cised about replacing cisplatin in combinations by carboplatin. Long-term data from comparative studies must equal the above reported results before a final decision can be made to replace cisplatin by carboplatin. So far, the CP regimen seems among the best regimens to be used for the treatment of epithelial ovarian cancer.

1. Neijt JP, ten Bokkel Huinink WW, van der Burg MEL, *et al.* Randomised trial comparing two combination chemotherapy regimens (Hexa-CAF vs CHAP-5) in advanced ovarian carcinoma. *Lancet* 1984, **ii**, 594–600.
2. Neijt JP, ten Bokkel Huinink WW, van der Burg MEL, *et al.* Randomised trial comparing two combination chemotherapy regimens (CHAP-5 vs CP) in advanced ovarian carcinoma. *J Clin Oncol* 1987, 5, 1157–1168.
3. Anderson JR, Crowley JJ, Propert KJ. Interpretation of survival data in clinical trials. *Oncology* 1991, 5, 104–110.
4. Smith JP, Day GT. Review of ovarian cancer of the University of Texas Systems Cancer Center M.D. Anderson Hospital and Tumor Institute. *Am J Obstet Gynecol* 1979, **135**, 984–990.
5. Sturgeon JFG, Fine S, Gospodarowicz MK, *et al.* A randomized trial of melphalan alone versus combination chemotherapy in advanced ovarian cancer. *Proc Am Soc Clin Oncol* 1982, **1**, 108.
6. van Houwelingen JC, ten Bokkel Huinink WW, Van der Burg MEL, Neijt JP. Predictability of the survival of patients with advanced ovarian cancer. *J Clin Oncol* 1989, 7, 769–773.

Eur J Cancer, Vol. 27, No. 11, pp. 1372–1375, 1991.
Printed in Great Britain
0277–5379/91 $3.00 + 0.00

Prognostic Value of c-*erb*B-2 Protein Expression in Human Lung Adenocarcinoma and Squamous Cell Carcinoma

Masahiro Tateishi, Teruyoshi Ishida, Tetsuya Mitsudomi, Satoshi Kaneko and Keizo Sugimachi

203 primary human lung tumours, of which 119 were adenocarcinoma and 84 were squamous cell carcinoma, were investigated immunohistochemically for the expression of c-*erb*B-2 protein. Positive staining was evident in 33 (28%) of adenocarcinomas and 2 (2%) of squamous cell carcinomas. In cases of adenocarcinoma, c-*erb*B-2 was present in 18% of those with stage I disease. In stage IIIA, stage IIIB and stage IV cases, c-*erb*B-2 was present in 39%, 50% and 60%, respectively (I vs. IIIA and I vs. IIIB: $P < 0.05$, I vs. IV: $P < 0.01$). The 5-year survival rates of c-*erb*B-2 positive patients and those who were negative were 30% and 52%, respectively, with a statistically significant difference ($P < 0.01$). These observations suggest that when the expression of c-*erb*B-2 correlates with invasiveness of the tumour, this correlation may serve as a prognostic indicator, particularly in cases of adenocarcinoma of the lung.
Eur J Cancer, Vol. 27, No. 11, pp. 1372–1375, 1991.

INTRODUCTION

THE c-*erb*B-2 oncogene is related to the *neu* oncogene and was first identified in a chemically induced rat neuroblastoma [1]. This oncogene codes for a 185 kD transmembrane glycoprotein [2] with tyrosine kinase activity [3, 4] and is structurally similar to, but distinct from, epidermal growth factor receptor (EGFR) [2–5]. Amplification of the gene has been noted in various sites of adenocarcinoma [6] and is associated with lymph node involvement, relapse and survival in human breast cancer [7–9].

Recent investigations revealed that amplification of the c-*erb*B-2 gene correlates with immunohistological staining for c-*erb*B-2 protein [10, 12]. We have now examined c-*erb*B-2 protein expression, using an immunohistochemical method. For this we used adenocarcinoma and squamous cell carcinoma tissues of the human lung and searched for possible prognostic factors and different expressions of c-*erb*B-2.

Correspondence to T. Ishida.
The authors are at the Department of Surgery II, Faculty of Medicine, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan.
Revised 31 May 1991; accepted 9 July 1991.

