TABLE II - CHEMOSENSITIVITY TO PROTEIN KINASE INHIBITORS[1]

Inhibitor	Target	IC_{50} values (μM)		RR[2]
		PC-9	PC-9/ZD	
AG-1478	EGFR	0.052 ± 0.02	6.0 ± 0.8	117
RG-14620	EGFR	13 ± 1.0	13 ± 2.5	1.0
Lavendustin A	EGFR	20 ± 4.6	27 ± 2.6	1.3
Genistein	TK	18 ± 1.5	27 ± 1.5	1.5
K252a	PKC	0.47 ± 0.17	0.63 ± 0.04	1.3
Staurosporin	PKC	0.0036 ± 0.0019	0.004 ± 0.0014	1.1
AG-825	HER2	>50	>50	

[1]Assessed by MTT assay in PC-9 and PC-9/ZD cells. Values are the mean ± SD of >3 independent experiments.–[2]Relative resistance value (IC_{50} of resistant cells/IC_{50} of parental cells).

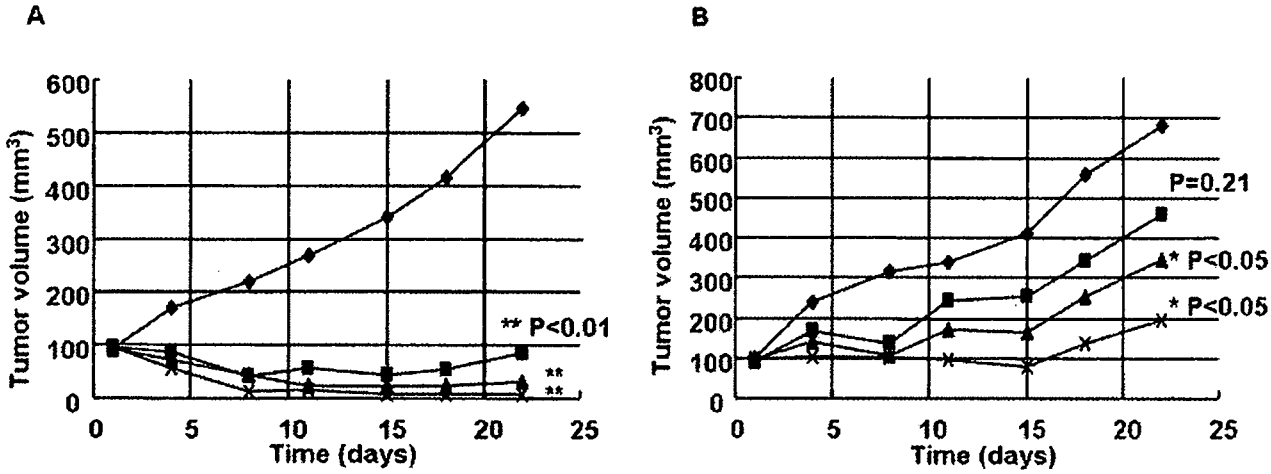

FIGURE 2 – Growth-inhibitory effect of gefitinib on PC-9 and PC-9/ZD cells xenotransplanted into nude mice. Ten days before gefitinib administration, 5 × 10^6 PC-9 (*a*) or PC-9/ZD (*b*) cells were injected s.c. into the back of mice. The mice were divided into 4 groups (◆, control group; ■, 12.5 mg/Kg group; ▲, 25 mg/Kg group; ×, 50 mg/Kg group). Gefitinib was administered p.o. to the tumor-inoculated mice on Days 1–21. Each group consisted of 6 mice. The statistical analysis was carried out by using the unpaired *t*-test.

ATP-binding site of the Bcr-Abl, the target of the drug.[24–27] We analyzed the sequences of the cDNAs of *EGFR*, *HER2*, and *HER3*, but found no differences in their sequences between PC-9 and PC-9/ZD cells. We did detect a deleted position of *EGFR* in both cell lines that results in deletion of 5 amino acids (Glu722, Leu723, Arg724, Glu725, and Ala726) (Fig. 4). Our findings indicate that the deletion does not directly contribute to the cellular resistance.

Inhibitory effect of gefitinib on autophosphorylation of EGFR in PC-9/ZD cells

Phosphorylation of EGFR is necessary for EGFR-mediated intracellular signaling. Although the EGFR phosphorylation levels of tumors were thought to be correlated with sensitivity to gefitinib, the basal level of phosphorylated EGFR in PC-9 and PC-9/ZD cells is almost the same. Gefitinib inhibited EGFR autophosphorylation in a dose-dependent manner and completely inhibited its phosphorylation at 0.2–2 μM in PC-9 cells (Fig. 5*a*), but its inhibitory effect on autophosphorylation of EGFR in PC-9/ZD cells was less than in PC-9 cells (Fig. 5*a*). Because each phosphorylation site of EGFR has a different role in the activation of downstream signaling molecules, we examined the inhibitory effect of gefitinib on site-specific phosphorylation of EGFR. Phosphorylation of several different EGFR tyrosine residues (Tyr845, Tyr992 and Tyr1068) was dose-dependently inhibited by gefitinib in PC-9 cells, whereas no clear inhibitory effects of gefitinib on phosphorylation at Tyr 845 and Tyr1068 residues in PC-9/ZD cells was observed (Fig. 5*b*,*c*,*e*). The most marked difference of in inhibition between the cells was observed at Tyr1068 (Fig. 5*e*). Tyr1045 showed resistance to inhibition of autophosphorylation by gefitinib in both PC-9 and PC-9/ZD cells (Fig. 5*d*).

Complex formation of EGFR and its adaptor proteins

Tyr1068 of EGFR is the tyrosine that is most resistant to inhibition of autophosphorylation by gefitinib in PC-9/ZD cells. Because the Tyr 1068 is a direct binding site for the GRB2/SH2 domain, and its phosphorylation is related to the complex formation of EGFR-adaptor proteins and their signaling, we examined complex formation between EGFR and the adaptor proteins GRB2, SOS, Shc, and PI3K by immunoprecipitation. The level of expression of these proteins in PC-9 and PC-9/ZD cells were similar (Fig. 3*a*). A smaller amount of EGFR-GRB2 complex was observed in PC-9/ZD cells and no EGFR-SOS complex was detected at all (Fig. 6). The amount of HER2- or HER3-GRB2 complex in PC-9 and PC-9/ZD cells was similar, and no decreases in complex formation were observed after exposure to gefitinib. A decreased amount of HER2-SOS complex and inability to detect HER3-SOS complex were also observed in PC-9/ZD cells. HER2-PI3K complex increased in PC-9/ZD. There are no significant differences in complex formation between SHC and EGFR, HER2, or HER3 between PC-9 and PC-9/ZD cells. These results suggest that GRB2-SOS-mediated signaling may be inactivated in PC-9/ZD cells.

Heterodimerization of HER family member in PC-9/ZD cells

Dimerization of members of the HER family is essential for activation of their catalytic activity and their signaling. We examined the effect of gefitinib on the dimerization of HER family members by immunoblotting, immunoprecipitation and chemical cross-linking analysis (Figs. 3*a*, 5*a*, 7*a*). The expression levels of EGFR and HER2 were similar and the HER3 level was lower in PC-9/ZD cells by immunoblotting (Fig. 3*a*). A chemical cross-

