The Over-Expression and Clinical Significance of EGF and EGFR in Non-Small Cell Lung Cancer

Xinju Li, Jingren Liang , Junke Fu

Department of Thoracic Surgery, First Hospital of Xi'an Jiaotong University, Xi'an, Shan Xi Province 710061, China

Abstract **Objective** To investigate the relationship between the over-expressions of epidermal growth factor (EGF), its receptor (EGFR)and clinical biological factors and prognosis of patients with non-small cell lung cancer (NSCLC). **Methods** The expression of EGF and EGFR were examined in 33 specimens of non-small cell lung cancers with immunohistochemical method, and the significance of over-expression of EGF and EGFR was analyzed statistically. **Results** Of the 33 NSCLC cases, the over-expression rates of EGF and EGFR were 57.6% and 63.6% respectively. The over-expression of EGFR was positively related with TNM stages and lymph node metastasis ($P<0.05$). The mean survival time was significantly shorter in patients with high EGF and/or EGFR level than the patients with low EGF and/or EGFR level ($P<0.01$). **Conclusion** EGF and EGFR may play an important role in the development and malignant potential of NSCLC, and their expressions may be of prognostic significance.

Key words lung neoplasms; epidermal growth factor; epidermal growth factor receptor; immunohistochemistry

Lung cancer is the most common malignant tumors because of high morbidity and mortality, but the molecular changes leading to lung cell transformation remain largely unknown. In recent years, more and more studies pay great attention to growth factors and its receptors which are related with the clinical biological behaviour of lung cancer. The abnormity of the autocrine/paracrine circles of Epidermal Growth Factor (EGF) and its receptor (EGFR) are common in some malignant tumors, which suggest they may be important for the development of tumors [1, 4]. It has been proved that the mean survival time was significantly shorter in patients with high EGFR level than the patients with low EGFR level in non-small cell lung cancer [5, 6]. But studies about the relationship between lung cancer and the autocrine/paracrine pathway of EGF and EGFR are few, and the result is unclear. We evaluated the EGF and EGFR protein level in NSCLC tissue by immunohistochemical method, and discussed the important significance of EGF and EGFR in the biological behavior and prognosis of lung cancer.

MATERIALS AND METHODS

Materials

The surgically resected samples were collected in thirty-three patients with non-small cell lung cancer in our department between Jul 1994 and Jun 1999, including 19 cases with squamous cell carcinoma and 14 cases with adenocarcinoma. There were 27 male patients and 6 female patients, aged 34~71 (mean 56.3) years old. Pathological grades (WHO): 5 cases in grade Ⅰ, 11 cases in grade Ⅱ, 17 cases in grade Ⅲ. Pathological stages (pTNM): 11 cases in stage Ⅰ, 2 cases in stage Ⅱ, 10 cases in stage Ⅲ. 21 cases had lymph node metastasis. 7 specimens of nonneoplastic lung tissue were used as control group (including 5 male patients and 2 female patients). There were 2 cases of mixed tumor harmatomas, 2 cases of tuberculosis granuloma and 3 cases of bronchiectasis.

Methods

Immunohistochemical SP method was used to detect the expression of EGF and EGFR. Rabbit polyclonal antibodies of EGF and EGFR (1:70) were from the Wuhan Boster Biotech Company(China). Taken known breast cancer slices as positive contrast and the substitution of PBS buffer for primary antibody act as negative contrast . Sections(5 μm) were mounted on glass microscope slides. In order to block the endogenous enzyme activation of peroxide, the sections were put into the methyl alcohol hydrogen peroxide solution to seal 30

