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Epidermal Growth Factor Receptor Expression in Human Lung Cancer Cell Lines[1]

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ABSTRACT

Non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) cell lines were studied for epidermal growth factor (EGF) receptor expression. All NSCLC cell lines tested (eight of eight) had specific EGF binding sites, whereas only five of 11 SCLC cell lines bound EGF. NSCLC and SCLC cell lines expressed the same type of high affinity EGF binding sites with a K_d of 0.5 to 4.5 nM; however, NSCLC cells bound significantly more EGF than SCLC cell lines. The amount of binding sites in NSCLC cells ranged between 71 and 1,000 fmol/mg of protein and in SCLC cells, between 26 and 143 fmol/mg of protein. The two SCLC cell lines with EGF binding values within the range of NSCLC belonged to the variant subtype of SCLC. By means of an anti-*erb*B serum and indirect radioimmunoprecipitation, a strong M_r ~170,000 protein band could be detected in the NSCLC cell lines. This protein corresponds to the EGF receptor molecule. Its identity was proven by competition with excess *erb*B antigen for the antibody during the radioimmunoprecipitation. Furthermore, this M_r 170,000 protein exhibited protein kinase activity as evidenced by *in vitro* autophosphorylation. The radioactivity incorporated into the M_r 170,000 band in radioimmunoprecipitation and protein kinase assays was 10 to 100 times lower in these SCLC cell lines which were positive in the EGF binding assay compared to the NSCLC cell lines. We conclude that NSCLC in contrast to SCLC expresses high levels of EGF receptors which may be used to facilitate the differential diagnosis in some cases of lung cancer. These data suggest that EGF may play a role in growth and differentiation of NSCLC.

INTRODUCTION

Growth factor research and oncogene research are closely linked based on the hypothesis of autocrine secretion; *i.e.*, cancer cells can produce and respond to their own growth factors (1). The sequence homology between the v-*erb*B oncogene product and the cytoplasmic and membrane part of the EGF[3] receptor hints toward a relation between oncogenic stimulation of a cell and normal growth-regulatory mechanisms (2). The EGF receptor is a glycoprotein with a molecular weight of 170,000 to 180,000 with an intrinsic tyrosine-specific protein kinase, which is stimulated upon EGF binding. The extracellular EGF binding domain of 621 amino acids is separated from the intracellular protein kinase domain of 542 amino acids by a short hydrophobic transmembrane part of 26 amino acids (3).

Since the EGF receptor may play a central role in cancer growth control, we examined a series of human SCLC and NSCLC cell lines for the presence of EGF receptors using two approaches: indirect by determining EGF binding sites and direct by identifying the protein in metabolically labeled cells and by using autophosphorylation reaction. Concerning NSCLC, squamous cell carcinoma, adenocarcinoma, large cell carcinoma, and mesothelioma were studied. Here we provide data on the number and kinetics of membrane EGF binding sites and evidence for their nature as membrane receptors.