PATIENTS AND METHODS

Surgical specimens

We examined paraffin-embedded tissues obtained surgically from 203 patients with primary lung cancer, 119 of adenocarcinoma and 84 of squamous cell carcinoma. All patients had been diagnosed and treated in the Department of Surgery II of Kyushu University between 1974 and 1986. Patients who had died within the first post operative month or who had undergone

exploratory thoracotomy were excluded from the analysis. The stage of the disease was classified according to the TNM classification of UICC [13], including a review of the surgical and pathological reports of the resected specimens. The present study was based on 57 patients with stage I, 11 with stage II, 31 with stage IIIA, 10 with stage IIIB and 10 with stage IV adenocarcinoma. In cases of squamous cell carcinoma, 34 patients had stage I, 10 stage II, 34 stage IIIA and 6 stage IIIB of the disease. Of these 203 patients, ages ranged from 33 to 85 years (mean 63); 163 were men and 40 were women. The WHO [14] histological degree of differentiation was used. The clinical charts were reviewed in January 1990 and survival time taken as that from the time of operation. Only tumour-related deaths were considered for the purposes of the present study.

Immunostaining

Expression of c-*erb*B-2 protein was observed in sections of routine 10% formalin-fixed paraffin-embedded blocks, after use of a polyclonal antibody and immunising rabbits with synthetic C-terminal 14 peptides (Nichirei, Tokyo, diluted 1:50) [15, 16].

The avidin–biotin peroxidase (ABC) method [17] was used to observe the antigen. The staining procedures were as follows. The deparaffinised sections were treated with 0.03% hydrogen peroxidase in methanol for 30 min at room temperature to eliminate the endogenous peroxidase activity. After washing in phosphate-buffered saline (PBS) and incubating with normal serum (diluted 1:200, 30 min, PK-4001; Vector Laboratories Burlingame, California), the sections were incubated at room temperature overnight with polyclonal rabbit anti-c-*erb*B-2 protein. For the second and third phase reagents of the ABC method, Vecstain ABC kit (PK-4001, Vector) was used. After these treatments, the diamino-benzidine method was used for visualisation of the peroxidase. Counterstaining was done with methyl green. Each section was then examined under a transmission light microscope and the findings compared with the haematoxylin and eosin (HE) stained sections. Omission of the primary antibody resulted in a negative staining. Staining reactions were evaluated as positive or negative, and a positive reaction was considered only when strong brown deposits were visible.

Immunoblotting

Using the lysates derived from the A-415 human tumour cell line which is transfectant of the c-*erb*B-2 oncogene, a sample was subjected to electrophoresis on a 7.5% sodium dodecyl sulphate polyacrylamide gel. The fractionated proteins were electrotransferred to nitrocellulose filters. The filters were blocked with 0.7% acetic acid, and reacted with polyclonal anti-c-*erb*B-2 rabbit antibody against the C-terminal 14 peptides [4]. The filters were reacted by the ABC method. Immunoblot analysis of the sample revealed the anti-c-*erb*B-2 rabbit antibody to react specifically with one protein band with an apparent molecular weight of 185 000 (Fig. 1). To determine specific binding, non-immune rabbit sera were substituted for primary antibody, and immunostaining was prevented by preincubation of primary antibody with an excess of the C-terminal peptides. There was no crossreaction with EGFR [15, 16].

Statistical analysis

Relations between staining results and variable clinicopathological factors were assessed using the χ^2 test. Survival curves were prepared by the Kaplan–Meier method [18]. Comparisons among survival rates were made using the generalised Wilcoxon test [19]. The difference was considered to be statistically significant when P was less than 0.05.

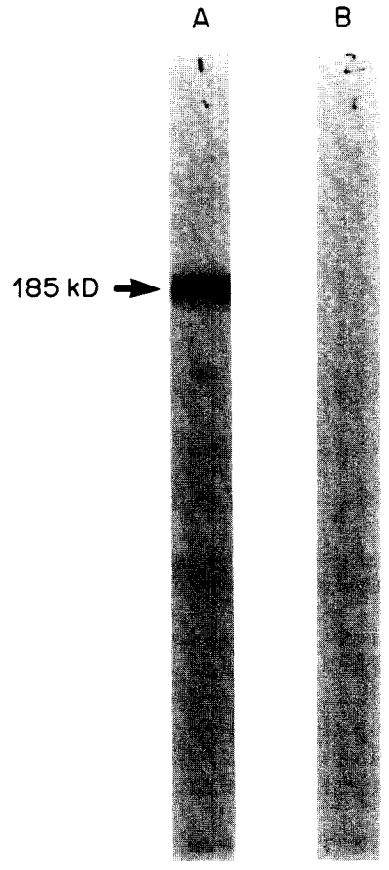

Fig. 1. Western immunoblot analysis. Lane A: reaction with anti-c-*erb*B-2 polyclonal antibodies. The antigenic protein has an apparent molecular weight of 185 000. Lane B: reaction with the same antibodies absorbed by the C-terminal 14 peptides, showing disappearance of the antigenic protein.