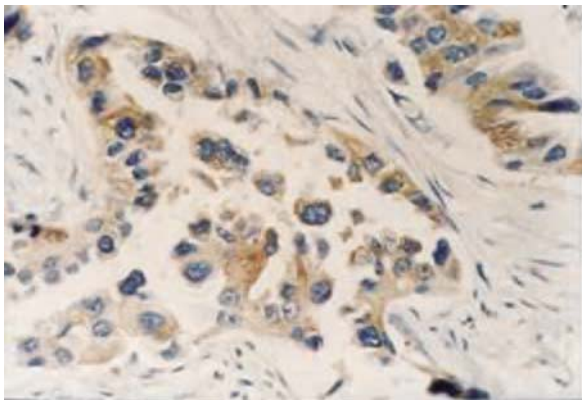

Fig. 1A Overexpression of EGF in non−small cell lung cancer. SP×400

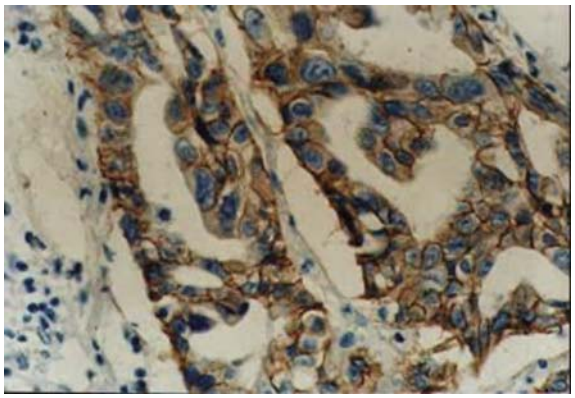

Fig. 1B Over−expression of EGFR in non−small cell lung cancer. SP×400

min with 100ml/L sheep serum. Subsequent the primary antibody were added into wet box for about 120 min, then washed twice with PBS and added biotin labeled secondary antibody. Tissue sections were incubated in streptavidin−biotinylated peroxidase for 20 min, washed in PBS liquid 3 times, and stained in DAB liquid for 5 min. Finally the sections were lightly counterstained with hematoxylin, dehydrated through graded alcohols and xylene, and mounted in Permount.

Criteria of judgement

Cells containing brown particle in cell membrane and cytoplasm were considered as positive cells. The percentage of positive cells was semiquantitatively classified into the following categories: no positive cell was 0 point, positive cells≤25% was 1 point, 26%−50% was 2 points, ≥50% was 3 points. The intensity of staining were also read: the cells no staining was 0 point, yellowish was 1 point, pale brown was 2 points, brown was 3 points.

Multiply the two results of each and got a last value to represent the positive reaction degree for the every case: 0~2 points was considered as(−); 3~6 points as (+).

Statistical analysis

Statistical analysis were performed by SPSS 10. 0 for windows software. The expressions of EGF and EGFR were determined by Fisher accurate probability method. The correlation among different groups were determined by non−parameter Kendall grade method. We made a surviving curve according to Kaplan−Meier method and tested it by Log−Rank method. P <0. 05 was considered as significantly.

RESULTS

Expression of EGF and EGFR in NSCLC

The expression rate of EGF is 57. 6% in NSCLC tissues

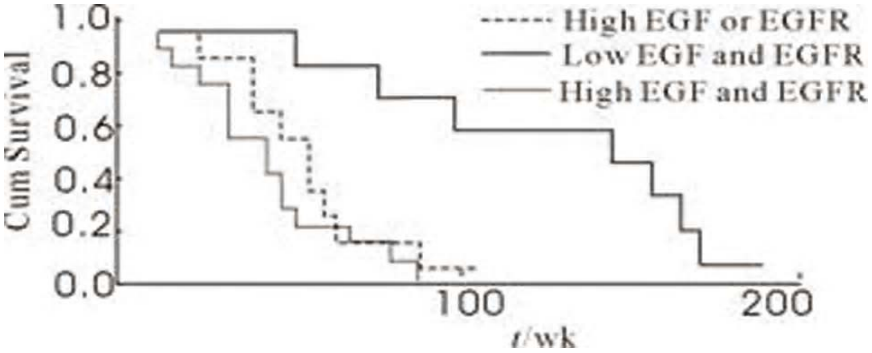

Fig. 2 Survival curves of patients with NSCLC according to EGF and EGFR

Table1 Correlation between EGF level and NSCLC pathology

Pathology	n	EGF		Overexpression rate(%)
		Low	High	
Grade 1, 2	16	8	8	50.0
Grade 3, 4	17	6	11	64.7
Stage Ⅰ, Ⅱ	22	11	11	50.0
Stage Ⅲ	11	3	8	72.7
Lymph node N_0	12	6	6	50.0
Lymph node N_X	21	8	13	61.9

