ANTICANCER RESEARCH *25*: 4693-4698 (2005)

Combined Overexpression of EGFR and Estrogen Receptor α Correlates with a Poor Outcome in Lung Cancer

HIDEKI KAWAI[1], AKIRA ISHII[2], KIYOTADA WASHIYA[2], TOSHIKO KONNO[2], HIROTO KON[2], CHIHARU YAMAYA[2], IWAO ONO[2] and JUNICHI OGAWA[1]

[1]Division of Thoracic Surgery, Department of Surgery, Akita University School of Medicine, 1-1-1 Hondo, Akita 010-8543; [2]Department of Pathology, Nakadori General Hospital, Akita, 3-15 Minamidori Misonocho, Akita 010-8577, Japan

Abstract. *Background: The aim of the present study was to determine the prognostic significance of the combined overexpression of epidermal growth factor receptor (EGFR) and estrogen receptor α (ER-α) in non-small cell lung cancer (NSCLC). Patients and Methods: An immunohistochemical assessment of EGFR and ER-α expression was carried out in tumor specimens from 122 consecutive NSCLC patients after surgery. Results: Of the 122 tumors examined, 57 (46.7%) overexpressed EGFR, 63 (51.6%) overexpressed ER-α, while 33 (27%) overexpressed both EGFR and ER-α. Univariate analyses showed that the overexpression of EGFR or ER-α correlated with the histological grade (p=0.01 and p=0.03, respectively) and a poorer prognosis (p=0.007), while overexpression of ER-α also correlated with smoking (p=0.02). Multivariate analysis showed the combined overexpression of EGFR and ER-α to be an independent indicator of poorer prognosis (p=0.0006). Conclusion: The combined overexpression of EGFR and ER-α in NCLSC patients is predictive of poor outcome and, thus, represents a valuable prognostic factor.*

Epidermal growth factor receptor (EGFR) is a product of the *erbB* gene family, which encodes a group of transmembrane receptor-type tyrosine-protein kinases. It is expressed in a variety of cell types, where it is involved in the regulation of cell proliferation and differentiation. Overexpression of EGFR has been detected in about 40～90% of non-small cell lung cancers (NSCLCs), with especially high levels being found in squamous cell carcinomas (1, 2). Evidence suggests that such overexpression of EGFR is involved in cancer progression and is indicative of a poor prognosis (3, 4).

Estrogen exerts most of its effects in breast cancer *via* its receptors, estrogen receptor α (ER-α) and ER-β. In breast cancer, the expression of ER-α is a useful marker that provides information about prognosis and the potential efficacy of hormonal therapy (5, 6). Recent studies have shown that ER-α is also expressed in NSCLC (7-9), though its function remains unknown.

Some recent studies have suggested that bidirectional cross-talk between EGFR and cellular pools of ER contribute to reproductive organ physiology and pathophysiology (10-12). In that regard, estrogen has been shown to stimulate the transactivation of EGFR in breast cancer cells, leading to up-regulation of cAMP and ERK (13), while ER-α directly associates with the membrane-tethered p85 subunit of phosphatidylinositol 3-kinase (PI3K) in endothelial cells (14). EGFR is also known to associate with PI3K (15), suggesting the possible existence of an ER/PI3K/EGFR multiprotein complex in some cell types.

With this background, the aim of the present study was to evaluate the expressions of EGFR and ER-α in 122 surgical specimens from NSCLC patients and to correlate those expressions with long-term survival.

Patients and Methods

Patients. This retrospective study was approved by the Human Ethics Committee of Nakadori General Hospital, Akita, Japan. One hundred and thirty-two lung tumors were obtained from patients who underwent surgical resection for NSCLC in the Department of Thoracic Surgery, Nakadori General Hospital between January 1995 and December 1997. The patient characteristics are summarized in Table I. All patients were staged using standard UICC criteria. Ten tumors were excluded from the study because the patients were administered postoperative radiotherapy. Adjuvant chemotherapy was not administered in all cases.