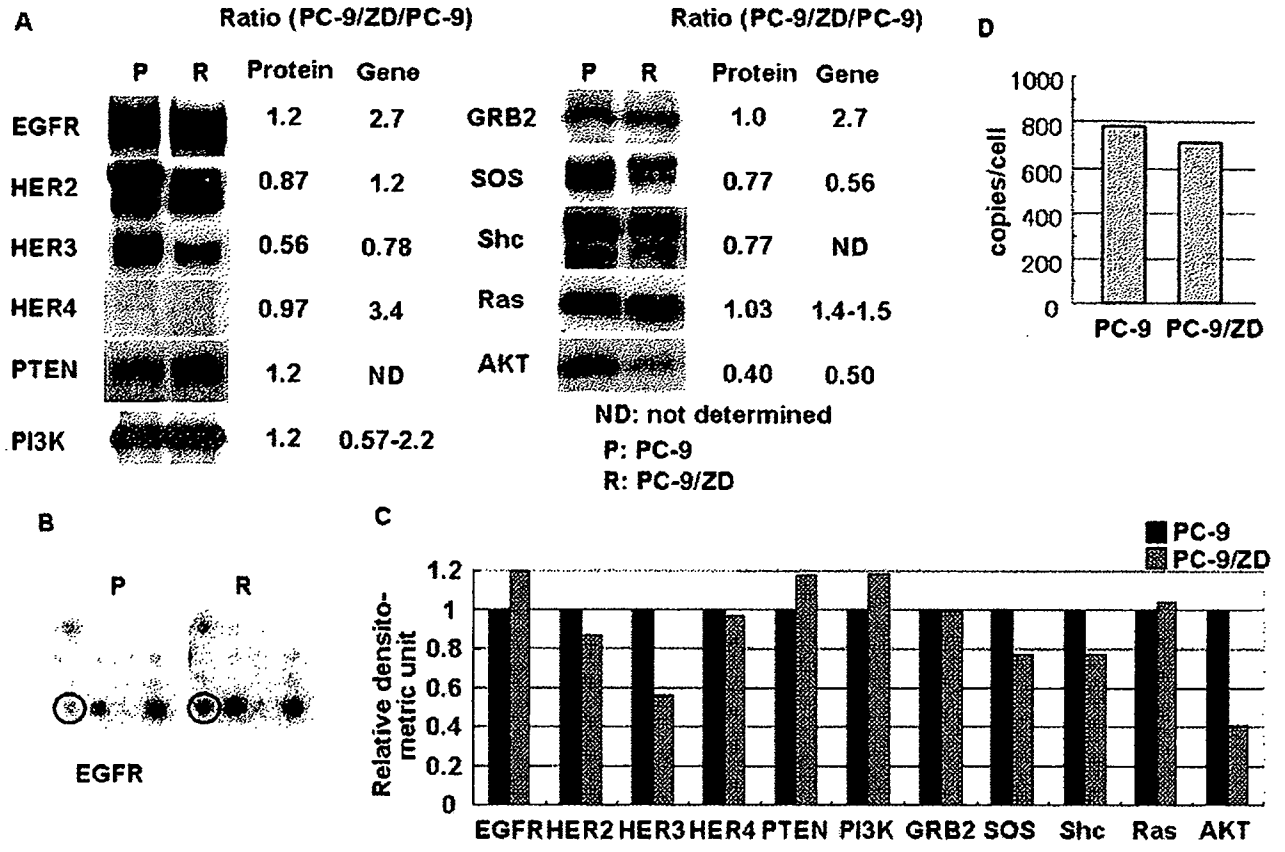

FIGURE 3 – Expresssion of HER family members and related molecules in PC-9 (P) and PC-9/ZD (R) cells. (*a*) Western blot analysis; a 20 μg sample of total cell lysates was separated by SDS-PAGE, transferred to a PVDF membrane, and incubated with a specific anti-human antibody as the first antibody and then with horseradish peroxidase-conjugated secondary antibody. The ratios of the levels of expression of proteins and genes in PC-9 cells to the levels in PC-9/ZD cells are shown. (*b*) cDNA expression array; Poly A RNA was converted into ^{32}P-labeled first-strand cDNA with MMLV reverse transcriptase. The ^{32}P-labeled cDNA fraction was hybridized to the membrane on which fragments of 777 genes were spotted. The close-up view shows *EGFR* mRNA expression. (*c*) Each band was quantified by a densitometry and with NIH image software. The levels of protein expression are shown in a graph. (*d*) Absolute amounts of *EGFR* transcripts of PC-9 cells and PC-9/ZD cells measured by real-time quantitative RT-PCR. The values were calculated back to the initial cell numbers for RNA extraction in Material and Methods.

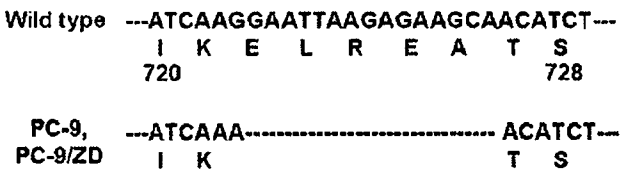

FIGURE 4 – Detection of a deleted position of EGFR. Direct sequencing of a PC-9 and PC-9/ZD-derived, amplified cDNA fragment containing the ATP-binding site of EGFR. *Top*, wild-type EGFR; *bottom*, PC-9 and PC-9/ZD.

linking assay showed that in the absence of gefitinib the amount of high molecular weight complexes (~400 kDa) that are recognized by anti-EGFR antibody (EGFR dimers), including formations of homodimers and heterodimers (EGFR-EGFR, EGFR-HER2 or EGFR-HER3), was almost the same in PC-9 and PC-9/ZD cells, whereas HER2 dimerization detected by anti-HER2 antibody was remarkably lower in PC-9/ZD cells (Fig. 7*a*). Increased EGFR/HER2 (and EGFR/HER3) heterodimer formation was detected in PC-9/ZD cells by immunoprecipitation analysis (Fig. 5*a*). The proportion of EGFR heterodimer to homodimer is increased significantly in PC-9/ZD (Fig. 7*b*). When exposed to gefitinib at a concentration of 0.2 μM for 6 hr the amount of dimer-formation increased similarly in PC-9 and PC-9/ZD cells (Fig. 7*a*), whereas marked induction of hetero-dimerization of EGFR-HER2 was observed only in PC-9 cells (Fig. 5*a*). These results suggest that a difference in hetero- or homo-dimerization is a possible determinant factor of gefitinib sensitivity.

AKT and MAPK pathways in PC-9/ZD cells

Because phosphorylation at Tyr 1068 of EGFR plays an important role for transduction of the signal to downstream of MAPK and AKT pathway,[28,29] we examined the difference between PC-9 and PC-9/ZD cells in downstream signaling. The basal level of phosphorylated AKT is higher in PC-9 cells than in PC-9/ZD cells, and although gefitinib inhibited AKT phosphorylation in a dose-dependent manner (Fig. 8*a*), the inhibitory effect of gefitinib on phosphorylation of AKT in PC-9/ZD cells was significantly less than in PC-9 cells (Fig. 8*a*). This difference in the inhibitory effect of gefitinib on AKT phosphorylation between PC-9 and PC-9/ZD cells is very similar to the difference in effect on EGFR autophosphorylation. No inhibition of phosphorylation of MAPK by gefitinib was observed in either cell line (Fig. 8*b*). These results suggest that downregulation of activated AKT is closely correlated with the cellular sensitivity to gefitinib, but that inhibition of the MAPK pathway does not contribute to drug sensitivity.

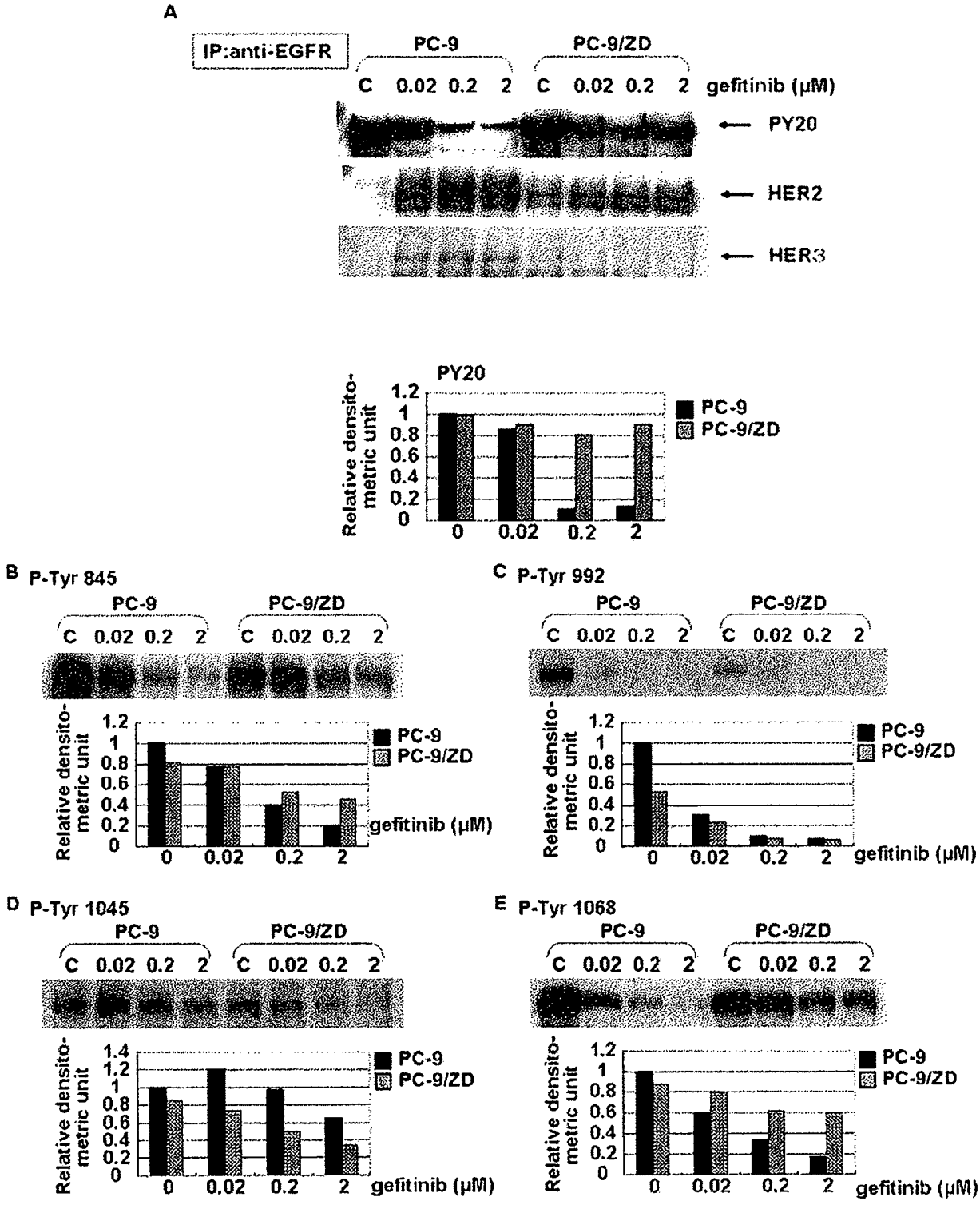

FIGURE 5 – Effect of gefitinib on autophosphorylation of EGFR. (*a*) PC-9 and PC-9/ZD cells (5×10^6) were exposed to 0.02, 0.2 or 2 μM gefitinib for 6 hr. The 1,500 μg of total cell lysate was immunoprecipitated with an anti-EGFR antibody. The immunoprecipitates were subjected to gel electrophoresis and Western blotting with anti-phosphotyrosine, anti-HER2 and anti-HER3 antibodies. Tyrosine-phosphorylated EGFR was determined with an anti-phosphotyrosine antibody. Heterodimer formation of EGFR was analyzed with anti-HER2 and anti-HER3 antibodies. The expression levels have been plotted in a graph. (*b–e*) PC-9 and PC-9/ZD cells were exposed to 0.02, 0.2 and 2 μM gefitinib for 6 hr. A 20 μg of protein of each sample was analyzed by Western blotting by using anti phospho-EGFR (Tyr845, Tyr992, Tyr 1045, Tyr 1068) antibodies.

