ONCOLOGY

Clinical and Prognostic Importance of Expression of Epidermal Growth Factor Receptors in Non-Small-Cell Lung Carcinoma

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Radioligand assay demonstrated that in patients with non-small-cell lung cancer, expression of epidermal growth factor receptors in the tumor is higher than in non-tumor tissue. Expression of epidermal growth factor receptors in tumor and non-tumor lung tissue does not correlate with the stage and metastasizing of the disease and tumor histology. It is also shown that expression of epidermal growth factor receptors in tumor and non-tumor tissue is associated with lower survival rate.

Key Words: *epidermal growth factor receptors; non-small-cell lung cancer; total and recurrence-free survival*

Lung cancer affects about one million people per year and is the most common cause of death among cancer patients in the majority of countries. It is now accepted that the outcome of treatment can be predicted by a number of clinical and morphological factors [2]. Biochemical parameters reflecting biological activity of tumor cells and tumor process in the whole are also very important.

Previous studies of the mechanisms regulating tumor cell growth in the tracheobronchial tree demonstrated that lung cancer cells produce polypeptide growth regulators acting via a auto/paracrine mechanism [6-8,12,15-18]. Among these polypeptides, epidermal (EGF) and transforming (a-TGF) growth factors are highly active and most studied regulators. These factors belong to the EGF-like peptide family and interact with common receptors on the membrane of target cells. It was shown that EGF control the growth of not only normal cells, but also their transformed malignant variants. This factor is a potent *in vivo* and *in vitro* mitogen for epitheliocytes and connective tissue cells [3,4].

Receptor of EGF (EGFR) is now considered as a prognostic tissue marker for tumors of various histogenesis [1,10]. However, despite extensive experimental and clinical investigation, the role of EGF-like peptides and their receptors in different tumors, in particular non-small-cell lung cancer (NSCLC) remained poorly understood. The aim of the present study was to evaluate the expression of EGFR in tumor and normal lung tissue and determine the importance of these parameters for NSCLC prognosis.

MATERIALS AND METHODS

Sixty-three patients aged 22-78 years with NSCLC observed and treated at the Department of Thoracic Surgery, N. N. Blokhin Oncology Research Center, from May 1994 to May 1997 were examined.

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Histological preparations were analyzed in accordance with the Cancer Staging System of American Joint Committee (4th ed., 1988). Forty-nine patients had squamous cell carcinoma and 14 patients had lung adenocarcinoma.

Tumor samples and normal lung tissue (500 mg) obtained during surgery were placed on ice. The samples were minced in liquid nitrogen and homogenized at 4°C in TED buffer (pH 7.4, 1 ml per 100 mg tissue) containing 10 mM Tris-HCl (Serva), 1.5 mM EDTA (Sigma), 0.5 mM dithiothreitol (Serva), 10% glycerol (extra-pure grade). The homogenate was centrifuged on an Optima TM TLC centrifuge (Beckman) at 105,000*g* and 4°C for 4 min and the pellet was used for isolation of a crude membrane fraction of tumor cells, where the content of EGFR was measured by radioligand assay. To this end, the pellet was homogenized at 4°C in K,Na-phosphate buffer (pH 7.4) containing 1 g/liter BSA (receptor grade, Sigma) and 0.07 g/liter bacitracin (Sigma) and centrifuged at 2000*g* and 4°C for 10 min. The supernatant presented by crude membrane fraction was transferred to Eppendorf tubes, frozen in liquid nitrogen and stored at -70°C for 12 months before experiments.

The content of EGFR in the membrane fraction of tumor cells was determined by modified radioligand assay [5] using mouse EGF (receptor grade, Sigma) iodinated with $Na^{125}I$ (specific activity 40-122 Ci/mmol) in the presence of Chloramine-T. The membrane preparations (0.2-1 mg/ml protein) were incubated (1 h, room temperature) with 3.5 nM ^{125}I-EGF in the presence or absence of a 200-fold excess of unlabeled EGF. The reaction was stopped by adding ice-cold 75% hydroxyapatite suspension (DNA-grade, Sigma) in K,Na-phosphate buffer (pH 7.4). The samples were washed 3 times with K,Na-phosphate buffer at 4°C, the radioactivity was counted on a Cobra-II-γ counter (Packard), and the amount of specifically bound ^{125}I-EGF was calculated. Tissues containing no less than 5 fmol EGFR/mg membrane protein were considered as EGFR-positive.