Correspondence to: Hideki Kawai, Division of Thoracic Surgery, Department of Surgery, Akita University School of Medicine, 1-1-1 Hondo Akita 010-8543, Japan. Tel: +81-18-884-6132, Fax: +81-18-836-2615, e-mail: hkawai@doc.med.akita-u.ac.jp

Key Words: EGFR, estrogen receptor α, lung cancer, prognostic factor.

Table I. *Clinical characteristics of the NSCLC patients in the study.*

	EGFR			ER-α		
Variables	low	high	*P*	low	high	*P*
Age			0.06			0.26
Median	66	68		67	68	
Range	42-77	38-81		38-81	42-81	
Gender			0.91			0.22
Male	36	31		29	38	
Female	29	26		30	25	
Smoking			0.82			0.02
Smoker	34	31		25	40	
Non-smoker	31	26		34	23	
UICC stage			0.93			0.21
Ia	27	21		24	24	
Ib	11	8		9	10	
IIa	5	4		6	3	
IIb	6	7		6	7	
IIIa	10	12		6	16	
IIIb	4	2		5	1	
IV	2	3		3	2	
Histology			0.11			0.35
Adenocarcinoma	52	40		48	44	
SCC	11	17		10	18	
LC	2	0		1	1	
Grade			0.01			0.03
Well	33	14		29	18	
Moderate	22	27		22	27	
Poor	10	16		8	18	

Assay for EGFR and ER-α. The expressions of EGFR and ER-α in specimens of resected lung cancer tissue were analyzed immunohistochemically. The specimens were fixed in formalin, embedded in paraffin and cut into 5-μm sections. The sections were then deparaffinized in xylene and ethanol, placed in 0.1 mol/L citrate buffer (pH 6.0) and irradiated for 15 min with microwaves (750 W). The primary antibodies used were mouse monoclonal anti-EGFR (H11, DAKO, Kyoto, Japan, diluted 1:100 in phosphate-buffered saline (PBS)) and anti-ER-α (HC-20, Santa Cruz Biotechnology, Santa Cruz, CA, diluted 1:50 in PBS) antibody. The distributions of the antibodies were visualized by immunostaining the specimens using the EnVision method (DAKO), according to the manufacturer's instructions. The EGFR expression was then scored according to the intensity of staining and the number of stained tumor cells. A score of 0 indicated staining in less than 10% of considered cells; 1, light staining in more than 10% of considered cells; 2, moderate staining in more than 10% of considered cells; and 3, strong staining in more than 10% of considered cells. For statistical analysis, scores of 0 or 1 were defined as no overexpression; scores of 2 and 3 as overexpression. The ER-α expression was categorized into eight grades according to previously described immunohistochemical scores (16-18). Briefly, entire slides were each examined under a light microscope. Initially, a proportional score was assigned, which represented the estimated proportion of positive tumor cells (0, none; 1, <1/100; 2, 1/100 to 1/10; 3, 1/10 to 1/3; 4, 1/3 to 2/3; and 5, >2/3). Next, an intensity score was assigned, which represented the average intensity of the positive tumor cells (0, none; 1, weak; 2, intermediate; and 3, strong). The proportional and intensity scores were then added to obtain a total score, which ranged from 0 to 8. For statistical analysis, scores of 0 to 4 were defined as no overexpression; scores of 5 to 8 as overexpression. The slides were scored by pathologists with no knowledge of the ligand-binding results or patient outcomes.

Statistical analysis. Two groups were compared using the χ^2 test. The probabilities of overall survival were calculated using the Kaplan-Meier method and compared using the log-rank test. A Cox proportional hazard model was used to determine factors related to overall survival. These analyses yielded hazard ratios, their 95% confidence intervals and *p*-values. Values of $p<0.05$ were considered significant.