MATERIALS AND METHODS

Cell Lines

The cell lines used in this study were the SCLC cell lines SCLC-16HV, SCLC-21H, SCLC-22H, SCLC-24H, NCI-H69, NCI-H82, NCI-H146, NCI-N417, NCI-H526, NCI-N592, and DMS-79, and the NSCLC cell lines U-1810 and LCLC-103H (large cell carcinoma), EPLC-32M1, EPLC-32M5, EPLC-65H, EPLC-65M2, and U-1752 (squamous cell carcinoma), A549 and NCI-H23 (adenocarcinoma), and MSTO-211H (mesothelioma). Details concerning the characteristics of these cell lines have been described elsewhere (4–10). In brief, cell lines SCLC-22H, SCLC-24H, NCI-H69, NCI-H146, and NCI-N592 had detectable activities of L-DOPA decarboxylase and were classified as classic SCLC cell lines (8). All other cell lines of SCLC origin had absent or very low activities, a characteristic feature of the variant SCLC subtype (8). Neuron-specific enolase and creatine kinase BB levels were high in the listed SCLC cell lines and low in NSCLC cell lines. Cell line A431, a human vulva carcinoma cell line, and cell line 5637, a human bladder carcinoma cell line, were used as reference cell lines of nonlung origin (4, 11). Cell lines of small cell origin grew as floating cell aggregates, and all other cell lines were substrate adherent. They were kept in RPMI 1640 medium (No. 041-1876; Gibco, Paisly, United Kingdom) supplemented with 10% fetal bovine serum (No. 011-6290; Gibco) in a well-humidified atmosphere of 5% CO_2 at 37°C and were free of *Mycoplasma* contamination. For receptor binding studies, adherent growing cells were seeded in 60-mm Falcon plastic Petri dishes (No. 3006 optical; Falcon, Oxnard, CA) and used during logarithmic growth phase just before they reached confluency. Floating cell lines were kept in 75-cm² culture flasks (No. 658170; Greiner, Nürtingen, West Germany) and used during logarithmic growth phase 48 h after medium change.

Reagents

EGF (from mouse submaxillary glands) was purchased from Sigma, Deisenhofen, West Germany (No. E-7755) and ^{125}I-EGF from Amersham International, Buckinghamshire, United Kingdom (Code IM. 124). EGF was labeled by chloramine-T-mediated iodination of mouse EGF and purified by gel chromatography. The specific activity was approximately 100 μCi/μg of total EGF. The protein assay (Coomassie Brillant Blue G-250) was obtained from Bio-Rad Laboratories, München, West Germany (No. 500-0006). Antisera used in the indirect immunoprecipitation assay and protein kinase assay were described elsewhere (12).

Protein Assay

Cells were washed twice with 4-ml portions of Hanks' BSS (No. 041-4175; Gibco). To precipitate the proteins, 2 ml of 10% TCA in double-distilled water were added to the NSCLC monolayer or to the centrifuged SCLC cell lines (150 × *g*, 5 min). After 30 min at 4°C, the TCA solution was decanted. The proteins were dissolved in 1 ml of 0.2 N NaOH at room temperature. The protein content was measured with the Bio-Rad standard protein assay. There was no interfering effect

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[3] The abbreviations used are: EGF, epidermal growth factor; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; L-DOPA, 3,4-dihydroxy-L-phenylalanine; BSS, balanced salt solution; TCA, trichloroacetic acid; DTT, dithiothreitol; PK, protein kinase; RIP, radioimmunoprecipitation.

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with NaOH up to 25 μg of protein/sample (corresponding to 100 to 500 μg of protein/ml).

Indirect Immunoprecipitation Assay (RIP)

On a 9-cm Petri dish, 2 to 4 × 10^6 cells of each cell line were grown in logarithmic phase to about 50% confluency. Cells were washed to remove medium and serum and incubated for 90 min with RPMI 1640 medium free of unlabeled methionine supplemented with dialyzed serum and [^{35}S]methionine (500 μCi/ml). Cells were lysed immediately in lysis buffer (50 mM Tris-HCl (pH 7.4):150 mM NaCl:1% Triton X-100:10% glycerol:1 mM DTT:10 mM NaF:100 units/ml of trasylol:1 mM phenylmethylsulfonyl fluoride) and treated with antiserum and Protein A for indirect immunoprecipitation. The antiserum against bacterially expressed *erb*B was prepared in rabbits as described (12). The precipitates were analyzed on 10% sodium dodecyl sulfate-polyacrylamide gels, dried, and expressed for autoradiography (exposure, 48 h). The specificity of the reaction was tested by competition with the antigen, *i.e.*, by absorbing the antibodies with excess of the bacterially expressed *erb*B which had served as the antigen. After preincubation (10 μl of serum:20 μg of protein, 30 min, 4°C), the complex was applied to the lysate, and the immunoprecipitation procedure was performed as described above. Details have been published elsewhere (12).