Protein concentration in membrane fraction of NSCLC samples and normal lung tissue was measured by the method of Lowry on a DU 650 spectrophotometer (Beckman).

The mean EGFR content in different samples was compared using Student *t* test and frequency distribution by χ^2-test. The total and recurrence-free survival were analyzed by the Kaplan—Meyer method, the significance of differences were determined using *F* test for small samples.

RESULTS

The lung is developed under regulatory control of EGF, α-TGF, and other EGF-like local regulators of cell proliferation and differentiation. Until now there is no consensus on the interrelation of EGFR with known clinical and morphological characteristics of lung cancer.

TABLE 1. Content of EGFR in Tumor and Histologically Unchanged Lung Tissue from Patients with NSCLC of Different Stage According to the TNM System (*M*±*m*)

Parameter		Number of samples	Content of EGFR, fmol/mg protein	
			tumor	normal lung tissue
Stage	I	25	113.6±45.1 (72)	74.3±17.9 (60)
	II	4	15.5±2.5 (50)	30.0±10.4 (100)
	III	30	57.8±14.8 (83)	44.3±12.1 (72)
	IV	4	37.0±21.8 (75)	105.0±79.6 (75)
Primary tumor	T_1	9	232.4±121.0 (67)	78.9±32.2 (78)
	T_2	35	45.2±10.2 (74)	60.4±14.3 (63)
	T_3	14	77.7±30.6 (79)	38.4±15.6 (79)
	T_4	5	37.5±18.1 (80)	59.3±46.8 (60)
Regional lymph nodes	N_0	32	97.6±36.2 (72)	63.3±14.9 (59)
	N_1	11	59.1±24.3 (73)	66.6±27.7 (91)
	N_2	18	49.8±20.1 (78)	35.3±12.1 (72)
	N_3	2	68 and 90 (100)	153.0 (50)
Distant metastases	M_0	59	78.7±20.3 (75)	54.1±9.5 (68)
	M_1	4	37.0±21.7 (75)	105.0±79.6 (75)

Note. Here and in Table 2: % of EGFR-positive samples in shown in parentheses.

TABLE 2. Content of EGFR in Tumor and Normal Lung Tissue in Patients with Different Histological Variants of NSCLC ($M \pm m$)

Histological variant	Content of EGFR, fmol/mg protein	
	tumor	normal lung tissue
Squamous cell cancer (n=49)	83.7±24.3 (72)	65.9±13.1 (64)
Adenocarcinoma (n=14)	55.2±17.9 (86)	32.9±9.2 (86)

The difference between the mean concentration of EGFR in membrane fractions of tumor and normal tissue were insignificant (76.0±19.1 and 57.7±10.1 fmol/ml protein, respectively). This finding agrees with previously reported hyperexpression of EGFR in tumors in comparison with normal lung [9,13,14]. However, a tendency (χ^2=3.27, p=0.07) toward a more frequent expression of EGFR in tumor tissues (75%) in comparison with normal tissue (68%) was revealed.

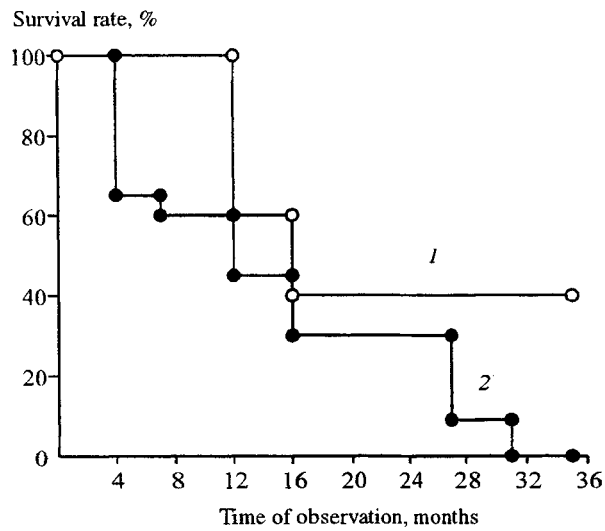

Fig. 1. Recurrence-free survival rate of patients with non-small-cell lung cancer and tumor content of epidermal growth factor below (*1*) and above (*2*) 50 fmol/mg protein.

There were no significant differences between the content of EGFR in the tumor and normal lung tissue at all stages of tumor process (Table 1). Expression of EGFR in tumor and normal tissue did not correlate with the stage of the disease and histological variant of the tumor.