Results

The patient characteristics and demographics are summarized in Table I. Univariate analyses showed that overexpression of EGFR or ER-α correlated with histological grade ($p=0.01$ and 0.03, respectively), and that overexpression of ER-α also correlated with smoking ($p=0.02$). There was no correlation between the overexpressions of EGFR or ER-α with any of the other clinical variables, which included age, gender, histological stage and histological type. Of the 122 specimens

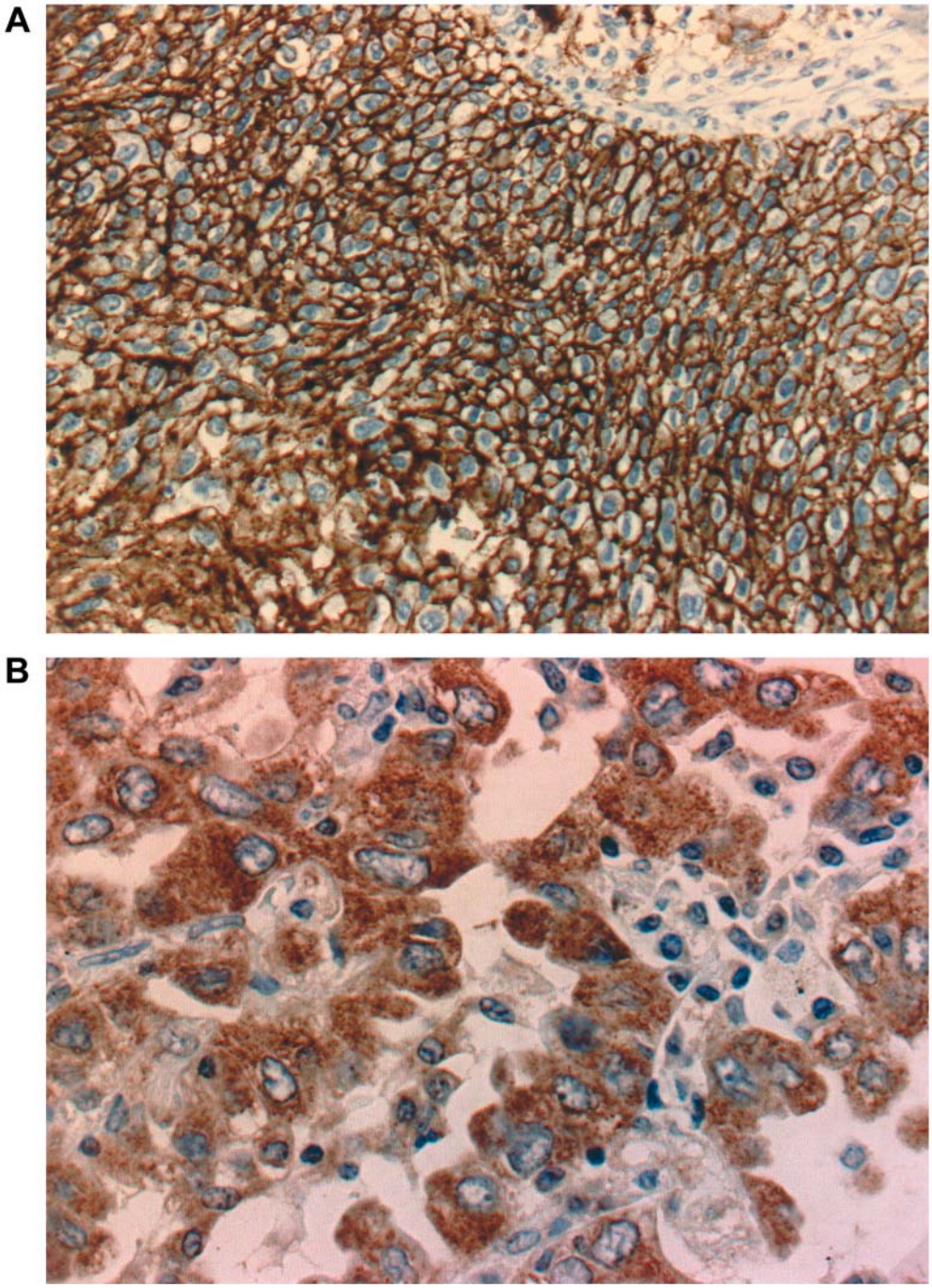

Figure 1. *Immunohistochemical staining of lung cancer specimens expressing EGFR and ER-α. A, Squamous cell carcinoma cells showing EGFR immunoreactively localized at the plasma membrane. B, Adenocarcinoma cells showing ER-α immunoreactivity localized in the cytoplasm.*