PK Assay

Immunoprecipitates were prepared from 10^7 cells as described for the indirect immunoprecipitation procedure except that cells were not labeled with the isotope. The immunoprecipitates were washed 4 times with lysis buffer as described above except that Triton X-100 was 0.1%. The final wash was performed in a kinase buffer containing 50 mM Tris-HCl (pH 7.5), 50 mM NaCl, 0.1% Triton X-100, 10% glycol, 1 mM NaF, and 1 mM DTT. The protein kinase reactions were performed in 100-μl total volume with 10 mM $MnCl_2$ added and with 20 μCi of [γ-^{32}P]ATP (3000 Ci/mmol; Amersham, United Kingdom) without unlabeled ATP. Incubation was performed for 10 min at 0°C and terminated by the addition of sodium dodecyl sulfate-containing gel electrophoresis buffer. The reaction products were directly applied to sodium dodecyl sulfate-polyacrylamide gels and processed for autoradiography (exposure, 30 min).

For determining the relative protein kinase activity, the M_r 170,000 band was cut off the gel, and the incorporated radioactivity was counted. The cpm values obtained were expressed as relative values using SCLC-22H or SCLC-24H as standard.

^{125}I-EGF Binding Assay

NSCLC. Monolayer cultures were washed twice with 4-ml portions of prewarmed Hanks' BSS. One ml of the prewarmed binding medium consisting of RPMI 1640 and 0.1% bovine serum albumin was added to each dish. Labeled and unlabeled EGFs (both dissolved in phosphate-buffered saline; M_r 6100) were added, and a final volume of 1.5 ml/dish was obtained. The corresponding cell density was about 10^6 cells/dish as calculated with a Neubauer chamber after harvesting cells by trypsin treatment. After incubation at 37°C, unbound ^{125}I-EGF was removed by washing the cells 5 times with 4-ml portions of cold Hanks' solution containing 0.1% bovine serum albumin. To solubilize the cells, 2 ml of 0.1 N NaOH were added to each culture dish. After 30 min at 37°C, the content of each dish was transferred into counting vials, and the radioactivity was measured. Nonspecific binding was determined by measuring the cell-bound radioactivity in the presence of 10 μg/dish (1 μM) unlabeled EGF. Specific binding was calculated from the difference between cell-bound radioactivity in the presence and absence of unlabeled EGF and analyzed by the method of Scatchard (13).

SCLC. Cells were washed twice with Hanks' BSS by centrifugation at 150 × *g* for 5 min and adjusted to 4 to 6 × 10^6 cells/ml in RPMI 1640 with 0.1% bovine serum albumin. One hundred μl of labeled and unlabeled EGF were added to 300 μl of the concentrated cell suspension. After incubation at 37°C, unbound radioactivity was removed by washing the cells twice with cold Hanks' solution containing 0.1% bovine serum albumin in a filtration apparatus (Millipore, Bedford, MA) with GF/B filters (Whatman, Maidstone, United Kingdom). The filters were dried and transferred into counting vessels, and the radioactivity was measured. Specific and nonspecific binding was determined as described for NSCLC.

Radioactivity Measurements

Radioactivity was determined with a gamma counter (Hydrogamma 16; Oakefield Instruments, Ltd., Eynsham, Oxford, United Kingdom). The efficiency was 70%.

RESULTS

Effect of Temperature on Time Course of ^{125}I-EGF Binding. At 37°C maximum binding was achieved after a 60-min incubation period in NSCLC and SCLC cell lines. After longer incubations the cell-bound radioactivity decreased to 65% of the maximum value (5-h incubation). At 0°C the maximum binding (65% of the maximum value at 37°C) occurred after an incubation period of 90 min. No loss in cell-bound radioactivity was detected up to 5 h of incubation. Based on these results the ^{125}I-EGF binding assays were performed with an incubation period of 60 min at 37°C.