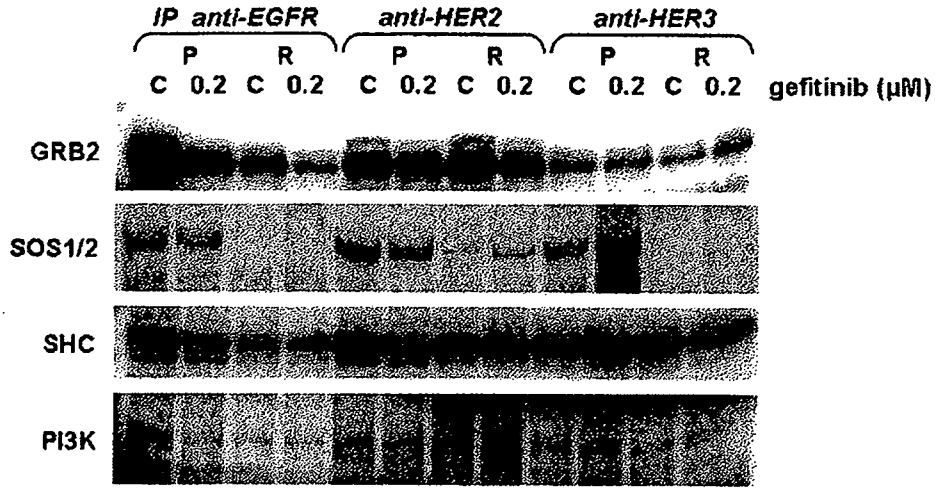

FIGURE 6 – Protein interaction between EGFR and its adaptor proteins. Cells (P: PC-9, R: PC-9/ZD) were exposed to 0 and 0.2 μM of gefitinib for 6 hr. The cells were lysed and immunoprecipitated with anti-EGFR, anti-HER2, and anti-HER3 antibodies, and the amounts of the Grb2, SOS1/2, SHC and PI3K precipitated were monitored by immunoblotting with their specific Abs.

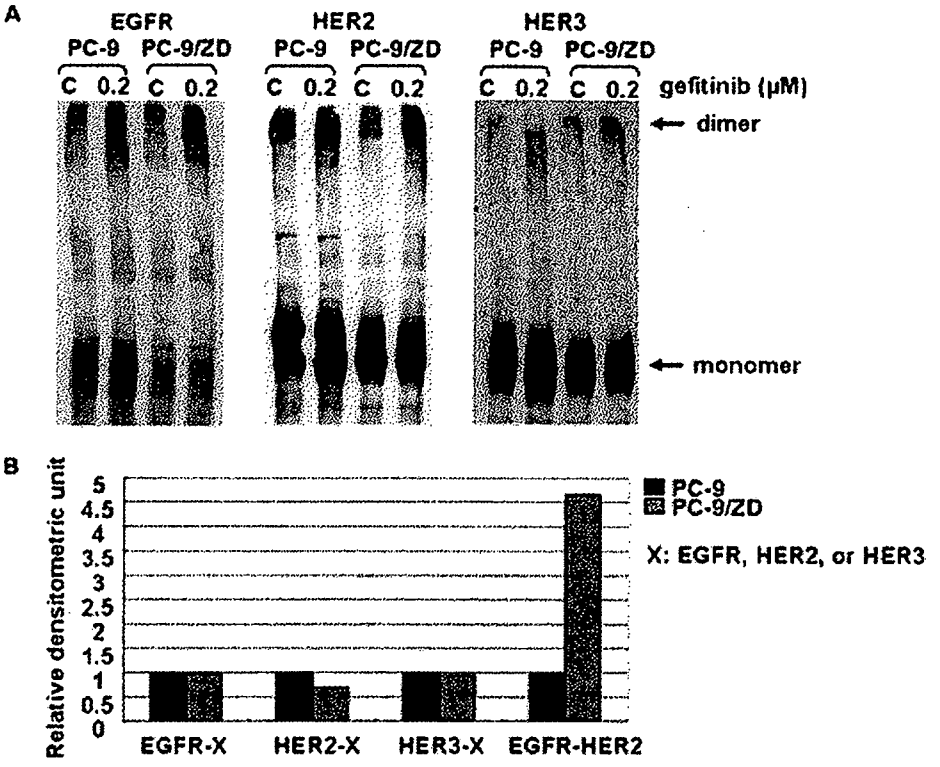

FIGURE 7 – Chemical cross-linking of PC-9 and PC-9/ZD cells. (*a*) After 6 hr exposure to 1.5 mM bis (sulfosuccinimidyl) substrate dissolved in PBS as indicated in Material and Methods. The cross-linking reaction was quenched and the cell lysates were prepared and subjected to immunoblot analysis of EGFR, HER2 and HER3. (*b*) Ratio of dimmers formed by PC-9 cells to those by PC-9/ZD cells in the absence of gefitinib. The density of the bands in (*a*) for EGFR-X, HER2-X and HER3-X were quantified densitometrically. The ratio of EGFR-HER2 was calculated by the band density obtained in Figure 5*a*. X = EGFR, HER2 or HER3.

Discussion

Interest in resistance to target-based therapy (TBT) has been growing ever since clinical efficacy was first demonstrated.[11–13] Although CML patients respond to STI-571 well at first, most patients eventually relapse in the late stage of the disease.[25–27] It has been reported that some patients in whom treatment with gefitinib is effective at first, ultimately become refractory.[30] Resistance is likely to remain a hurdle that limits the long-term effectiveness of TBT. PC-9 had a deletion mutation within the kinase domain of *EGFR* and is highly sensitive. These characters are similar to those of NSCLC with clinical responsiveness to gefitinib. Analyzing the mechanism of resistance of PC-9/ZD subline might be clinically meaningful.

The mechanism of drug resistance is thought to be multifactorial. Because the growth-inhibitory assay in our present study showed no cross resistance to a variety of cytotoxic agents, the mechanism of the resistance differs from the mechanism of multidrug resistance patterns. Although expression of BCRP, one of the multidrug-resistance-related proteins has been reported to contribute to the resistance to gefitinib,[31] expression of *BCRP* mRNA is observed only in PC-9 cells (data not shown). Although mutations in the ATP-binding pocket of *BCR-ABL* gene have been identified recently in cells from CML patients who were refractory to STI-571 treatment or relapse,[25–27] there have been no reports of any such mutations for gefitinib resistance. PC-9/ZD also became refractory to gefitinib without secondary mutation in *EGFR* cDNA. These suggest the possibility of refractory tumor after treatment of gefitinib including this kind of phenotype.

There is no significant difference in expression level of EGFR between PC-9 and PC-9/ZD. Does the antitumor effect of gefitinib

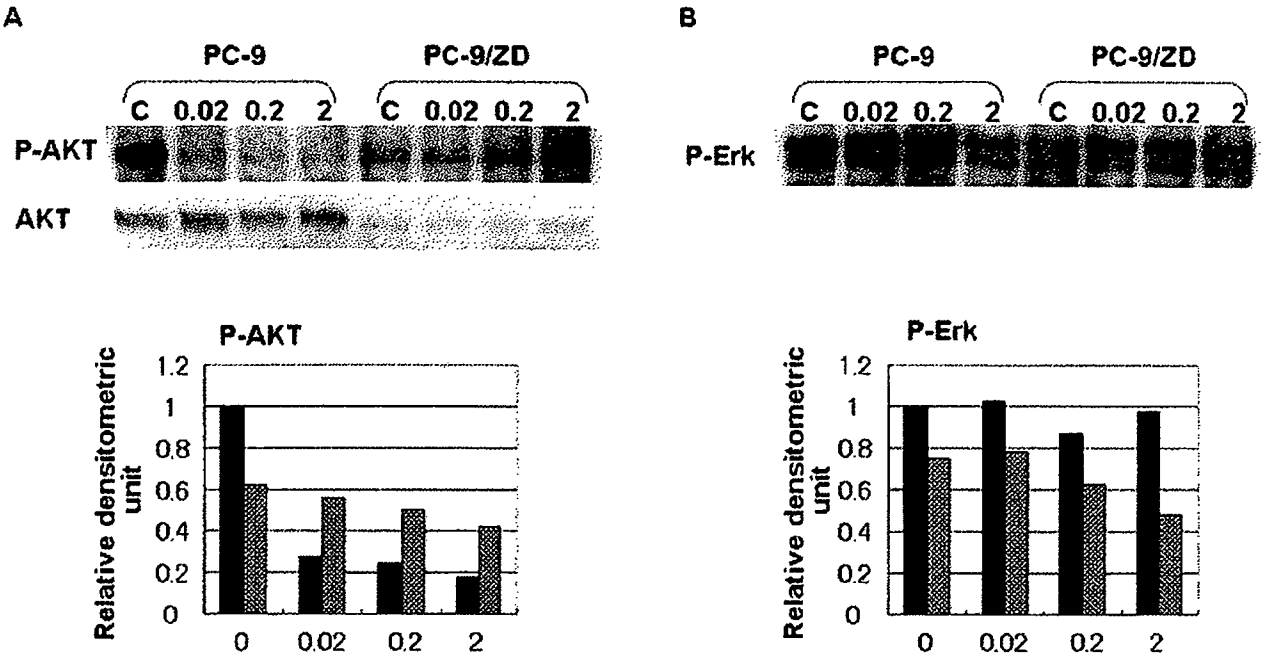

FIGURE 8 – Effect of gefitinib on the MAPK and AKT pathway. Cells were placed in medium containing 0, 0.02, 0.2 or 2 μM of gefitinib for 6 hr and harvested in EBC buffer. Total cellular lysates were separated on SDS-PAGE, transferred to a membrane and blotted with (*a*) anti-phospho-AKT (Ser473) and (*b*) anti-phospho-Erk (p44/42) antibodies. The expression levels are shown in a graph.

require EGFR expression? Naruse *et al.*[32] suggested that the high sensitivity of K562/TPA to gefitinib is due to acquired EGFR expression. In their study autophosphorylation of EGFR in K562/TPA cells was inhibited by 0.01 μM gefitinib, and the IC_{50}-value of gefitinib in parental K562 cells, which do not express EGFR, was approximately 400-fold higher than that in the K562/TPA subline. Furthermore, most patients who responded to gefitinib therapy have *EGFR* mutation in lung tumor.[18,19] These findings suggest strongly that gefitinib exerts its antitumor effect through an action on EGFR. Our present study showed similar EGFR expression and autophosphorylation levels in PC-9 and PC-9/ZD cells. The inhibitory effect of gefitinib on phosphorylation of EGFR is different. PC-9/ZD did not show cross-resistance to the specific EGFR TK inhibitors RG-14620 and Lavendustin A in an MTT assay, nor did inhibit the phosphorylation of EGFR at the cellular level (data not shown). Paez *et al.*[18] reported that phosphorylation of EGFR in gefitinib-resistant cell lines was inhibited only when gefitinib was present at high concentration. These findings suggest that the difference in the inhibitory-effect on EGFR phosphorylation may determine the efficacy of the drug.