studied, 57 (46.7%) overexpressed EGFR, 63 (51.6%) overexpressed ER-α, while 33 (27%) overexpressed both EGFR and ER-α. The expressed EGFR was localized to the plasma membrane (Figure 1A), whereas ER-α was localized in the cytoplasm (Figure 1B).

The overall Kaplan-Meier survival curves for the EGFR and ER-α expressions are shown in Figure 2. Univariate analyses revealed that expression of EGFR was linked to poor overall survival ($p=0.007$) (Figure 2A), as was expression of ER-α ($p=0.007$) (Figure 2B). Furthermore, EGFR (high)/ER-α

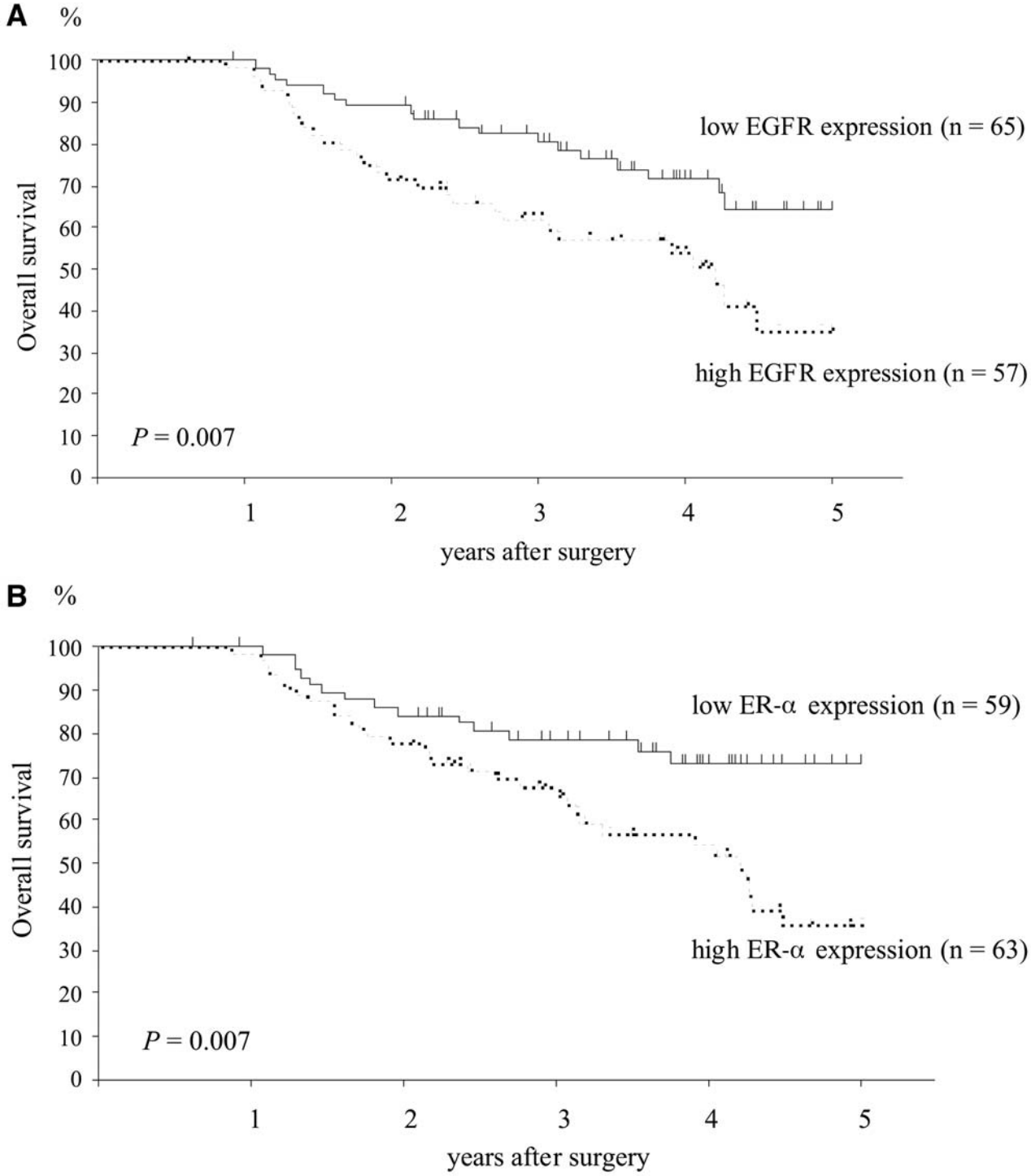

Figure 2. *A, B, Kaplan-Meier analysis showing the overall survival of NSCLC patients categorized according to their EGFR (A) or ER-α (B) expression status. P-values were calculated using the log-rank test.*

(high) patients had a significantly poorer prognosis (5-year survival rate: 25.4%) than EGFR (low)/ER-α (low) patients (5-year survival rate: 81.8%) (p=0.0003) (Figure 3).

Multivariate analysis of overall survival using the Cox proportional hazard model revealed that, of the factors listed in Table II, only stage and combined overexpression