Competition of ^{125}I-EGF. Different NSCLC and SCLC cell lines were studied in displacement experiments with a constant amount of the labeled derivative (0.5 nM) and varying quantities of unlabeled EGF in the concentration range of 0.1 to 2 × 10^3 nM. The effect of increased concentrations of unlabeled EGF on ^{125}I-EGF binding is shown in Fig. 1*A* for three NSCLC cell lines (A549, EPLC-32M5, NCI-H23) and in Fig. 1*B* for two SCLC cell lines (NCI-H146 and SCLC-22H). As can be seen from Fig. 1, differences in the maximum cell-bound radioactivity for NSCLC and SCLC cell lines and among different NSCLC cell lines existed. The competition of labeled EGF with unlabeled was completed when unlabeled EGF was added in concentrations >100 nM for all NSCLC cell lines tested (list of cell lines tested in Table 1). No displacement could be obtained in the SCLC cell line NCI-H146. The residue level of cell-bound radioactivity was low, especially for NSCLC cell lines, suggesting low unspecific binding.

Scatchard Analysis of ^{125}I-EGF Binding. In Fig. 2 the effect of increased concentrations of labeled EGF on binding in NSCLC and SCLC cell lines is shown for cell lines EPLC-65M2 and DMS-79, respectively. Nonspecific and specific binding was determined as described in "Materials and Methods." As anticipated from competition experiments, nonspecific binding was very low for EPLC-65M2 and slightly higher for DMS-79. To analyze binding characteristics, Scatchard plots were evaluated (*inserts* in Fig. 2). The maximal amount of binding sites (B_{max}) was achieved from the *x*-intercept of the Scatchard plot. About 487 fmol of EGF/mg of protein were maximally bound by EPLC-65M2 and about 143 fmol of EGF/mg of protein by DMS-79. Identical data were obtained regardless of whether the NSCLC cell line EPLC-65M2 was investigated by the usual method for NSCLC cell lines or by the filtration method after solubilizing the cells by scraping. EGF binding characteristics were established for 8 NSCLC and for 11 SCLC cell lines. Evaluations of all cell lines tested are summarized in Table 1. EGF binding sites were found in 8 of 8 NSCLC cell lines and 5 of 11 SCLC cell lines. The dissociation constants were calculated from Scatchard plots and are very similar to those for NSCLC and SCLC cell lines. However, a marked difference in the maximally bound EGF amounts was found between NSCLC and SCLC cell lines. The range of bound EGF/mg of protein was 71 to 606 fmol/mg of protein in

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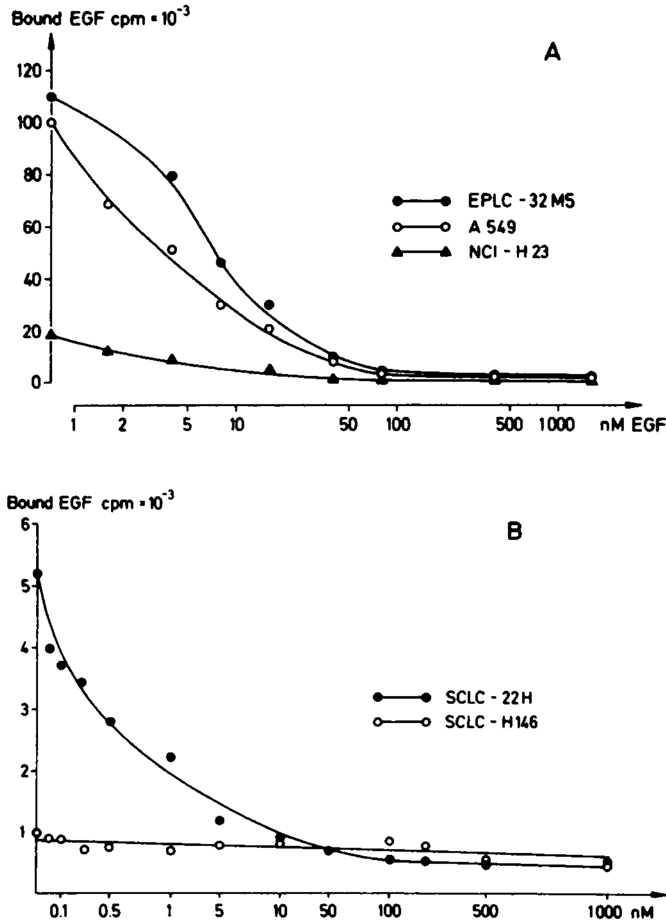