The inhibitory effect of gefitinib on EGFR phosphorylation is not significant in PC-9/ZD cells despite the absence of differences in the sequences of *EGFR*, *HER2*, and *HER3*. There are several possible explanations for the difference in inhibitory effect. First, the avidity of gefitinib for the ATP-binding site of EGFR may be decreased in PC-9/ZD cells due to a protein-protein interaction, *i.e.*, EGFR and a certain protein prevent gefitinib from binding to EGFR. Second, a change in the activity of specific protein-tyrosine kinase or phosphatase of EGFR in PC-9/ZD cells, especially after exposure to gefitinib, may result in resistance to inhibition of EGFR phosphorylation. The phosphorylation level is maintained in exquisite balance by the reciprocal activities of kinase and phosphatase,[33,34] and Wu reported that phosphatase plays a role in STI571-resistance.[35] Third, increased heterodimer formation by EGFR with other members of the HER family results in the limited inhibition. Heterodimer formation is increased in PC-9/ZD cells under basal conditions, and no increase in formation was observed after exposure to gefitinib, although marked heterodimer induction was observed in PC-9 cells. Calculations in *in vitro* studies have shown that the IC_{50}-value for inhibition of the tyrosine kinase activity of EGFR is 0.023–0.079 μM, whereas the IC_{50}-value for inhibition of HER2 is 100-fold higher.[36] We estimate that the inhibitory effect of gefitinib depends on the ratio of homodimer formation to heterodimer formation, and the heterodimer may be one of the routes of escape from the action of gefitinib.

Signal transduction by the HER family member is mediated by 2 major pathways, the MAPK signaling pathway and the AKT signaling pathway, which regulate cell proliferation and survival. Because phosphorylated AKT was inhibited completely by gefitinib in PC-9 cells, but inhibition of phosphorylated MAPK was not significant, inhibition of the AKT pathway may be more important to cell sensitivity than inhibition of MAPK. Moasser *et al.*[37] reported consistent results, showing that downregulation of AKT activity is predominantly seen in tumors that are sensitive to gefitinib. The phosphorylation of AKT and MAPK was not inhibited significantly by gefitinib in PC-9/ZD cells. This finding might be attributable to inactivation of Tyr 1068-GRB2-SOS-mediated signaling.

Based on the results of this comparative study, EGFR-GRB2-SOS complex formation, phosphorylation of Tyr1068, the ratio of the amount of homodimer formation to heterodimer formation, and the AKT signaling pathway are possible predictive biomarkers for gefitinib sensitivity. As a different approach, we are now looking for the genes associated with gefitinib resistance in PC-9/ZD cells compared to PC-9 cells by subtractive cloning.

Acknowledgements

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Expression pattern of the scaffold protein IQGAP1 in lung cancer

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Abstract. IQGAP1 is a scaffold protein whose function relates to signal transduction, cell adhesion, local invasion, and distant metastasis of cancer cells. We examined the expression patterns of this protein and clinicopathologic features of lung cancer, and the antibody against IQGAP1 was used for immunohistochemical analysis. Of the 70 surgical specimens examined, there were 40 adenocarcinomas, 19 squamous cell carcinomas, 5 large cell carcinomas, 3 small cell carcinomas, 2 carcinoid tumors, and 1 mucoepidermoid carcinoma. The localization of IQGAP1 was classified into three types: 1) cytoplasmic, 2) membranous, and 3) reduced expression. In adenocarcinoma, the 3 types were observed equally, and differentiation grade was related to the expression pattern. The cytoplasmic type was common in well-differentiated adenocarcinomas, and membranous or reduced expression was frequently seen in moderately- or poorly-differentiated adenocarcinomas. In squamous cell carcinoma, the membranous type was most common. Although the staining pattern of IQGAP1 did not correlate with the positivity of regional lymph nodes, survival in those patients with a cytoplasmic type was significantly better than others with adenocarcinoma (p=0.0144). Expression typing of IQGAP1 in lung cancer was associated with histologic type and can be used to predict survival in patients with adenocarcinoma of the lung.

Introduction

In cancer cells, abnormal protein expression affects signal transduction, cell adhesion, local invasion, and distant metastasis. IQGAP proteins are multidomain molecules that contain several protein-interacting motifs, and IQGAP1 is a component of signaling networks that are integral to maintaining cytoskeletal architecture and cell adhesion (1). These functions include modulating the actin cytoskeleton (2), mediating signaling by the Rho family GTPases (3) and calmodulin (4), and regulating the E-cadherin and ß-catenin function (5,6).

Recent microarray analysis has revealed that highly-metastatic mouse melanoma cells have gene expression of IQGAP1 increased by >2.5-fold (7). In human clinical cases, IQGAP1 was overexpressed in colon cancer compared with normal tissue, and IQGAP1 tended to be expressed more at the invasive front (8). These reports imply that IQGAP1 may play an important role in tumor development and malignant behavior.

We performed an immunohistochemical analysis using a newly-developed specific antibody against IQGAP1 to elucidate the relationship between expression patterns of IQGAP1 and clinicopathologic features of patients with lung cancer.

Patients and methods

Patients. The patients included 45 men and 25 women with an average age of 63 years. Lobectomy and mediastinal lymph node dissection were performed in all cases, and no pre-surgical chemotherapy or radiotherapy was administered. The diagnosis of lung cancer was established by histologic examination of the surgical specimens (9), and TNM staging was performed using the latest criteria (10) with results showing pathologic stage IA in 19, IB in 15, IIA in 2, IIB in 12, IIIA in 13, IIIB in 5, and IV in 4 cases. Informed consent for immunohistochemical analysis of the primary lung cancer was obtained from all patients, and the median follow-up period of the censored cases was 60 months.

Tissue samples. The tissue samples were fixed in buffered formaldehyde and stored as paraffin-embedded blocks until use. Distribution of the IQGAP1 antigen in normal human tissues was examined using NormalGrid™ Multi-Tissue control slides (Biomeda Corp., Foster City, CA).

Generation of specific antibody against IQGAP1. We selected a cDNA clone (KIAA0051) coding IQGAP1 from the Kazusa cDNA library to generate a specific antibody for immunohistochemistry. We screened the HUGE database, which contains human novel large cDNAs identified in the Kazusa cDNA sequencing project (11) and is available at

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Key words: adenocarcinoma, carcinogenesis, cytoskeleton, immunohistochemistry, survival

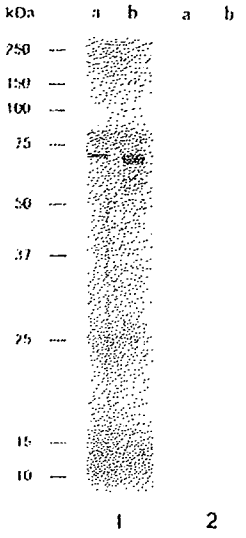

Figure 1. Western blot analysis of the reactivity of affinity-purified anti-IQGAP1 antibody (KD0019). Lane 1 indicates the results of SDS-PAGE stained by Coomassie Brilliant Blue. (a) Purified maltose-binding protein-fusion protein carrying the antigenic region of IQGAP1, and (b) control protein. Lane 2 indicates the results of Western blot analysis reacted with affinity-purified anti-IQGAP1 antibody, KD0019. (a) Purified maltose-binding protein-fusion protein carrying the antigen region of IQGAP1, and (b) control protein.

http://www.kazusa.or.jp/huge. Using KIAA0051 as a template, a DNA fragment coding 200 amino acids (E201-N400) was amplified by polymerase chain reaction (5' primer, CGC GGATCCGAAGAAATCAACAACATGAAGACTG; and 3' primer, CCCAAGCTTCTAGTTTGCAGCATCCACTCC AGACTGC), fused to the plasmid pDEST15 and propagated in *Escherichia coli*. The protein was purified and used as an immunogen. A rabbit was immunized with the purified IQGAP1, and antiserum was purified with N-hydroxy-succinimide (NHS)-activated Sepharose 4 Fast Flow (Amersham Biosciences, Piscataway, NJ) bound with the same antigen protein. The purified rabbit-specific antibody against IQGAP1 was named KD0019 and used for immunohistochemistry.

Western blot analysis confirms the reactivity of affinity-purified anti-IQGAP1 antibody, KD0019. To verify the specificity of KD0019, 1.2 μg of purified maltose-binding fusion protein carrying the antigen region of IQGAP1 or a control sequence was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by transfer onto a polyvinylidene fluoride (PVDF) membrane. The blotted membrane was reacted with KD0019 or affinity-purified control antibody, followed by alkaline phosphatase-conjugated anti-rabbit IgG antibody (7500-fold dilution) (Promega, Madison, WI). The immunoreactive proteins were visualized with the bromo-chloro-iodoryl phosphate (BCIP)/nitro-blue tetrazolium (NBT) color development substrate (Promega).

Immunohistochemistry. The streptavidin-biotin peroxidase complex (SABC) technique was used for immunohistochemical staining. Sections (4 μm thick) were cut, kept in xylene, rehydrated and washed with water, then treated with 0.3% hydrogen peroxide in methanol for 10 min to inhibit endogenous peroxidase and autoclaved in a citrate buffer solution (10 mM sodium citrate, pH 6.0) at 100°C for 20 min to retrieve antigenicity. After blocking non-specific binding with 5% normal rabbit serum, sections were incubated with primary antibody, KD0019 (500 ng/ml) at 4°C overnight. Slides were then washed and incubated with a second antibody, biotinylated anti-rabbit IgG (LSAB kit) (Dako, Copenhagen, Denmark) for 15 min at room temperature. Finally, the slides were washed and incubated with SABC Elite reagent (Dako) for 15 min at room temperature. Specific staining was developed with diaminobenzidine tetrahydrochloride supplemented with 0.03% hydrogen peroxide and counterstained with hematoxylin, and lung cancers were classified into three types according to the staining pattern of IQGAP1: 1) cytoplasmic (C-type), 2) membranous (M-type), and 3) reduced expression (R-type).

Table I. The staining pattern of IQGAP1 according to the histologic type of lung cancer.