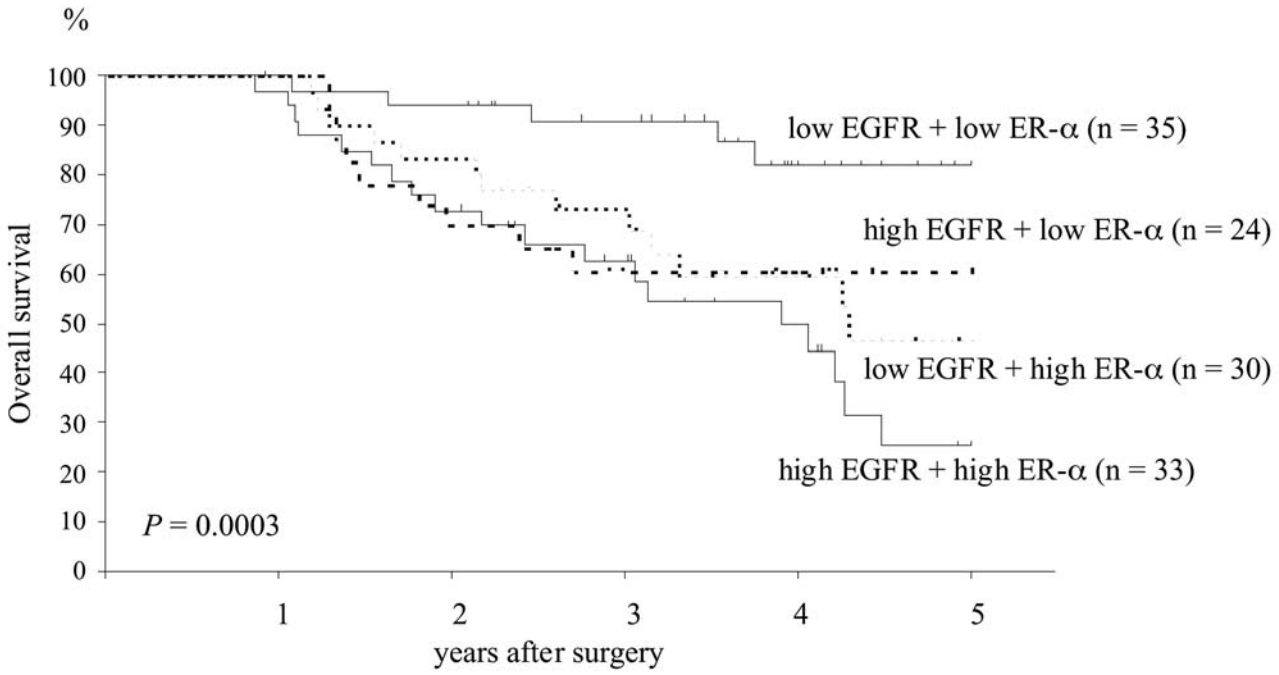

Figure 3. *Kaplan-Meier analysis showing overall survival of NSCLC patient subtypes defined by the combination of their EGFR and ER immunohistochemical status. P-values were calculated using the log-rank test.*

of EGFR and ER-α (double marker) were significant independent variables that correlated with overall survival ($p=0.003$ and $p=0.0006$, respectively); the double marker yielded a hazard ratio of 2.03 with a 95% confidence interval extending from 1.35 to 3.04.

Discussion

Although overexpression of EGFR has been observed and its prognostic value confirmed in multiple tumor types, comparatively little information is available on the role of EGFR in NSCLC. Still, Selvaggi *et al*. showed that overexpression of EGFR correlates with a poor prognosis in completely resected NSCLC (4). It is therefore disappointing that in the 2 years that Gefitinib, an EGFR receptor tyrosine kinase inhibitor, has been prescribed to treat lung cancer, it has proved ineffective in many patients, even those overexpressing EGFR. This may be related to the fact that Gefitinib is clinically effective in patients whose tumors express various EGFR mutants (19, 20). In addition, epidemiological analysis has shown that Gefitinib was more effective in non-smokers, women and those with adenocarcinomas than in others (21-23). We found levels of EGFR expression to be unaffected by gender, histological type, or whether or not the patient was a smoker. On the other hand, we found that ER-α was overexpressed significantly more often in smokers, which is noteworthy given that smokers tend to be relatively insensitive to Gefitinib.

Table II. *Multivariate Cox proportional hazard analysis of overall survival among the NSCLC patients in the study.*

Parameters	Hazard ratios	95% CI	*P*