RESULTS

Of 119 patients with adenocarcinoma, immunoreactivity for c-*erb*B-2 was obtained in 33 (28%) cases. Positivity for the c-*erb*B-2 showed as an intense brown granular staining located predominantly at the cell membrane (Fig. 2). Heterogeneity of

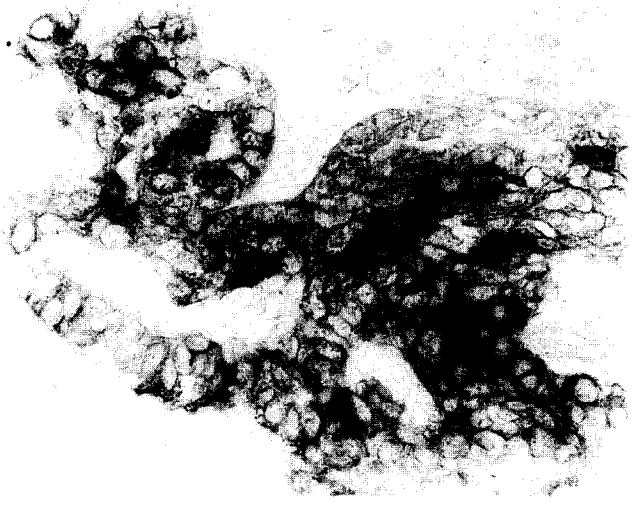

Fig. 2. Immunohistochemistry of adenocarcinoma of the lung. Note the intense membrane staining for c-*erb*B-2 (× 400).

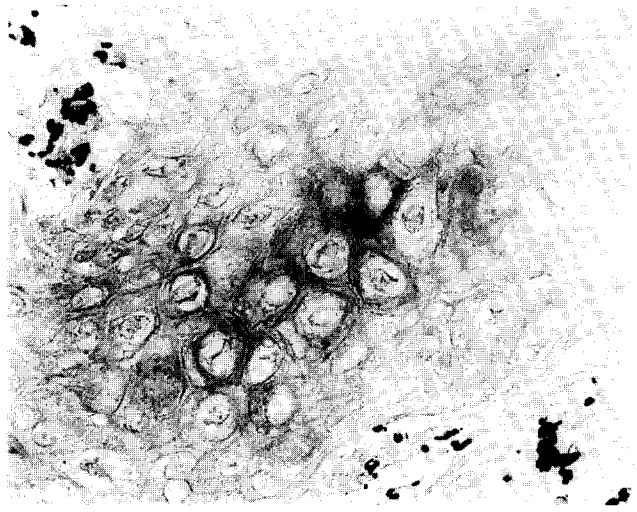

Fig. 3. Immunohistochemistry of squamous cell carcinoma of the lung. Note the heterogeneity of staining for c-*erbB*-2 (× 400).

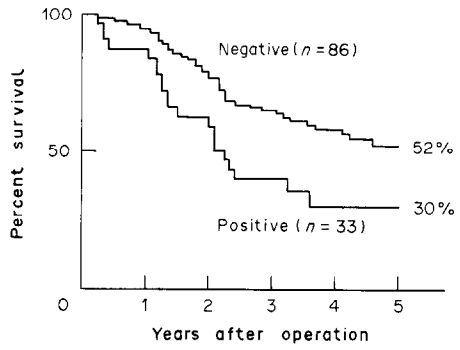

Fig. 4. Survival curves of the patients with adenocarcinoma of the lung, based on the c-*erb*B-2 status. The difference is statistically significant ($P < 0.01$).

the c-*erb*B-2 staining was seen in areas with a diffusely stained cytoplasm. In cases of a squamous cell carcinoma, there were immunoreactivities for c-*erb*B-2 protein in tissues from 2 patients only (Fig. 3). In the remaining cases of squamous cell carcinoma, a focal or diffuse cytoplasmic staining only was seen.

