stage, T stage and N stage. But histologic type showed significant correlation with EGFR overexpression. The others of histologic type were included 5 large cell carcinoma, 5 adenosquamous cell carcinoma and 4 sarcomatoid carcinoma. The rate of positivity was as follows: For sex (male:female, 78.4%:76.2%, p=0.831), for histologic type (squamous cell carcinoma : adeno carcinoma : others, 91.5%:67.6%:57.1%, p=0.005), for TNM stage (1:II:III:IV, 83.0%:77.3%:69.6%:66.7%, p=0.603), for T stage (1:2:3:4, 81.0%:77.8%:80.0%:0%, p=0.300) and for N stage (0:1:2, 81.4%:77.8%:66.7%, p=0.421). Only difference of histologic type was statistically significant.

Survival

Survival analysis demonstrated no association between EGFR expression and poor outcome when the proportion of positive neoplastic cells was considered (Fig. 2). Mean survival time according to EGFR negativity and positivity were 42.0 and 53.84 months, respectively (p=0.507). We also evaluated 3-year survival time were 21.5 and 25.8 months for EGFR negative and EGFR positive, respectively, which was statistically not significant (p=0.416). We also multivariate analysis to identify which factors would be significantly related to survival time in patients, using sex, histologic type, stage, T stage, N stage. This analysis showed no significant correlation.

Discussion

In current study, overexpression of EGFR was found in 74 of 95 specimens of NSCLC (78%), which was somewhat high rate, compared with previous reports.²⁻⁰ with absolutely higher rate in squamous cell carcinomas than
adenocarcinomas and other types ($p=0.005$). Theoretically, high EGFR expression in squamous cell carcinomas is expected because EGF promotes the proliferation and differentiation of epidermal-like tissue. But, in previous immunohistochemical studies of NSCLC, no consistent conclusion was reached with regard to the correlation between the EGFR overexpression and clinicopathologic features including histologic type, reflecting the large differences in reported immunohistochemical data. The heterogeneity of available reports could also be explained by differences in interpreting the intensity of expression and the localization of receptors and by the wide range of methods in use for EGFR detection. The immunohistochemistry relies on subjective judgement which represents an intrinsic limit of the technique: with immunohistochemistry some authors reported only cell membrane staining as opposed to cytoplasmic staining, while others did not report any preferential localization of the receptor. But in our study, we considered as positive staining both cytoplasmic and membranous staining. We considered that such interpretation method contributed to high rate of EGFR overexpression to some degree. In this study, EGFR was expressed at higher positive rate in squamous cell carcinomas than in adenocarcinoma and other types as has been found in other studies. The biological role of EGFR can explain its poor prognosis. Its expression is generally low in normal lung tissue and is only detected in the basal layer of the bronchial epithelium. EGFR expression is enhanced in metaplastic, preneoplastic and neoplastic lesions with a progressively increased intensity of staining. In these lesions, EGFR staining is also present in the superficial layers of the bronchial epithelium. Therefore, it has been proposed as an early marker of neoplastic transformation.

The ErbB family comprises four structurally related receptors: ErbB1 [more commonly known as EGFR and also called HER1], ErbB2 [HER2/neu], ErbB3 [HER3], ErbB4 [HER4]. On ligand stimulation, the receptors form either homodimers or heterodimers, which activate their cytoplasmic domain. The tyrosine-autophosphorylated region functions as a docking site for messenger mitogenic pathways. Inhibition of these pathways is facilitated by several newly developed compounds that have shown promising results in preclinical and clinical trials. Although EGFR expression may not be useful as a prognostic factor, it has potential clinical implications. The past few years have seen the rapid development of EGFR inhibitors, and an increasing body of evidence suggests that selective inhibitors of EGFR are potential therapeutic agents for treatment of NSCLC in adjuvant, metastatic and chemopreventive settings. Recently, it was reported that mutations of the EGFR gene have been detected in NSCLC (26–32), and these mutations were found in the tyrosine kinase domain in EGFR. These EGFR and are associated with sensitivity to EGFR inhibitor, gefitinib (26–28, 30), although tumors with the mutation T970M was resistant to gefitinib (30). These mutations were more frequent in women than in men, in adenocarcinoma than in other histologies, in nonsmoker than in smoker, and in patients from Japan than in patients from the United State. On the other hand, Huang et al (30) reported there was no correlation of EGFR mutation relate with gender and smoking history in Taiwanese patients. Therefore, the correlation between EGFR mutation and clinicopathologic features in NSCLCs needs studies of large numbers of patients.

**Conclusion**

This result indicated that EGFR expression in NSCLC showed no relationship with patient’s survival and most...
of clinicopathologic features. Therefore, EGFR expression could not be used as a poor prognostic marker down to date. But further studies of large numbers of patients will be needed because it has potential clinical implications.

References


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Fig. 1. Representative sections of non-small cell lung carcinoma with negative (left) and positive (right) immunoreactivity for EGFR

Fig. 2. Survival curve for all 95 patients according to positivity and negativity of EGFR expression.

= 국문초록 =

배경: 비소세포 폐암종에서 epidermal growth factor receptor (EGFR)의 발현이 예후에 미치는 영향에 대해서는 아직 일치된 결론에 이르지 못하고 있으며, 비소세포 폐암종의 조직 병리학적 소견으로는 종양의 진행 및 예후에 대해 예측이 불가능하다. 이에 저자들은 종양의 EGFR 발현과 임상 및 병리학적 특징과의 관계를 관찰하였다.

재료 및 방법: 1990년부터 2004년까지 순천향 대학교 친안병원에서 수술로 절제된 95명 환자의 포르말린에 고정한 파라핀 포매 조직을 이용한 EGFR 항체를 이용한 면역조직화학 검사를 시행하였으며 이의 결과를 환자의 임상양상과 병리학적 소견과 비교 분석하였다.

결과: EGFR 발현과 환자의 생존기간을 비롯한 성별, TNM 임상병기, T-병기, N-병기와는 관련이 없었으나 조직학적 유형증 편평상피암종은 47예중 43예가 양성 반응을 보여 통계학적으로 의미 있는 상관관계를 보였다(p=0.005). 그 외에 성별, 조직학적 유형, TNM 임상병기, T-병기, N-병기에서 어떠한 변수도 생존기간과의 연관성을 보이지 않았다.

결론: 비소세포 폐암종에서 EGFR의 발현은 환자의 생존기간 및 임상병리학적 소견과 유의한 상관관계를 보이지 않으므로 예후인자로서의 이용은 현재로서 불가능하며 좀 더 많은 증례를 통한 연구가 필요함 것으로 사료된다.

간추린 제목: 비소세포 폐암종에서 EGFR의 발현