Staining pattern	Histologic type					
	Ad	Sq	La	Sm	Mis	Total
C-type	15	4	1	3	2	25
M-type	14	13	1	0	0	28
R-type	11	2	3	0	1	17
Total	40	19	5	3	3	70

Ad, adenocarcinoma; Sq, squamous cell carcinoma; La, large cell carcinoma; Sm, small cell carcinoma; Mis, miscellaneous tumors; C-type, cytoplasmic type; M-type, membranous type; R-type, reduced expression type.

Statistical analysis. Differences between groups were evaluated using the χ^2 test, the survival rate was calculated by the Kaplan-Meier method, and survival differences were compared using the log-rank test as a univariate analysis. $p<0.05$ was considered significant.

Results

Specificity of anti-IQGAP1 antibody. Affinity-purified anti-IQGAP1 antibody, KD0019, strongly reacted with the maltose-binding IQGAP1 fusion protein, but not the control molecule (Fig. 1).

Localization of IQGAP1 in normal human tissues. In normal lung tissues, strong staining was observed in alveolar macrophages, bronchial epithelium, and bronchial glands. In bronchial glands, localization of IQGAP1 was limited to the cytoplasm and cell boundaries of serous glands; no mucous glands were stained (Fig. 2A). Skin, epithelium of the external glands of the prostate, Kupffer cells, and distal urinary tubules in the kidney all showed relatively strong staining (Fig. 2B-E). Skin showed the strongest positive reaction in both the cell membrane and cytoplasm. Only weak staining was observed in the spleen, uterus, placenta,

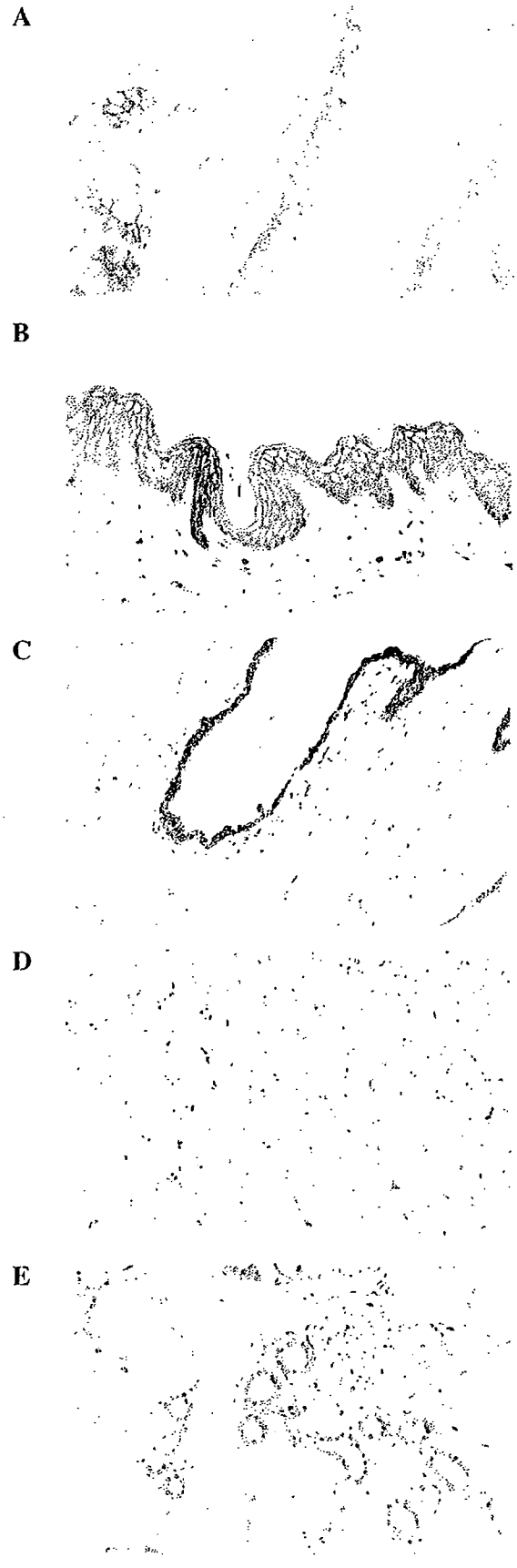

Figure 2. The distribution of IQGAP1 in normal lung. IQGAP1 strongly stained (A) bronchial epithelium and serous glands in normal lung, (B) normal skin, (C) external glands of the prostate, (D) Kupffer cells, and (E) distal urinary tubules in the kidney.

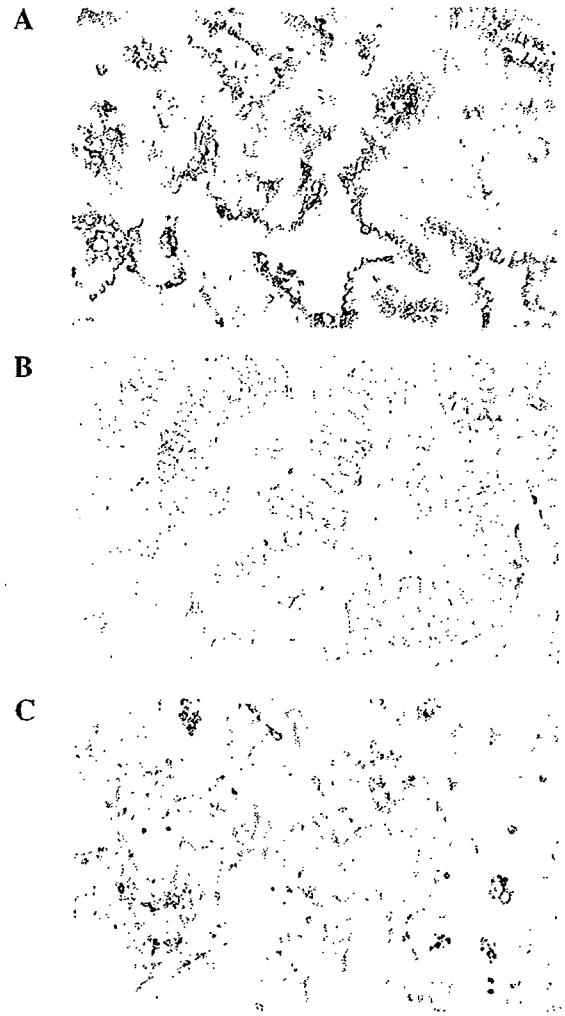

Figure 3. Representative staining patterns of IQGAP1 in lung cancer. Adenocarcinomas showing (A) cytoplasmic, (B) membranous, and (C) reduced expression type.

tonsil, testis, ovary, pancreas, breast, heart, stomach, small and large intestine, brain, pituitary gland, and adrenal gland.

Localization of IQGAP1 in lung cancer

Expression pattern according to histologic type. Of the 70 surgical specimens examined, there were 40 adenocarcinomas, 19 squamous cell carcinomas, 5 large cell carcinomas, 3 small cell carcinomas, 2 carcinoid tumors, and 1 mucoepidermoid carcinoma. A representative staining pattern is shown in Fig. 3A-C. In squamous cell carcinoma, M-type was frequently seen (68.4%, 13/19); in adenocarcinomas, the 3 types were equally observed (Table I). All 3 small cell lung cancers and 2 carcinoids showed weak cytoplasmic staining.

Expression pattern and grade of differentiation of lung cancer. In adenocarcinoma, differentiation grade was related to the expression pattern (Table II). C-type was common in well-differentiated adenocarcinoma, and M- and R- types were frequently seen in moderately- and poorly-differentiated adenocarcinoma ($p=0.0004$). In squamous cell carcinoma, a difference in staining pattern according to differentiation grade was not observed ($p=0.3960$).

Table II. The staining pattern of IQGAP1 according to the differentiation grade of lung cancer.

Staining pattern	Ad			Sq			Total
	WD	MD	PD	WD	MD	PD	
C-type	10	5	0	1	1	2	19
M-type	2	9	3	3	9	1	27
R-type	1	6	4	0	0	2	13
Total	13	20	7	4	10	5	59

Ad, adenocarcinoma; Sq, squamous cell carcinoma; La, large cell carcinoma; Sm, small cell carcinoma; Mis, miscellaneous tumors; C-type, cytoplasmic type; M-type, membranous type; R-type, reduced expression type.

Table III. The staining pattern of IQGAP1 according to nodal status.

Staining pattern	Ad		Sq		Total
	N(-)	N(+)	N(-)	N(+)	
C-type	12	3	2	2	19
M-type	7	7	6	7	27
R-type	4	7	0	2	13
Total	23	16	8	11	59

N(-), node negative; N(+), node positive; Ad, adenocarcinoma; Sq, squamous cell carcinoma; C-type, cytoplasmic type; M-type, membranous type; R-type, reduced expression type.

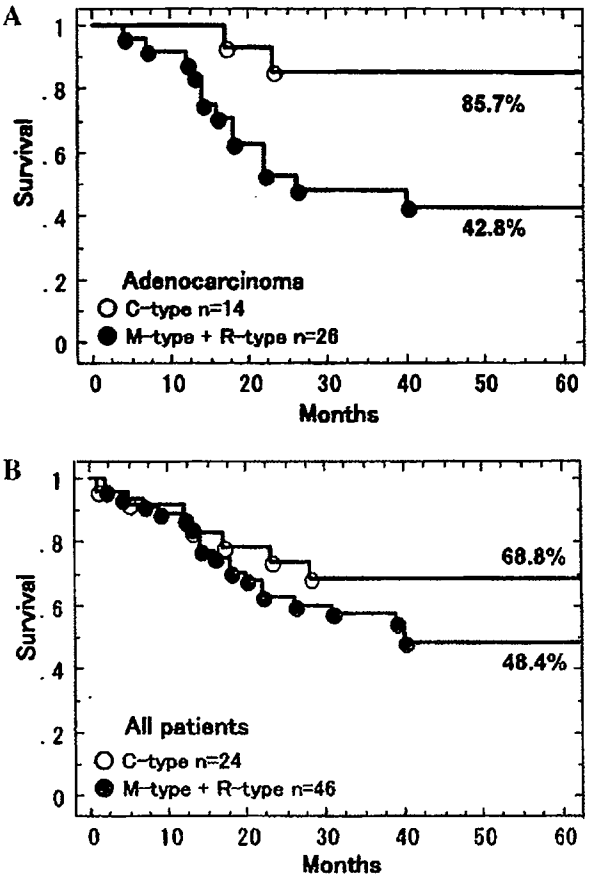

Figure 4. Kaplan-Meier survival curves after resection of lung cancer. (A) Staining pattern of IQGAP1 was significantly related to survival in patients with adenocarcinoma (p=0.0144). (B) Staining pattern of IQGAP1 was not related to survival when all patients were analyzed together (p=0.2363).