Table 1 shows data on patients with adenocarcinoma, including T (tumour), N (node) and M (metastasis) status and stage and pathological grade of histology, according to the c-*erb*B-2 status. There were statistically significant differences in T and M status and stage ($P < 0.05$).The 5-year survival rates of patients with a c-*erb*B-2 positive adenocarcinoma and those with negative lesions were 32% and 50%, respectively (Fig. 4), with a statistically significant difference ($P < 0.01$).

Table 1. *Relationship among the immunoreactivity of* c-*erb*B-2 *and various clinicopathological factors in patients with lung adenocarcinoma*

	c-*erb*B-2 (%)	
	Positive	Negative
Sex		
Male	19 (26)	54 (74)
Female	14 (30)	32 (70)
T		
1	9 (18)	42 (82)
2	8 (19)	33 (81)
3	9 (60)	6 (40)
4	7 (58)	5 (42)
N		
0	20 (26)	56 (74)
1	2 (14)	12 (86)
2	11 (38)	18 (62)
M		
0	27 (25)	82 (75)
1	6 (60)	4 (40)
Stage		
I	10 (18)	47 (82)
II	0	11 (100)
IIIA	12 (39)	19 (61)
IIIB	5 (50)	5 (50)
IV	6 (60)	4 (40)
Differentiation		
Well	21 (32)	44 (68)
Moderate	9 (24)	28 (76)
Poorly	3 (19)	13 (81)
Unknown		1
Total	33 (28)	86 (72)

T = tumour, N = nodal and M = metastatic stage. *$P < 0.01$, †$P < 0.05$

Of 84 patients with a squamous cell carcinoma, only tissues from 2 (2%) patients were positive for c-*erb*B-2 protein, including those with T3N0M0 and T2N0M0 diseases. The prognosis of these 2 patients was poor; the former died 8 months after operation, the latter 11 months later.

DISCUSSION

Berger *et al.* noted a statistically significant correlation between c-*erb*B-2 protein expression and the nodal status or nuclear grade in patients with breast carcinoma [20]. Recent multivariate analyses revealed that c-*erb*B-2 protein expression could serve as an independent prognostic indicator in case of breast carcinoma [7–9]. It was concluded that c-*erb*B-2 protein expression is associated with a poor prognosis in these patients.

In lung cancer, alterations of c-*erb*B-1 (which codes for EGFR) and c-*erb*B-2 correlated with the histology. c-*erb*B-1 is common in squamous cell carcinoma and c-*erb*B-2 was detected in adenocarcinoma [21]. The expression of c-*erb*B-2 is consistently high in lung adenocarcinomas [22]. However, there seems to be no documentation on the prognostic significance of c-*erb*B-2 protein expression in lung cancer.

We examined immunohistochemically c-*erb*B-2 protein in 119 cases of adenocarcinoma and in 84 of squamous cell carcinoma of the lung. C-*erb*B-2 protein positive immunoreactivities were obtained in 28% of cases of adenocarcinoma, yet in only 2% of those with squamous cell carcinoma. Our results provide positive support for data that c-*erb*B-2 gene amplification is often present in the histological type of adenocarcinoma. The prognosis of the patients with a positive c-*erb*B-2 adenocarcinoma was poor ($P < 0.01$).

Holmes and Gail found that adjuvant chemotherapy led to a significantly better prognosis in surgically resected non-small cell lung cancer patients [23]. However, Evans stated that the prolonged disease-free or overall survival period, even with adjuvant chemotherapy, was about 6 months [24]. Other randomised trials showed no significant benefit of adjuvant chemotherapy for such patients [25].