Fig. 1. Competition of labeled and unlabeled EGF for binding to NSCLC cell lines A549, EPLC-32M5, and NCI-H23 (*A*) and to SCLC cell lines NCI-22H and NCI-H146 (*B*). Indicated amounts of unlabeled EGF and 0.5 nM ^{125}I-EGF (140,000 cpm/ng) were added simultaneously to the culture dish. *Ordinate*, bound radioactivity (cpm); *abscissa*, unlabeled EGF (nM).

Table 1 *EGF binding sites in human lung cancer cell lines*

Cell line	Maximum binding (fmol/mg)	K_d (nM)
Squamous cell carcinoma		
EPLC-32M1	486	0.50
EPLC-65H	570	2.60
EPLC-65M2	487	1.44
U-1752	606	1.29
Adenocarcinoma		
A549	553	4.54
NCI-H23	71	0.59
Large cell carcinoma		
LCLC-103H	522	2.63
U-1810	252	1.48
Mesothelioma		
MSTO-211H	1000	3.10
Small cell carcinoma, classic subtype		
SCLC-22H	26	0.58
SCLC-24H	33	0.50
NCI-H69	—[a]	—
NCI-H146	—	—
NCI-N592	—	—
Small cell carcinoma, variant subtype		
SCLC-16HV	100	2.60
SCLC-21H	—	—
NCI-H82	—	—
NCI-N417	26	0.84
NCI-H526	—	—
DMS-79	143	0.95
Nonlung carcinoma		
A431	2370	1.78
5637	1430	3.25

[a] —, defined as negative.

NSCLC cell lines and 26 to 143 fmol/mg of protein in SCLC cell lines. Both cell lines of nonlung origin, the urinary bladder carcinoma cell line 5637 (14) and the vulvar carcinoma cell line A431 (15) used as controls, had an even higher binding potential.

Analysis of the EGF Receptor and Its Associated Protein Kinase Activity. EGF binding to tumor cells only indirectly suggests that binding involves EGF receptors. In order to demonstrate EGF receptor molecules directly in the appropriate cells, antibodies which recognize the EGF receptor specifically were used for its identification in an indirect immunoprecipitation procedure. The antibody was directed against the oncogene protein *erb*B, which represents a truncated EGF receptor (3). Properties of this antibody have been described elsewhere (12). [^{35}S]Methionine-labeled cells were treated for indirect immunoprecipitation with the anti-*erb*B serum. The result is shown in Fig. 3. A strong band with an approximate molecular weight of 170,000 was detected with anti-*erb*B serum in the control A431 and was also detected in the NSCLC cell lines EPLC-65M2, EPLC-32M1, and MSTO-211H cells. To demonstrate more clearly that the prominent band represents the EGF receptor molecule, the specificity of the reaction was proven by competition of the excess antigen which was the bacterially expressed *erb*B protein for the antibody. Presence of the competing antigen is indicated by *+C* in Fig. 3*A*. Clearly its presence completely erased the precipitation band.