Expression pattern and nodal status. Staining patterns of IQGAP1 did not correlate with positivity of the regional lymph nodes (Table III).

Expression pattern and survival of patients with lung cancer. In adenocarcinoma, a survival difference was observed between the C group and the M and R groups (p=0.0144; Fig. 4A). This difference was not observed in all cases of lung cancer or other histologic types (p=0.2363; Fig. 4B).

Discussion

IQGAP1 is a scaffold protein that plays an important role in molding the cytoskeleton, signal transduction, and intercellular adhesion, and co-localizes with actin filaments in the cell cortex. It binds *in vitro* to F-actin and several signaling proteins, including calmodulin, Cdc42, Rac1, and ß-catenin (12,13). F-actin binding activity of IQGAP1 is regulated by its reversible association with these signaling molecules, but the mechanism is unclear (14).

Previously, localization of IQGAP1 in malignant tumors had only been examined in adenocarcinomas, and gastric, colon, and endometrial cancer. In gastric cancer, IQGAP1 was frequently observed diffusely in the cytoplasm in intestinal-type, well-differentiated tumors, but it was expressed at the cell membrane in diffuse-type, poorly-differentiated tumors (15). In that report, E-cadherin was localized to the cell membrane of the well-differentiated type and in the cytoplasm of poorly-differentiated tumors; thus, subcellular localization of IQGAP1 was inversely correlated with E-cadherin localization. Movement of IQGAP1 from the cytoplasm to the cell membrane could be correlated with E-cadherin dysfunction and dedifferentiation in gastric carcinogenesis. In colorectal cancer, IQGAP1 seemed to be expressed more at the invasion front of the tumor, and this expression pattern was most apparent in advanced disease (8). In endometrial cancer, α-catenin and IQGAP1 were absent from cell adhesive sites in well-differentiated adenocarcinomas (16). All of these results suggest that the differential grade of adenocarcinomas is associated with abnormal intracellular localization of IQGAP1.

Lung cancer has four major histologic types: 1) adenocarcinoma, 2) squamous cell carcinoma, 3) small cell carcinoma, and 4) large cell carcinoma, unique from other cancers. Thus, we were interested in IQGAP1 expression according to the histologic type of lung cancer. In normal tissues, we demonstrated that stratified squamous epithelium showed strong staining in both cytoplasm and cell boundaries in skin. Therefore, it is not surprising that squamous cell

carcinoma was positive for IQGAP1. However, squamous cell carcinoma frequently showed membranous staining, whereas normal stratified squamous epithelium showed both cytoplasmic and membranous staining. This altered localization of IQGAP1 may relate to the dysfunction of cell adhesion or signal transduction during carcinogenesis in squamous cell carcinomas.

Concerning glandular cells, the cytoplasm of normal bronchial serous glands were specifically stained, whereas mucous glands were not. Well-differentiated adenocarcinoma frequently showed a cytoplasmic staining pattern, whereas moderately- or poorly-differentiated adenocarcinoma showed membranous staining or reduced expression. Thus, our findings are similar to gastric (15) and endometrial (16) cancer. This might result in a worse prognosis for patients with lung adenocarcinoma showing membranous or reduced staining patterns. An inverse correlation of membranous expression of IQGAP1 and either E-cadherin or α-catenin (15,16) might explain some dedifferentiated features of cancer cells. Since the most common cause of death in this series of patients was distant metastasis, abnormal localization of IQGAP1 might play a role in local invasion and distant metastases.

In summary, the expression pattern of IQGAP1 in lung cancer was different according to histologic type, which may reflect the features of cancer cell origin. In adenocarcinoma, cytoplasmic staining was frequently observed in the well-differentiated type, and survival of patients with this type was better than those with membranous or reduced expression. Therefore, the expression pattern of IQGAP1 can predict survival in patients with lung adenocarcinoma.

Acknowledgements

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Frequent loss of E-cadherin and/or catenins in intrabronchial lesions during carcinogenesis of the bronchial epithelium

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Early-stage bronchial squamous cell carcinoma;
Immunohistochemistry

Summary Inactivation of the cadherin-mediated cell–cell adhesion system is believed to play a role in the initial steps of cancer invasion and metastasis. Expression of E-cadherin and its intracytoplasmic binding molecules (α-catenin, β-catenin, and plakoglobin) was examined immunohistochemically in 84 cases of intrabronchial precancerous lesions (bronchial squamous metaplasia (BSM) without atypia, BSM with atypia, dysplasia), and 21 cases of carcinoma in situ, and 4 cases of microinvasion to the bronchial wall, and 32 cases of stage I well differentiated squamous cell carcinoma (squamous cell carcinoma) to investigate the association between expression of E-cadherin and/or catenins and cancer progression. Reduced expression of E-cadherin and/or catenins was closely correlated with an atypical grade of dysplasia in the basal layer ($p < 0.05$). In particular, downregulation of E-cadherin and/or catenins was associated with an atypical grade of BSM with atypia in intrabronchial lesions ($p < 0.01$). We conclude that downregulation of α-catenin and/or β-catenin, which may reflect dysfunction of the cadherin-mediated cell–cell adhesion system, is an important marker for atypical grade during carcinogenesis of the bronchial epithelium.

1. Introduction

Cadherins are a family of cell–cell adhesion molecules that are essential for tight junctions

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between cells [1,2], and E-cadherin is the form most strongly expressed in epithelial cells. Cadherins form a complex with cytoplasmic proteins, known collectively as catenins. This molecular complex, together with other cytoskeletal components such as actin, constitutes the intercellular adherence junction [2–4]. The catenins are classified into two groups, α-catenins and β-catenins, and the latter group includes plakoglobin and *Drosophila* Armadillo protein as well as β-catenin itself [5,6]. Plakoglobin is isolated from the desmosomal fraction [7] and is present in both desmosomes and adherence junctions [8], and may therefore be a common regulatory molecule in cell junctions.

Cadherin-mediated cell adhesion acts as a suppressor of the invasion of cancer cells in vitro [9–11], and dysfunction of the E-cadherin system correlates with cancer cell invasion in human cancers [12,13]. The role of α-catenin in the cadherin adhesion system has been revealed by studies with cancer cells. The human lung cancer cell line PC9 expresses an aberrant α-catenin mRNA and shows very loose cell–cell association [14,15]. PC9 cells become much more closely associated and acquire an epithelioid arrangement after transfection with cDNA for a subtype of α-catenin and αN-catenin [16]. These results suggest that α-catenin is indispensable for cadherin-mediated cell–cell adhesion.

Previous immunohistochemical studies have revealed many examples of reduced and/or heterogeneous expression of E-cadherin [17–19] and α-catenin [20] in undifferentiated invasive cancers, and impaired expression of E-cadherin or α-catenin has been reported to be associated with high incidences of lymph node metastasis of human breast [21], esophageal [22], and head and neck [23] cancers. However, there have been few studies on the relationship between reduced E-cadherin expression and the prognosis of cancer patients [24–28].

The role of β-catenin and plakoglobin in determining the fate of cells has been suggested by work on a *Drosophila* homologue of this protein, Armadillo [29,30]. Moreover, it has been revealed that the association between E-cadherin and α-catenin is mediated by β-catenin [31], and that β-catenin in turn mediates the interactions of the cadherin–catenin complex with the c-erbB-2 gene product and epidermal growth factor receptor (EGF-R) [32–34]. A tumor suppressor gene product, APC protein, has been shown to interact with β-catenin and plakoglobin and to play important roles in the E-cadherin-mediated cell adhesion system and to participate in tumor invasion and metastasis.

In a previous study, we divided primary lung cancers into two groups on the basis of their expression of E-cadherin and catenins, as detected by immunohistochemistry [35]. In addition, we demonstrated a close relationship between E-cadherin-associated cell–cell adhesion, catenins, and cytologic features, in particular the formation of cellular clusters and the frequency of solitary cells. Preoperative evaluation of both cytologic features and E-cadherin-associated cell–cell adhesion may be useful for predicting the malignant characteristics of lung cancer [36].

E-cadherin and α-catenin, and also β-catenin and plakoglobin, play important roles in the cadherin-mediated cell adhesion system in various cancers. However, in the context of carcinogenesis of the bronchial epithelium, expression of E-cadherin, α-catenin, β-catenin, and plakoglobin in intrabronchial precancerous lesions has not yet been reported. In order to investigate a possible dysfunction of the E-cadherin-mediated cell adhesion system in intrabronchial lesions, we used immunohistochemistry to examine the expression of E-cadherin, α-catenin, β-catenin, and plakoglobin in biopsy specimens.

2. Materials and methods

2.1. Biopsy specimens

The biopsy samples were obtained from 109 patients with intrabronchial lesions resected between 1991 and 2000 at the Department of Surgery of Tokyo Medical University Hospital. These lesions were diagnosed pathologically as BSM without atypia in 32 cases, BSM with atypia in 25 cases, dysplasia in 5 cases, carcinoma in situ in 21 cases, microinvasion to the bronchial wall in 4 cases, and stage I well differentiated squamous cell carcinoma in 32 cases. The specimens were fixed with 10% formalin and embedded in paraffin.

2.2. Immunohistochemistry

Mouse monoclonal antibodies against human E-cadherin (HECD-1; Takara, Kyoto, Japan), α-catenin and β-catenin (anti-α-catenin and anti-β-catenin; Transduction Laboratories, Lexington, KY), and plakoglobin (CBL175; Cymbus Bioscience, Southampton, UK) were used for immunohistochemical staining. Four-micrometer-thick tissue sections were prepared from all paraffin-embedded specimens and collected on silane-coated glass slides. After deparaffinization, the formalin-fixed paraffin-embedded sections were treated with

0.01% trypsin and subjected to microwave antigen retrieval [37].