Table 2 Correlation between EGFR level and NSCLC pathology

Pathology	n	EGF		Overexpression rate(%)
		Low	High	
Grade 1, 2	16	7	9	56.3
Grade 3, 4	17	5	12	70.6
Stage Ⅰ, Ⅱ	23	11	12	52.2
Stage Ⅲ	10	1	9	90.0[a]
Lymph node N_0	12	7	5	41.7
Lymph node N_X	21	5	16	76.2[c]

[a]$P<0.05$ vs Ⅰ, Ⅱ; [c]$P<0.05$ vs N_0.

(Fig.1A), there was no expression of EGF in 7 nonneoplastic lung tissues. It was higher than those in normal contral group($P<0.01$). But the expression of EGF had nothing to do with the different histological types, grades, TNM stages and lymph node metastasis of NSCLC ($P>0.05$)(Table 1). The expression rate of EGFR is 63. 6 % in NSCLC tissues (Fig.1B). It was higher than those in normal contral group($P<0.01$). In addition, there was strong relationship between EGFR expression and TNM stage and lymph node metastasis($P<0.05$), and have nothing to do (Table 2) with the histology type and grade.

EGF and EGFR Expression and Survival Rate

EGF expression were closely related with EGFR expression level in patients with NSCLC ($P<0.05$). In 8 patients with EGF negative expression, their EGFR expression were negative too, and in 15 patients with positive EGF expression, their EGFR expression were also positive. The survival rates of the patients in the group of EGF and EGFR positive xpression was obviously lower than those in the group of EGF and EGFR negative expression ($P<0.05$). We also observed the mortality in patients with overexpression of one or two factors were significantly higher($P<0.01$, Fig. 2).

DISCUSSION

The occurrence and development of tumor is a complicated course in which the adjustment of the metabolism of DNA has been changed abnormally during cell cycle as a result of the common effect of cell growth factor and its receptor, cell factors, and numerous oncogene , antioncogene , and so on [1, 2]. The binding of ligand EGF and EGFR causes the activation of the receptor, and then leads to a series of reactions of signal transduction pathways. Finally the copying level of intranuclear gene increases and goes on developing the multiplication, transformation and the malignancy of the cells [1~3]. The expression level of EGF and EGFR in NSCLC is very high, it is very low or negligible in small cell lung cancer and normal lung tissues. In our research we found that there were no expression of EGF and EGFR in 7 nonneoplastic lung tissues, while in 33 NSCLC cases their expression level were 57.6% and 63.6% respectively ($P<0.05$), which suggested that the over-expression of EGF and EGFR may play an important role in the occurrence of NSCLC. Therefore, the preoperative survey of expression level of EGF and EGFR in serum and lung tumor tissues of patients with highly doubtful lung cancer is one of the reference markers of the diagnosis of NSCLC. It was also observed that the expression level of EGF had nothing to do with the histological types, grades, TNM stages and lymph node metastasis of NSCLC ($P>0.05$). In addition, there was strong correlation between EGFR expression level and TNM stages and lymph node metasta-

sis (P <0. 05), and have nothing to do with the histological types and grades of NSCLC(P> 0.05). It suggests that the binding of EGF and EGFR, the activation of EGFR after binding and a series of signal transduction pathways may play an important role in the invasion and metastasis of NSCLC. In addition, our studies show that EGF and EGFR over-expression were closely related with each other(P <0. 05) in NSCLC patients. By comparing the survival rate in positive EGF and/or EGFR expression groups, we find it obviously shorter than that in the negative groups (P <0. 05), which suggests EGF and EGFR may play an important role in the malignant potential of NSCLC, and their over-expressions may be of useful prognostic marking [5, 6]. Therefore, EGF and EGFR oncogene-protein may provide a new target for the early diagnosis, effective treatment, preparation of anti-tumor vaccine, and prognostic judgement of non-small cell lung cancer [5, 7, 8].

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