The EGF receptor is associated with a tyrosine-specific protein kinase. This property is a further independent proof for the identification of the receptor. Therefore a protein kinase assay was performed using immunoprecipitated proteins from various cell lines. Incorporation of radioactively labeled ATP into the EGF receptor itself occurs in an autocatalytic fashion. Radioactively labeled EGF receptor was detected in A431, EPLC-65M2, EPLC-32M1, and MSTO-211H cells. The result is shown in Fig. 3*B*. The appearance of autophosphorylated EGF receptor is in accordance with the presence of EGF receptors as evidenced by [^{35}S]methionine-labeled M_r 170,000 molecules shown in Fig. 3*A*. These results indicate that indeed EGF receptors are highly expressed in NSCLC cells.

While the incorporated radioactivity at a molecular weight of 170,000 in RIP and in the even more sensitive PK was quite strong for NSCLC cell lines, it was markedly reduced in the SCLC cell lines positive in the binding assays. Table 2 gives a comparison between the cpm values obtained for SCLC and NSCLC cell lines after the M_r 170,000 band was cut off the gel. When the cpm values obtained are expressed as relative values (relative protein kinase activity), it can be seen that they are 10 to 100 times smaller in the five SCLC cell lines positive in EGF binding experiments compared to the values obtained for NSCLC cell lines and 1,000 times smaller compared to the control A431.

DISCUSSION

We have studied EGF receptor expression in human lung cancer cell lines. Displacement experiments demonstrated competition of labeled EGF with unlabeled EGF in all NSCLC cell lines tested but not in all SCLC cell lines. Time course experiments for NSCLC and SCLC cell lines demonstrated differ-

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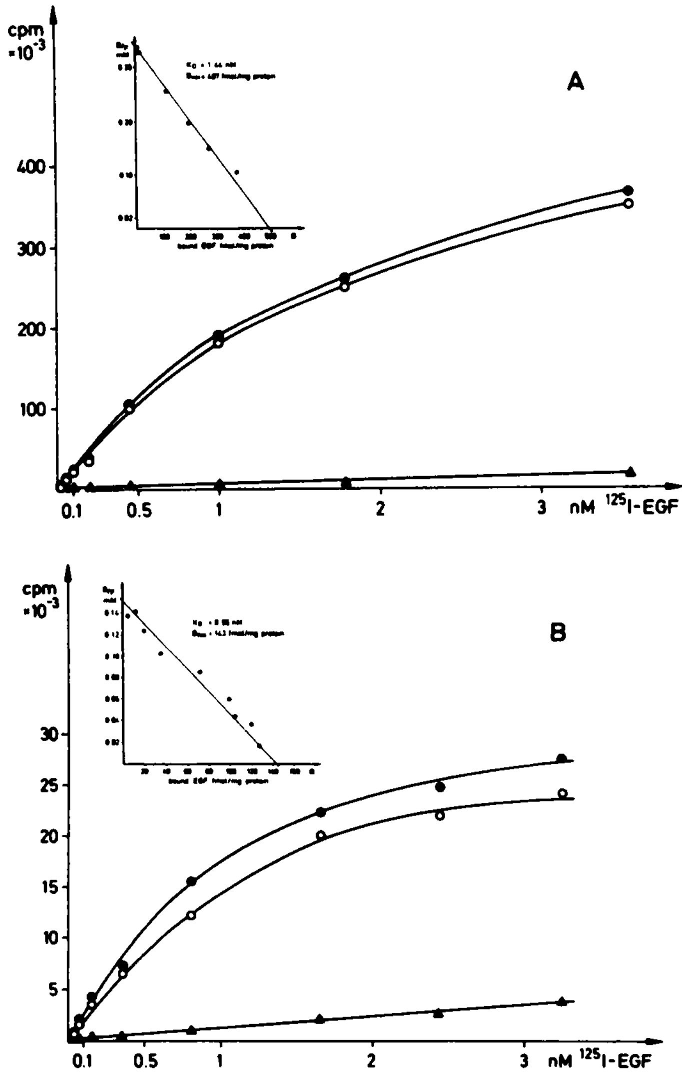

Fig. 2. Effect of EGF concentration on binding of NSCLC cell line EPLC-65M2 (*A*) and SCLC cell line DMS-79 (*B*). Labeled EGF (150,000 cpm/ng) was added as indicated to the culture dish without (●) and with (▲) an excess amount of unlabeled EGF (1 μM). ○, specific binding. *Ordinate*, bound radioactivity (cpm); *abscissa*, ^{125}I-EGF (nM). *Inserts*, Scatchard plot of binding data.