The immunohistochemical method using the avidin-biotin-peroxidase complex was described previously [35]. The reaction products were visualized with diaminobenzidine and the sections were counterstained with hematoxylin.

Negative control staining, which was performed with the same class of immunoglobulin instead of the first antibody, yielded negative results in all cases. The intensity and pattern of immunostaining with HECD-1, anti-α-catenin, anti-β-catenin, and CBL175 in intrabronchial lesions were compared with those of normal bronchial epithelium, and the immunohistochemical staining results were evaluated as described previously [35]. Levels of immunostaining were evaluated in separate compartments of the bronchial epithelium: the basal layer (the first two-fifths of the distance between the basement membrane and the free surface), the intermediate layer, and the superficial layer (the upper one-fifth of this distance). Expression of E-cadherin, α-catenin, β-catenin, and plakoglobin in each layer was judged to be normal if more than 90% of the intrabronchial lesion cells were positively stained by the appropriate antibodies. If staining was distinctly weaker than that of normal epithelium, or if less than 90% of the intrabronchial lesion cells were positively stained, the expression was judged to be reduced. Immunohistochemical staining was scored independently by two observers (Y.K., Y.E.).

2.3. Statistical analysis

The data were analyzed using the Cochran–Armitage test [38], which was conducted by a stepwise method excluding E-cadherin, α-catenin, β-catenin, and plakoglobin, since these four variables are the variables of interest. Differences at $p < 0.05$ were considered to be statistically significant.

3. Results

In bronchial epithelium, E-cadherin and all catenins were expressed at a high level. Immunohistochem-

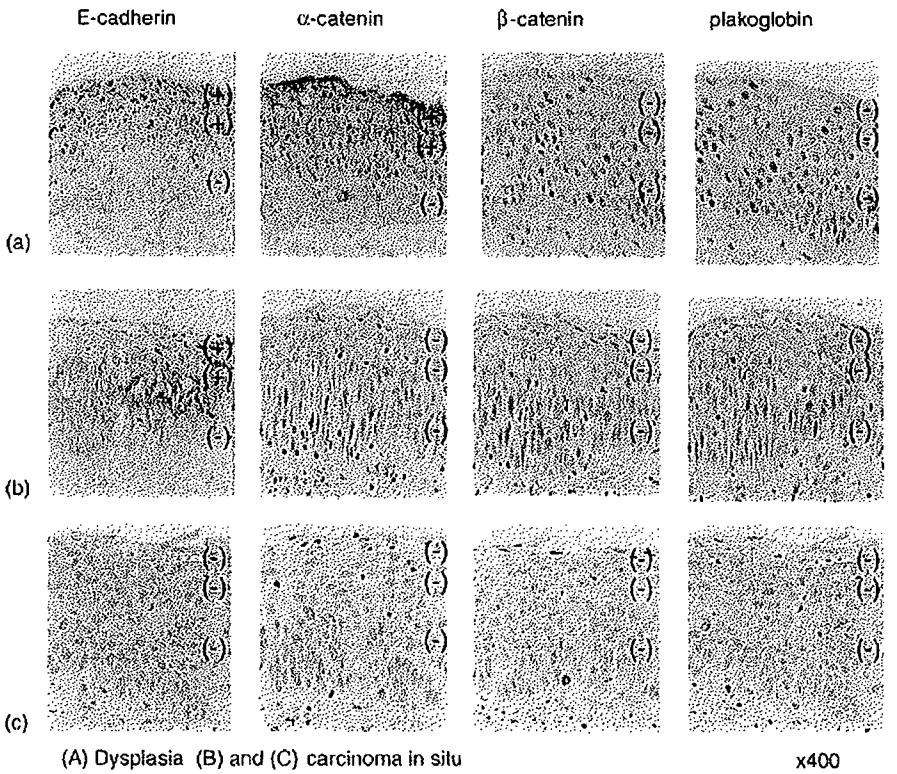

Fig. 1 (A) Representative immunohistochemical staining for E-cadherin, α-catenin, β-catenin, and plakoglobin in biopsy specimens of dysplasia (a) and carcinoma in situ (b and c). Evaluation for each layer of the intrabronchial lesions is shown at the right side of each picture ×400. (B) A borderline area between carcinoma in situ and dysplasia. Evaluation for each layer of the carcinoma in situ area is shown at the left side of each picture, and that for each layer of the dysplasia area at the right side of each picture.

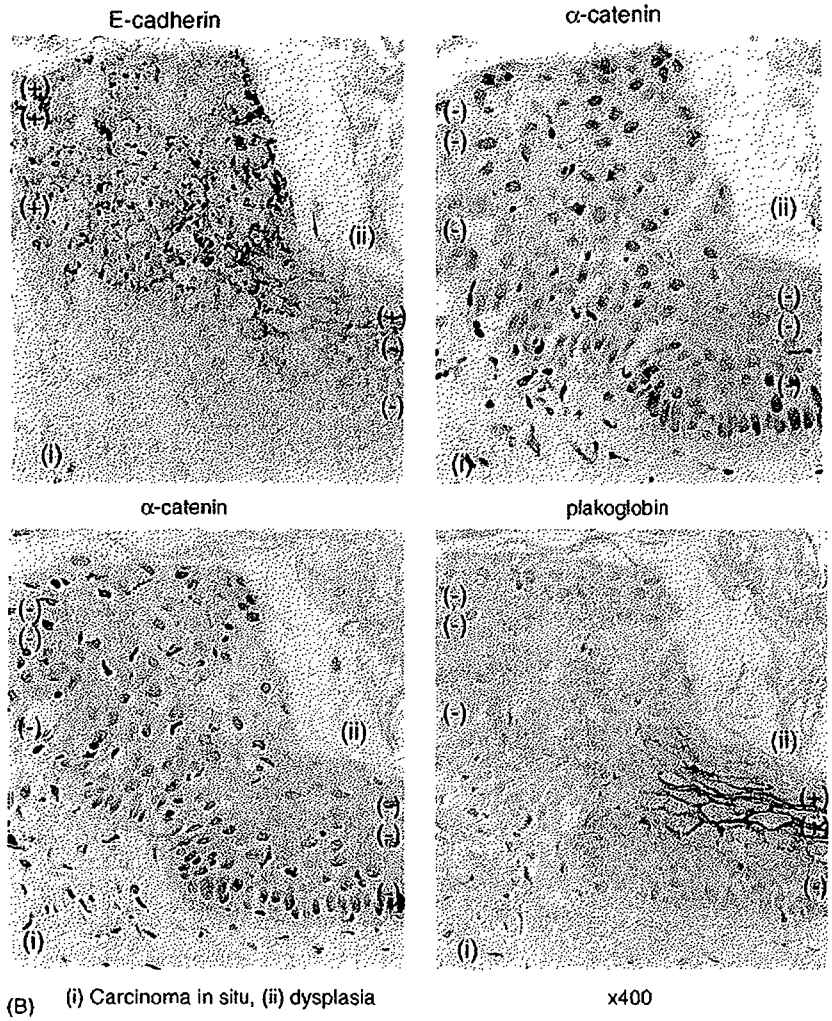

Fig. 1 (*Continued*).

ical findings for representative intrabronchial lesions are shown in Fig. 1. Cases with reduced expression of either E-cadherin or catenins in intrabronchial lesions are summarized in Table 1. Reduced expression of E-cadherin and/or catenins was closely correlated with an atypical grade of dysplasia in the basal layer ($p < 0.05$). In particular, down-regulation of E-cadherin and/or catenins was associated with an atypical grade of BSM with atypia in intrabronchial lesions ($p < 0.01$). Additionally, reduced expression of E-cadherin and catenins was observed in squamous cell carcinoma, as shown in Table 2.

In BMS without atypia ($n = 32$ cases), loss of expression of α-catenin, β-catenin or plakoglobin was observed in the basal layer in six cases (18%), in the intermediate layer in two cases (6%), and in the superficial layer in three cases (9%). In BSM with atypia ($n = 25$ cases), loss of expression of E-cadherin, α-catenin, β-catenin or plakoglobin was observed in the basal layer in seven cases (28%), in the intermediate layer in seven cases (28%), and in the superficial layer in five cases (20%). In dysplasia ($n = 5$ cases), loss of expression of these molecules was observed in the basal layer in two cases (40%), in the intermediate layer in one case (20%), and in the superficial layer in one case (20%). In carcinoma in situ ($n = 21$ cases), loss of expression was observed in the basal layer in 10 cases (48%), in the intermediate layer in 9 cases (43%), and in the superficial layer in 8 cases (38%). In microinvasion to bronchial wall ($n = 4$), loss of expression was observed in the basal layer in four cases (100%), in the intermediate layer in three cases (75%), and in the superficial layer in two cases (50%). These results are presented in Fig. 2 and Table 3.

Table 1 Aberrant expression of E-cadherin and catenins in intrabrounchial lesions

	E-cadherin			α-Catenin			β-Catenin			Plakoglobin			Rate[a] (%)
	B	I	S	B	I	S	B	I	S	B	I	S	
BSM without atypia $n=32$	+	+	+	−	−	+	+	+	+	−	−	−	21
	+	+	+	+	+	+	−	+	+	+	+	+	
	+	+	+	+	+	+	−	+	+	+	+	+	
	+	+	+	+	+	+	+	+	−	+	+	+	
	+	+	+	+	+	+	+	+	+	−	+	+	
	+	+	+	+	+	+	+	+	+	−	+	+	
	+	+	+	+	+	+	+	+	+	−	−	−	
BSM with atypia $n=25$	−	−	+	+	+	+	+	+	+	+	+	+	28
	+	+	+	−	+	+	−	−	−	+	+	+	
	+	+	+	−	−	−	−	−	−	+	+	+	
	+	+	+	+	+	+	−	−	−	+	+	+	
	+	+	+	+	+	+	−	−	−	+	+	+	
	+	+	+	+	+	+	−	−	+	−	−	+	
	−	−	−	−	−	−	−	−	−	−	−	−	
Dysplasia $n=540\%$	−	+	+	−	+	+	−	−	−	−	−	−	40
	−	+	+	−	+	+	+	+	+	+	+	+	
Carcinoma in situ $n=21$	+	+	+	+	+	+	−	−	+	+	+	+	48
	+	+	+	+	+	+	−	+	+	−	+	+	
	+	+	+	−	−	−	−	−	−	−	−	−	
	+	−	+	−	−	+	−	−	−	+	+	+	
	−	+	+	−	−	−	−	−	−	−	−	−	
	−	−	+	−	−	−	+	+	+	+	+	+	
	−	−	−	−	−	+	−	−	−	−	−	+	
	−	−	−	−	−	−	−	−	−	+	+	+	
	−	−	−	−	−	−	−	−	−	−	−	−	
	−	−	−	−	−	−	−	−	−	−	−	−	
Microinvasion to bronchial wall $n=4$	+	+	+	−	−	+	−	−	−	+	+	+	100
	+	+	+	−	−	+	−	−	+	+	+	+	
	+	+	+	+	+	+	−	−	−	−	−	−	
	+	+	+	+	+	+	+	+	+	−	+	+	

Variable	Contrast	*p*-Value
BSM without atypia	BSM with atypia	0.097
BSM without atypia	Dysplasia	0.043
BSM without atypia	Carcinoma in situ	0.003
BSM without atypia	Microinvasive to bronchial wall	0.001

B: basal layer; I: intermedtate layer; S: superficial layer.
[a] Reduced expession rate of either E-cadherin or catenins in intrabronchial lesions.