Non-smoker/smoker	0.87	0.43-1.73	0.69
Stage I, II/III, IV	2.56	1.39-4.73	0.003
SCC/adenocarcinoma	1.18	0.53-2.63	0.68
Well/others	0.82	0.44-1.51	0.52
Double marker	2.03	1.35-3.04	0.0006

Double marker: EGFR/ER-α – high expression *vs.* low expression

There have been several reports on the cross-talk between EGFR and ER in recent years (10-12). None of these studies, however, focused on whether patient prognosis is affected by the co-expression of EGFR and ER-α. In the present study, we found that the overexpression of either EGFR or ER-α is predictive of a poor outcome in NSCLC, and that combined overexpression of EGFR and ER-α is an independent prognostic factor in NSCLC, suggesting the cross-talk between EGFR and ER-α. We, therefore, further suggest that, when considering EGFR as a target for therapy

in NSCLC patients, it is also important to consider whether the patient is also overexpressing ER-α or is expressing a mutant form of EGFR.

References

1 Scagliotti GV, Selvaggi G, Novello S *et al*: The biology of epidermal growth factor receptor in lung cancer. Clin Cancer Res *10*: 4227s-4232s, 2004.

2 Hendler FJ and Ozanne BW: Human squamous cell lung cancers express increased epidermal growth factor receptors. J Clin Investig *74*: 647-651, 1984.

3 Fontanini G, De Laurentiis M, Vignati S *et al*: Evaluation of epidermal growth factor-related growth factors and receptors and of neoangiogenesis in completely resected stage I-IIIA non-small cell lung cancer: amphiregulin and microvessel count are independent prognostic indicators of survival. Clin Cancer Res *4*: 241-249, 1998.

4 Selvaggi G, Novello S, Torri V *et al*: Epidermal growth factor receptor overexpression correlates with a poor prognosis in completely resected non-small cell lung cancer. Ann Oncol *15*: 28-32, 2004.