It should be noted that NSCLC is a heterogeneous group of lung tumors including adenocarcinoma and squamous and large cell lung cancer, adenocarcinoma and squamous cell cancer being most frequent forms. EGFR were less frequently found in squamous cell cancer (72%) than in adenocarcinoma (86%), however, this difference did not reach the level of statistical significance because of low number of patients in this group (Table 2). Previous studies also revealed no significant differences in the content of EGFR in different morphological variants of NSCLC [9,16,17].

The role of auto/paracrine growth factors involved in autonomic regulation of tumor cell proliferation in the pathogenesis and progression of lung cancer is little studied. The prognostic value of the expression of EGFR and their ligands remains a matter of controversy. Published data suggest that 79-80% recurrences in patients with NSCLC occur during 18 month after surgery [2].

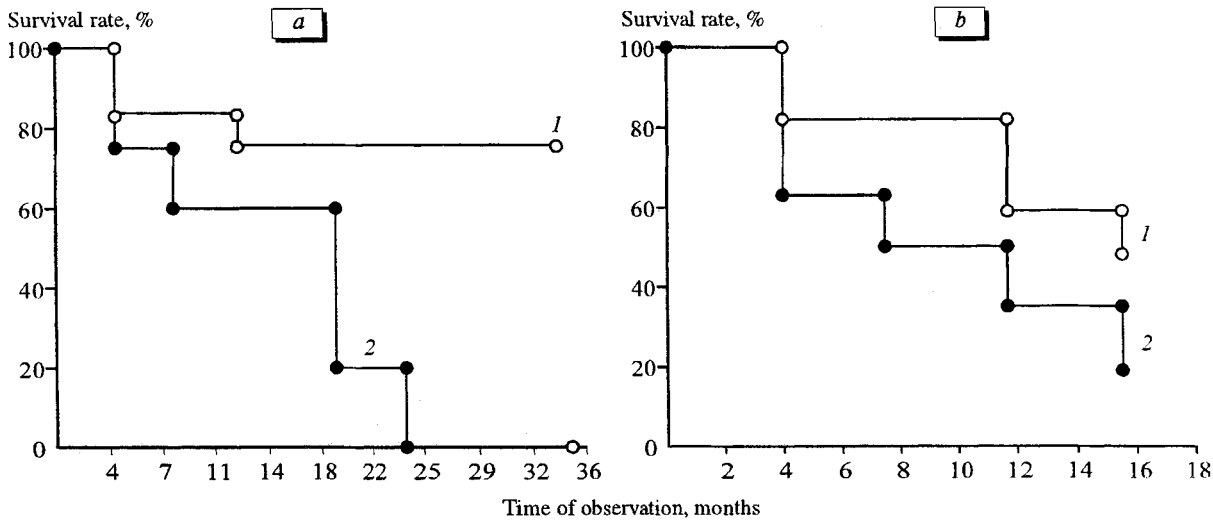

Fig. 2. Total (*a*) and recurrence-free survival rate (*b*) of patients with non-small-cell lung cancer and the content of epidermal growth factor in histologically unchanged lung tissue below (*1*) and above (*2*) 20 fmol/mg protein.

In our study 32 of 63 patients with NSCLC were followed up for 36 months. The total and recurrence-free survival did not correlate with EGFR expression in the tumor. However, more close inspection revealed a threshold level of EGFR expression (50 fmol/mg membrane protein) associated with a significant decrease in recurrence-free survival in patients with NSCLC (p=0.03, Fig. 1). Because of high survival rate in the group of patients with EGFR expression above 50 fmol/mg membrane protein (one patient died in this group) we did not construct total survival curves. When the patients were divided into groups in accordance with their age, stage of the disease, tumor size and metastases, the expression of EGFR did not correlate with the outcome.

For histologically unchanged lung tissue, the threshold concentration of EGFR (20 fmol/mg membrane protein) was determined. The content of EGFR surpassing this value was associated with a significant decrease in the total (p=0.04) and recurrence-free (p=0.05) survival rate in patients with NSCLC (Fig. 2).

Thus, our findings suggest that the expression of EGFR in NSCLC does not depend on histological variant, stage and metastases of the tumor. The correlation between EGFR expression and survival rate allow us to propose this parameter as a prognostic factor of patients with NSCLC.

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