Table 2 Reduced expression rate of E-cadherin and catenins in advanced stage of squamous cell carcinoma

	Squamous cell carcionoma, $n=32$
E-cadherin	21 (67%)
α-Catenin	26 (81%)
β-Catenin	27 (84%)
Plakoglobin	14 (44%)
Rate[a]	100%

[a] Reduced expression rate of either E-cadherin or catenins in squamous cell carcinoma.

4. Discussion

It has been established that malignant transformation can arise from an accumulation of genetic alterations. This stepwise transformation is known as multistep carcinogenesis. In general, it is known that primary lung carcinoma is one of the most malignant solid tumors, and that it has a wide range of invasive and metastatic behavior. There is a high possibility that alterations in genotype are reflected in the morphological phenotype of the bronchial epithelium. In this context, bronchial

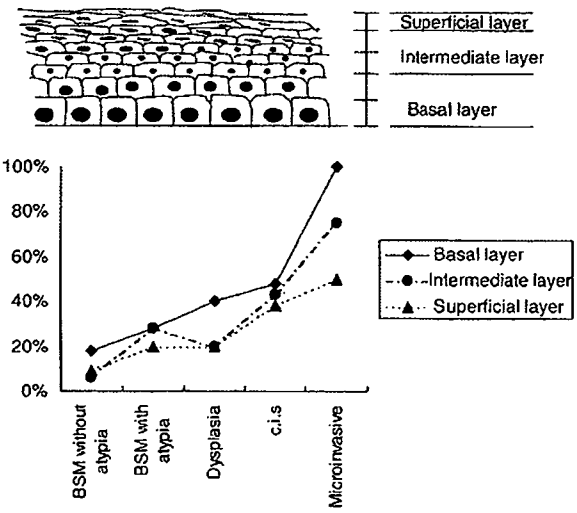

Fig. 2 Proportion of cases with reduced expression of either E-cadherin or catenins within the basal layer (♦), the intermediate layer (●) or the superficial layer (▲) of the intrabronchial lesions. The relative distribution of the different layers is shown in the upper part of the figure.

squamous metaplasia and dysplasia can be considered as precancerous lesions, mutation of the p53 tumor suppressor gene, and deletion of chromosome 17p have been reported in such lesions [39–42]. We have reported sequential changes in cell proliferation, DNA aneuploidy and accumulation of mutant p53 protein during carcinogenesis in the bronchial epithelium, and that these histochemical changes initially occurred in the basal layer [43]. We believe that the ability of cancerous cells to invade the bronchial wall will be acquired in a sequential manner during carcinogenesis. Therefore, we investigated the reduction of expression of E-cadherin and/or catenins in intrabronchial precancerous lesions and the early stages of bronchial squamous cell carcinoma. In intrabronchial lesions and squamous cell carcinoma, expression of either E-cadherin or catenins was reduced in 21% of BSM without atypia, 28% of BSM with atypia, 40% of dysplasia, 48% of carcinoma in situ, 100% of carcinoma microinvasive to the bronchial wall, and 100% of squamous cell carcinoma. We also demonstrated a positive correlation between the expression of these molecules and the grade of atypia of intrabronchial lesions. Our previous studies showed that reduced expression of E-cadherin and catenins occurs frequently in non-small cell lung carcinoma [35]. Hence, our present findings indicate that downregulation of E-cadherin and catenins may play an important role in the progression of human intrabronchial lesions and squamous cell carcinoma.

Studies on cell–cell adhesion molecules may help to clarify the mechanisms of local invasion and metastasis. Investigations of the cadherin–catenin complex have been carried out at the cellular and molecular levels [14,22,44]. It has already been reported that reduction of E-cadherin expression is caused by mutation and by inactivation of the E-cadherin gene by hypermethylation in the promoter region [45]. Dysfunction of the cadherin–catenin complex caused by reduction of the expression of these molecules implies an increased ability of cancer cells to disperse, which is the probable early step of local invasion and metastasis. Reduction of expression of E-cadherin and α-catenin is associated with local invasion and metastasis of scirrhous carcinoma in gastric cancer, breast cancer, and esophageal cancer [20,22].

In BSM with atypia and dysplasia, cells showing reduction of E-cadherin and/or catenin expression were localized mainly in the basal layer. As histological atypia increased, reduced expression of each molecule also became evident in the intermediate and superficial layers. This observation parallels the finding that proliferating cells and cells with accumulation of mutant p53 protein appeared from the basal layer to the superficial layer during carcinogenesis in the bronchus [43]. Therefore, we hypothesize that these cellular changes indicate an increased risk of eventual malignant transformation, and also that cells in the basal layer are the first to acquire the capacity for local invasion.

Our present study suggests that reduction of expression of E-cadherin and/or catenins is a rela-

Table 3 Aberrant expression rate of E-cadherin and/or catenins in intrabronchial lesions

	BSM without atypia	BSM with atypia	Dysplasia	c.i.s	Microinvasion to bronchial wall	Sq.c.ca.
Basal layer	6 (18%)	7 (28%)	2 (40%)	10 (48%)	4 (100%)	100%
Intermediate layer	2 (6%)	7 (28%)	1 (20%)	9 (43%)	3 (75%)	
Superficial layer	3 (9%)	5 (20%)	1 (20%)	8 (38%)	2 (50%)	
Whole layer	7 (21%)	7 (28%)	2 (40%)	10 (48%)	4 (100%)	
Total (cases)	32	25	5	21	4	32

tively early event in the genesis of bronchial squamous cell carcinoma, and that increasing histological atypia is accompanied by further diminution in the expression of these molecules. Finally, reduced levels of E-cadherin and/or catenins might play a critical role in local invasion beyond the basement membrane and the development of the advanced stage of squamous cell lung carcinoma.

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Gefitinib in the adjuvant setting: safety results from a phase III study in patients with completely resected non-small cell lung cancer

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Standard therapy for stage I–IIIA non-small cell lung cancer (NSCLC) is surgery, although adjuvant therapies are required to prevent disease recurrence and improve patient survival. This is the first study that planned to administer adjuvant gefitinib (Iressa) 250 mg/day or placebo to randomized patients with completely resected NSCLC (stage IB–IIIA) 4–6 weeks following surgery, for 2 years, until recurrence/withdrawal. However, recruitment was stopped after the randomization of 38 patients, because interstitial lung disease (ILD)-type events were being increasingly reported in Japan in the advanced disease setting. Finally, the trial was halted. Safety data for 38 recruited patients (18 gefitinib and 20 placebo) showed no unexpected adverse drug reactions (ADRs), with the most common being grade 1/2 gastrointestinal and skin disorders in 12 and 16 patients receiving gefitinib and in five and six patients receiving placebo, respectively. Grade 3/4 ADRs occurred in four patients receiving gefitinib and one patient receiving placebo. ILD-type events were reported in one patient receiving gefitinib (concomitantly with other ILD-inducing drugs) who died and two patients receiving placebo. Eight patients receiving gefitinib withdrew due to ADRs compared with three patients receiving placebo. Adverse events associated with surgical complications were reported for six patients receiving gefitinib and four patients receiving placebo. In the adjuvant setting there were no unexpected adverse events observed. Gefitinib had no impact on surgery-related complications when given within 4–6 weeks post-operatively. *Anti-Cancer Drugs* 16:1123–1128 © 2005 Lippincott Williams & Wilkins.

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Keywords: gefitinib, non-small cell lung cancer, phase III, safety

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Introduction

Non-small cell lung cancer (NSCLC) is generally not diagnosed until the disease is symptomatic, by which time more than two-thirds of patients are in the advanced stages of disease and have a poor prognosis [1]. Approximately 25% of patients with NSCLC are diagnosed when their disease is in the early stages; however, as many of these patients frequently have undetectable metastases, disease often recurs in distant sites [2]. Adjuvant therapies are therefore required to help prevent disease recurrence and as they will need to be given to patients post-operatively for a prolonged period, they should be well tolerated.

Although some clinical trials in NSCLC have shown a significant survival benefit with adjuvant uracil plus tegafur (UFT) and cisplatin-based chemotherapy [3–7], others have not observed a significant improvement in survival [5,8,9]. At the time of commencing this study, there were no standard adjuvant treatment regimens for NSCLC.

Gefitinib (Iressa), an orally active epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI), was approved in Japan for the treatment of inoperable or recurrent NSCLC in 2002. Two large phase II trials, IDEAL (Iressa Dose Evaluation in Advanced Lung cancer) 1 and 2, observed objective responses and stable disease in more than 40% of pre-treated patients with NSCLC receiving 250 mg/day gefitinib, with the majority of adverse events (AEs) being mild to moderate gastrointestinal and skin disorders [10,11]. Gefitinib was not associated with the well-recognized AEs observed with cytotoxic chemotherapy (e.g. bone marrow depression, neurotoxicity, nephrotoxicity). The tolerability profile of gefitinib has been confirmed by data from the