5 Osborne CK, Yochmowitz MG, Knight WA III *et al*: The value of estrogen and progesterone receptors in the treatment of breast cancer. Cancer *46*: 2884-2888, 1980.

6 Thorpe SM, Rose C, Rasmussen BB *et al*: Prognostic value of steroid hormone receptors: multivariate analysis of systemically untreated patients with node negative primary breast cancer. Cancer Res *47*: 6126-6133, 1987.

7 Mollerup S, Jorgensen K, Berge G and Haugen A: Expression of estrogen receptors α and β in human lung tissue and cell lines. Lung Cancer *37*: 153-159, 2002.

8 Nunno LD, Larsson LG, Rinehart JJ and Beissner RS: Estrogen and progesterone receptor in non-small cell lung cancer in 248 consecutive patients who underwent surgical resection. Arch Pathol Lab Med *124*: 1467-1470, 2000.

9 Stabile LP, Davis ALG, Gubish CT *et al*: Human non-small cell lung tumors and cells derived from normal lung express both estrogen receptor α and β and show biological responses to estrogen. Cancer Res *62*: 2141-2150, 2002.

10 Schiff R, Massarweh SA, Shou J *et al*: Cross-talk between estrogen receptor and growth factor pathways as a molecular target for overcoming endocrine resistance. Clin Cancer Res *10*: 331s-336s, 2004.

11 Lichtner RB: Estrogen/EGF receptor interactions in breast cancer: rationale for new therapeutic combination strategies. Biomed Pharmacother *57*: 447-451, 2003.

12 Levin ER: Bidirectional signaling between the estrogen receptor and the epidermal growth factor receptor. Mol Endocrinol *17*: 309-317, 2003.

13 Filardo EJ, Quinn JA, Frackelton AR Jr *et al*: Estrogen action *via* the G protein-coupled receptor, GPR30: stimulation of adenylyl cyclase and cAMP-mediated attenuation of the epidermal growth factor receptor-to-MAPK signaling axis. Mol Endocrinol *16*: 70-84, 2002.

14 Simoncini T, Hafezi-Moghadam A, Brazil DP *et al*: Interaction of oestrogen receptor with the regulatory subunit of phosphatidylinositol-3-OH kinase. Nature *407*: 538-541, 2000.

15 Rodrigues GA, Falasca M, Zhang Z *et al*: A novel positive feedback loop mediated by the docking protein Gab1 and phosphatidylinositol 3-kinase in epidermal growth factor receptor signaling. Mol Cell Biol *20*: 1448-1459, 2000.

16 Harvey JM, Clark GM, Osborne CK *et al*: Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. J Clin Oncol *17*:1474-1481, 1999.

17 Allred DC, Harvey JM, Berardo MD *et al*: Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol *11*:155-168, 1998.

18 Allred DC, Clark GM, Elledge R *et al*: Association of p53 protein expression with tumor cell proliferation rate and clinical outcome in node-negative breast cancer. J Natl Cancer Inst *85*: 200-206, 1993.

19 Paez JG, Jänne PA, Lee JC *et al*: EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science *304*: 1497-1500, 2004.

20 Lynch TJ, Bell DW, Sordella R *et al*: Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med *350*: 2129-2139, 2004.

21 Tokumo M, Toyooka S, Kiura K *et al*: The relationship between epidermal growth factor receptor mutations and clinicopathologic features in non-small cell lung cancers. Clin Cancer Res *11*: 1167-1173, 2005.

22 Kaneda H, Tamura K, Kurata T *et al*: Retrospective analysis of the predictive factors associated with the response and survival benefit of gefitinib in patients with advanced non-small-cell lung cancer. Lung Cancer *46*: 247-254, 2004.

23 Miller VA, Kris MG, Shah N *et al*: Bronchioloalveolar pathologic subtype and smoking history predict sensitivity to gefitinib in advanced non-small-cell lung cancer. J Clin Oncol *22*: 1103-1109, 2004.

Received July 5, 2005
Accepted August 29, 2005