1. Schechter AL, Stern DF, Vaidyanathan L, *et al.* The *neu* oncogene: an *erb*-B-related gene encoding a 185,000-Mr tumour antigen. *Nature* 1984, **316**, 513–516.
2. Downward J, Yarden Y, Mayes E, *et al.* Close similarity of epidermal growth factor receptor and v-*erb*-B oncogene protein sequences. *Nature* 1984, **307**, 521–527.
3. Coussens L, Yang-Feng TL, Liao Y, *et al.* Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene. *Science* 1985, **230**, 1132–1139.
4. Akiyama T, Sudo C, Ogawara H, Toyoshima K, Yamamoto T. The product of the human c-*erb*B-2 gene: a 185-kilodalton glycoprotein with tyrosine kinase activity. *Science* 1986, **232**, 1644–1646.
5. Yamamoto T, Ikawa S, Akiyama T, *et al.* Similarity of protein encoded by the human c-*erb*-B-2 gene to epidermal growth factor receptor. *Nature* 1986, **319**, 230–234.
6. Yokota J, Yamamoto T, Toyoshima K, *et al.* Amplification of the c-*erb*B-2 oncogene in human adenocarcinomas *in vivo*. *Lancet* 1986, **i**, 765–766.
7. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the *HER-2/neu* oncogene. *Science* 1987, **235**, 177–182.
8. Wright C, Angus B, Nicholson S, *et al.* Expression of c-*erb*B-2 oncoprotein: a prognostic indicator in human breast cancer. *Cancer Res* 1989, **49**, 2087–2090.
9. Tsuda H, Hirohashi S, Shimosato Y, *et al.* Correlation between long-term survival in breast cancer patients and amplification of two putative oncogene-coamplification units: *hst1/int-2* and c-*erb*B-2/*ear*-1. *Cancer Res* 1989, **49**, 3104–3108.
10. Yamada Y, Yoshimoto M, Murayama Y, *et al.* Association of elevated expression of the c-*erb*B-2 protein with spread of breast cancer. *Jpn J Cancer Res* 1989, **80**, 1192–1198.
11. Tsuda H, Hirohashi S, Shimosato Y, *et al.* Immunohistochemical study on overexpression of c-*erb*B-2 protein in human breast cancer: its correlation with gene amplification and long-term survival of patients. *Jpn J Cancer Res* 1990, **81**, 327–332.
12. Venter DJ, Tuzi NL, Kumar S, Gullick WJ. Overexpression of the c-*erb*B-2 oncoprotein in human breast carcinomas: immunohistological assessment correlates with gene amplification. *Lancet* 1987, **ii**, 69–72.
13. Mountain CF. A new international staging system for lung cancer. *Chest* 1986, **89** (Suppl.), 225–233.
14. WHO. Histological typing of lung tumors. *Am J Clin Pathol* 1982, **77**, 123–136.
15. Mori S, Akiyama T, Morishita Y, *et al.* Light and electron microscopical demonstration of c-*erb*B-2 gene product-like immunoreactivity in human malignant tumors. *Virchows Arch B* 1987, **54**, 8–15.
16. Mori S, Akiyama T, Yamada Y, *et al.* c-*erb*B-2 gene product, a membrane protein commonly expressed on human fetal epithelial cells. *Lab Invest* 1989, **61**, 93–97.
17. Hsu SM, Raine L, Fanger H. Use of avdin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem Cytochem* 1981, **29**, 577–580.
18. Kaplan EL, Meier P. Nonparametric estimation from incomplete observation. *J Am Stat Assoc* 1958, **53**, 457–481.
19. Gehan E. A generalized Wilcoxon test for comparing arbitarity singly-censored samples. *Biometrika* 1965, **52**, 203–224.
20. Berger MS, Locher GW, Saurer S, *et al.* Correlation of c-*erb*B-2 gene amplification and protein expression in human breast carcinoma with nodal status and nuclear grading. *Cancer Res* 1988, **48**, 1238–1243.
21. Cline MJ, Battifora H. Abnormalities of protooncogenes in non-small cell lung cancer; correlations with tumor type and clinical characteristics. *Cancer* 1987, **60**, 2669–2674.
22. Schneider PM, Hung M, Chiocca, SM, *et al.* Differential expression of the c-*erb*B-2 gene in human small cell and non-small cell lung cancer. *Cancer Res* 1989, **49**, 4968–4971.
23. Holmes EC, Gail M. Surgical adjuvant treatment for stage II and III adenocarcinoma and large cell undifferentiated carcinoma. *J Clin Oncol* 1986, **4**, 710–715.
24. Evans WK. Adjuvant therapy for non-small cell lung cancer. *Chest* 1989, **96** (Suppl.), 87–91.
25. National Cancer Institute of Canada Clinical Trial Group. *Proc Am Soc Clin Oncol* 1987, **6**, 168.

Acknowledgements—This work was supported in part by the Grant-in-Aid for General Scientific Research 03670659 from the Japanese Ministry of Education, Science and Culture. We thank M. Ohara for comments.