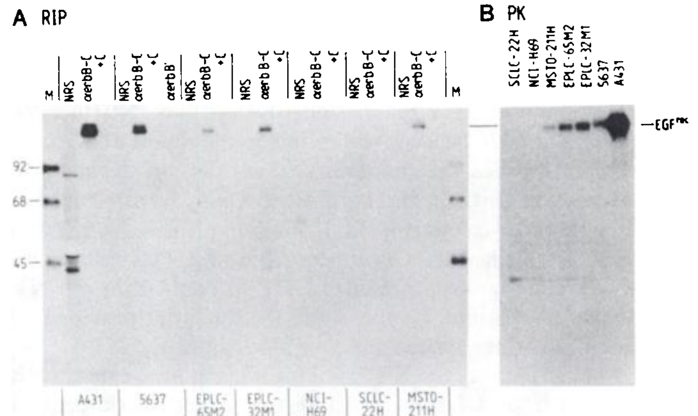

Fig. 3. RIP (*A*), indirect immunoprecipitation of EGF receptor proteins in [^{35}S]methionine (2 to 4 × 10^{6} cell each)-labeled cell lines. The sera used were 10 μl of normal rabbit serum (*NRS*), 10 μl of a negative rabbit hyperimmune serum 1317/5 (α-*erb*B'), 10 μl of an anti-*erb*B serum (α-*erb*B). Presence of purified bacterial MS2-*erb*B protein (50 μg) as competing antigen is indicated (+*C*). PK (*B*), analysis of protein kinase activity of the EGF receptor *in vitro*. Freshly harvested cells (10^{6}) were lysed and tested for protein kinase activity by the addition of radioactively labeled ATP to preformed immune complexes. The reaction products were analyzed by gel electrophoresis and autoradiography. For nomenclature of sera, see RIP.

Table 2 *Cpm values of incorporated radioactivity in PK and relative protein kinase activity in SCLC and NSCLC cell lines*

	cpm	Relative protein kinase activity
SCLC cell lines		
SCLC-16HV	2,192	10
DMS-79	377	1
NCI-N417	2,602	10
SCLC-22H	244	1
SCLC-24H	166	1
NSCLC cell lines		
EPLC-32M1	11,173	100
EPLC-65H	21,350	100
LCLC-103H	9,960	100
Nonlung carcinoma		
A431	127,553	1,000

ences in EGF binding at 37°C and 0°C, respectively. These results were in agreement with investigations on fibroblasts (16) and nonlung cancer cell lines (17). The decrease in cell-bound radioactivity after an incubation period of 1 h at 37°C may be explained by receptor internalization and degradation (16–18). At 4°C, internalization and degradation of receptor-bound EGF were negligible, but EGF binding was reduced, which may be caused by conformational changes of proteins and phospholipids at low temperature (18).

Detailed analysis of EGF binding sites in NSCLC and SCLC cell lines based on Scatchard analysis revealed a characteristic difference in the amount of binding sites between the two lung cancer groups (Table 1). The amount of binding sites in NSCLC cells ranged from 71 to 1000 fmol/mg of protein and was significantly lower in SCLC cells (26 to 143 fmol/mg of protein). In addition, all NSCLC cell lines tested (squamous cell carcinoma, adenocarcinoma, large cell carcinoma, mesothelioma) had EGF binding sites, and only 5 of 11 SCLC cell lines bound EGF (Table 1).

The amount of EGF molecules bound/cell (82,500 molecules for EPLC-65M2 and 17,000 for DMS-79) agreed with values found in other cancer cell lines (15). The dissociation constants were very similar and characteristic for EGF binding sites (16, 19, 20) and classify the EGF binding sites as high affinity sites. Nonspecific binding was low, especially for the NSCLC cell lines. For most of the cell lines tested, the low bound-free ratio at low growth factor concentrations found in Scatchard plots can be explained by a longer time required for maximum binding. This agrees with data published for EGF binding to human fibroblasts (16) and to the urinary bladder carcinoma cell line 5637. Both control lines A431 (squamous cell carcinoma) and the urinary bladder carcinoma cell line 5637 (adenocarcinoma) had even higher levels of EGF binding sites than NSCLC cells. This may be caused by an amplification of the EGF receptor gene, which is known for A431 but has not yet been investigated in 5637. The levels of EGF binding sites in NSCLC are not unusually high and do thus not suggest an EGF receptor gene amplification in this type of lung cancer. Within the group of SCLC cell lines, low levels of EGF binding sites were found in both subtypes of SCLC, classic and variant. The highest levels, however, were found in cell lines of the variant subtype. In light of the recently published inverse relationship between EGF receptor expression and differentiation in tumors of neuroectodermal origin (21), our data suggest that the variant SCLC subtype is less differentiated than the classic subtype. This is in agreement with the reported loss of neuroendocrine features (L-DOPA decarboxylase) in variant SCLC cell lines (8, 10).

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Our data generally confirm earlier results published by Sherwin *et al.* (22). In contrast to these authors, however, we found small amounts of EGF binding sites in 5 of 11 SCLC cell lines, including the only EGF binding-positive cell line (NCI-N417), which was stated to be a converter line, in the report of Sherwin *et al.* (22). Besides the possibility of conversion during prolonged passage *in vitro*, these differences may be attributed to the fact that all except one EGF binding-positive SCLC cell line were established in laboratories (Marburg, Federal Republic of Germany, and Dartmouth, NH) different from the National Cancer Institute (Bethesda, MD); the latter provided all the SCLC cell lines for the study of Sherwin *et al.* (22).

In order to classify the EGF binding sites in lung cancer cells as EGF receptors, various cell lines were tested in a protein kinase assay and in an indirect immunoprecipitation assay with an antibody directed against the internal part of the EGF receptor. In the indirect immunoprecipitation assay, a strong band with an approximate molecular weight of 170,000 could be detected in the NSCLC cell lines EPLC-65M2, EPLC-32M1, and MSTO-211H. The specificity of the reaction was proven by competition for the antibody by excess antigen. The appearance of autophosphorylated EGF receptors in the protein kinase assay confirmed the results obtained by the indirect immunoprecipitation assay and provided more evidence for the characterization of the EGF binding sites as EGF receptors. As to what to expect from results of the binding assay, the incorporated radioactivity at the M_r 170,000 band in RIP and PK was strongly reduced in the five SCLC cell lines positive for EGF binding sites. The relative protein kinase activity revealed that the cpm values obtained for the SCLC cell lines were 10 to 100 times decreased compared to the cpm values obtained for NSCLC cell lines (Table 2). Again, the even higher levels of EGF receptors in the control line A431 are demonstrable.

We are currently conducting experiments on the proliferative effects of exogenous EGF in serum-free growing cell lines. Preliminary data have shown that the growth of NCI-H69 and SCLC-16HV is independent of EGF. In contrast, U-1810 seems to require exogenous EGF for optimal serum-free culture growth. Data taken from the literature show that EGF stimulates epithelial growth in cells with moderate receptor levels but inhibits growth in malignant cells with high level receptor expression (23–25).

In summary, we have shown that NSCLC, in contrast to SCLC, expresses in all cases high levels of EGF receptors. This suggests that EGF may serve as a biological marker for NSCLC and may play a role in the growth and differentiation control of human lung cancer.

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